Single-molecule sequencing of the *Drosophila serrata* genome

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**Keywords:** *Drosophila, montium*, PacBio, Celera, long-reads, genome assembly
ABSTRACT

Long read sequencing technology promises to greatly enhance de novo assembly of genomes for non-model species. Although the high error rates of long reads have been a stumbling block, sequencing at high coverage permits the self-correction of many errors. Here we sequence and de novo assemble the genome of Drosophila serrata, a species from the montium subgroup that has been well studied for latitudinal clines, sexual selection, and gene expression but which lacks a reference genome. Using 11 PacBio SMRT cells, we generated 12 Gbp of raw sequence data comprising approximately 65x whole genome coverage. Read lengths averaged 8,940 bp (NRead50 12,200) with the longest read at 53 Kbp. We self-corrected reads using the PBDagCon algorithm and assembled the genome using the MHAP algorithm within the PBcR assembler. Total genome length was 198 Mbp with an N50 just under 1 Mbp. Contigs displayed a high degree of arm-level conservation with D. melanogaster and many could be sensibly placed on the D. serrata physical map. We also provide an initial annotation for this genome using in silico gene predictions that were supported by RNA-seq data.
INTRODUCTION

Second-generation sequencing (2GS) platforms, such as Illumina sequencing-by-synthesis, have dramatically reduced genome sequencing costs while increasing throughput exponentially (Shendure and Ji 2008). The relatively low cost and massive throughput of second-generation sequencing platforms have paved the way for sequencing and \textit{de novo} assembly of thousands of species’ genomes (Alkan et al. 2011). Second-generation sequencing methods generate short reads (less than a few hundred base pairs in length) that have limitations for \textit{de novo} genome assembly, where assembly is performed without the aid of a reference genome (Green 1997; Miller et al. 2008; Nagarajan and Pop 2013; Alkan et al. 2011). With short reads, \textit{de novo} assembly is an inherently difficult computational problem because repetitive DNA sequences are often much longer than the length of each read (Ukkonen 1992). For instance, it has been estimated that short read \textit{de novo} assemblies could be missing up to 20% of sequence information because repeat DNA sequences can increase the number of misassembled and fragmented regions (Schatz et al. 2010; Alkan et al. 2011; Ukkonen 1992). One way to alleviate the problem of repetitive DNA in the \textit{de novo} assembly process has been to incorporate a second set of mate-pair libraries with very long inserts (>2 kbp) (Li et al. 2010; Chaisson et al. 2009; Simpson et al. 2009; Alkan et al. 2011; Butler et al. 2008). Mate-pair libraries can resolve repeats (Treangen and Salzberg 2012; Wetzel et al. 2011) and improve scaffolding (van Heesch et al. 2013), but paired-end contamination and insert size mis-estimation can also lead to mis-assemblies (Phillippy et al. 2008; Sahlin et al. 2016).

More recently, third-generation (3GS) single-molecule sequencing technologies such as Pacific Biosciences’ (PacBio) SMRT sequencing and Oxford Nanopore’s MinION sequencing, which currently produce much longer reads, up to 54 kbp (Lee et al. 2014) and > 10 kbp (Quick et al. 2014), respectively, can overcome some of the shortcomings of 2GS assembly (Berlin et al. 2015). Although long-read sequencing technology produces reads with a high error rate, ranging from 82.1% (Chin et al. 2011) to 84.6% accuracy (Rasko et al. 2011), sequencing errors occur at more or less random positions across long-reads (Chin et al. 2013) and can be corrected with 2GS
short-read data (Koren et al. 2012) or by using excess 3GS reads for a self-correction (Chin et al. 2013).

In this paper, we use PacBio long-read sequencing to de novo assemble the genome of the fly, *Drosophila serrata*, which has been particularly well studied from an evolutionary standpoint. *D. serrata* is a member of the *Drosophila montium* subgroup, which split from the *D. melanogaster* subgroup approximately 40 Mya (Tamura et al. 2004), and consists of an estimated 98 species (Brake and Bächli 2008). At present, only one draft genome assembly (*D. kikkawai*) is available (Chen et al. 2014) from this species-rich subgroup. *D. serrata* has a broad geographical distribution, ranging from Papua New Guinea to south eastern Australia and has emerged as a powerful model for addressing evolutionary questions such as the evolution of species borders (Blows and Hoffman 1993; Hallas et al. 2002; Magiafoglou et al. 2002) and climate adaptation (Frentiu and Chenoweth 2010; Latimer et al. 2011; Kellermann et al. 2009). The species has also been used to investigate sexual selection (Hine et al. 2002; Gosden and Chenoweth 2011; Frentiu and Chenoweth 2008; Chenoweth et al. 2015), male mate choice (Chenoweth and Blows 2003; Chenoweth et al. 2007), mate recognition (Higgin et al. 2000), sexual dimorphism (Chenoweth et al. 2008; Yassin et al. 2016), sexual conflict (Delcourt et al. 2009) and indirect genetic effects (Chenoweth et al. 2010b). Its cuticular hydrocarbons, which serve as contact pheromones (Chung et al. 2014), have been extensively used to develop novel multivariate quantitative genetic approaches for exploring genetic constraints on adaptation (Blows et al. 2004; Chenoweth et al. 2010a; McGuigan et al. 2011b; Rundl et al. 2009).

Despite the importance of *D. serrata* as a model for evolutionary research, our poor understanding of its genome remains a significant limitation. Linkage and physical genome maps are available (Stocker et al. 2012), and an expressed sequence tag (EST) library has been developed (Frentiu et al. 2009), but the species lacks a draft genome. Here we report the sequencing and assembly of the *D. serrata* genome using exclusively Pacific Biosciences SMRT technology. We also provide an initial annotation of the genome based on in silico gene predictors and mRNA-seq data. Our de novo genome and its annotation will provide a resource for ongoing population
genomic and trait mapping studies in this species as well as facilitate broader studies of genome evolution in the family Drosophilidae.

MATERIALS AND METHODS

Fly Strains and DNA Extraction

We sequenced a mix of ~100 mg of males and females from a single inbred line that originated from Forster, Australia, and had been inbred via full-sib mating for 10 generations before being maintained at a large population size (N ~ 250 individuals) (McGuigan et al. 2011b). A single further generation of full-sib inbreeding was applied before extraction of DNA. This same inbred line was used for the D. serrata linkage map, was the founding line for previous mutation accumulation studies (Latimer et al. 2015; McGuigan et al. 2014a; McGuigan et al. 2014b; McGuigan et al. 2011a) and is fixed for the light female abdominal pigmentation phenotype mapped by Yassin et al. (2016). High molecular weight DNA was extracted from fly bodies (heads were excluded to reduce eye pigment contamination) using a Qiagen Gentra Puregene Tissue Kit (Cat #158667) which produced fragments > 100 kbp (measured using pulsed-field gel electrophoresis). Two phenol-chloroform extractions were performed by the University of California-Davis DNA Technologies Core prior to preparation of a standard sequencing library.

Genome Sequencing and Assembly

DNA was sequenced using 11 SMRT cells and P6-C4 chemistry on the Pacific Biosciences RS II platform. In total this produced ~13 billion base pairs spanning 136,119 filtered subreads with a mean read length of 8,840 bp and an N50 of 12,220 bp (Figure S1). The PacBio genome was assembled using the PbcR pipeline that implements the MHAP algorithm within the Celera Assembler (version 8.3rc2) (Berlin et al. 2015) and polished with Quiver (GenomicConsensus version 0.9.2 and ConsensusCore version: 0.8.8) (Chin et al. 2013) in three steps: (1) errors were corrected in reads using PBDagCon, which requires at least 50x genome coverage and utilizes the consensus of over-sampled sequences (Chin et al. 2013), (2) overlapping sequences were assembled using MHAP and the Celera Assembler (Berlin et al.
2015), and (3) contigs were polished with Quiver to correct for spurious SNP calls and small indels (Chin et al. 2013). The “sensitive” setting was used for both read correction and genome assembly (Berlin et al. 2015) whereas the default settings were used for polishing with Quiver (Chin et al. 2013). We elected to correct all reads as opposed to the default longest 40x. The longest 25x corrected reads were subsequently used for genome assembly. The PBDagCon correction was performed on a computer with 60 CPU cores and 1TB of RAM; 58 CPU cores were used for the assembly and the amount of RAM used, although not tracked, was far less than machine capacity. Error correction with PBDagCon took ~26 days. Assembly of corrected reads using MHAP and the Celera Assembler took ~19 hours using 28 CPU cores. Our initial runs using the much faster error correction algorithm (HGAP) produced a slightly shorter assembly (194 Mbp compared to 198 Mbp) with a slightly lower N50 (0.88 Mbp vs 0.95 Mbp). We therefore chose to use the more sensitive PBDagCon correction method.

**Transcriptome Sequencing and Assembly**

The same inbred fly strain that was the progenitor for DNA sequencing was also used for adult mRNA sequencing to annotate the *D. serrata* genome. Adult males and females were transferred to fresh vials shortly after eclosion and held in groups of ~25 where they were allowed to mate and lay eggs for 2 days. They were then sexed under light CO₂ anesthesia and snap frozen using liquid nitrogen in groups of 10, at the time of freezing all flies were assumed to be non-virgins. Total RNA was extracted from each pool of flies using the standard Trizol protocol. Initial quality assessment of the total RNA using a NanoDrop and gel electrophoresis indicated that the RNA was of high quality, with a RNA integrity number (RIN) greater than 7. RNA was stored at -80 degrees Celsius for several days before being shipped for sequencing.

One male and one female 75 bp paired-end sequencing library was prepared using the TruSeq Stranded mRNA Library prep kit and sequenced on an Illumina NextSeq500 at the Ramaciotti Centre for Genomics, University of New South Wales, Australia. In total 79 M and 88 M reads were produced for males and females respectively. Quality assessment of the RNA-seq data using FastQC (Andrews 2010) indicated that the reads were of a high quality and therefore no trimming of reads was performed. The
transcriptome was *de novo* assembled for each sex separately using Trinity version 2.1.1 (Grabherr et al. 2011) where all reads were used and the --jaccard_clip option was enabled to minimize gene fusion events caused by UTR overlap in high gene density regions.

**Annotation**

Maker version 2.31.8 (Campbell et al. 2014; Holt and Yandell 2011) was used to annotate the PacBio genome via incorporation of *in silico* gene models detected by Augustus (Stanke and Morgenstern 2005) and/or SNAP (Johnson et al. 2008), the *de novo* *D. serrata* male and female transcriptomes, and protein sequences from 12 *Drosophila* species genomes (*D. ananassae* r1.04, *D. erecta* r1.04, *D. grimshawi* r1.3, *D. melanogaster* r6.07, *D. mojavensis* r1.04, *D. persimilis* r1.3, *D. pseudoobscura* pseudoobscura r3.03, *D. sechellia* 1.3, *D. simulans*, r2.01, *D. virilis* r1.03, *D. willistoni* r1.04, and *D. yakuba* r1.04) obtained from FlyBase (McQuilton et al. 2012; Attrill et al. 2016). Repeat masking was performed based on *D. melanogaster* training (Smit et al. 1996). Maker was run with default settings apart from allowing Maker to take extra steps to identify alternate splice variants and correct for erroneous gene fusion events.

**Data Availability Statement**

All sequence data including PacBio and RNA-seq reads have been submitted to public repositories and are available via the *D. serrata* genome NCBI project accession PRJNA355616. The genome assembly and annotation tracks are available in gff formats from [http://www.chenowethlab.org](http://www.chenowethlab.org). We also supply a list of *D. melanogaster* orthologs in Supplementary Table S1.

**RESULTS & DISCUSSION**

To assemble a draft *D. serrata* genome we sequenced DNA from a pool of adult males and females that originated from a single inbred line to a coverage of approximately 65x using Pacific Bioscience (PacBio) long-read, single-molecule real-time (SMRT) sequencing technology. We produced 136,119 filtered subreads with a
mean read length of 8,940 bp and a read N50 of 12,200 bp that spanned greater than ~13 Gbp (Figure S1). The PacBio reads were assembled using the MHAP algorithm within the Celera Assembler (Miller et al. 2008; Berlin et al. 2015) after self-correction using PBDagCon (Chin et al. 2013). The final genome was polished with a single iteration of Quiver (Chin et al. 2013) and consisted of 1,360 contigs containing more than 198 Mbp with a GC content of 39.13% (Table 1). The longest contig was ~7.3 Mbp and the N50 of all contigs was ~0.95 Mbp. Flow cytometry studies suggest that species of the montium subgroup commonly have genome lengths over 200 Mbp (Gregory and Johnston 2008) with the estimate for the female *D. serrata* genome being approximately 215 Mbp (0.22 pg). This estimate is in broad agreement with our assembly length of 198 Mbp for the female genome.

Completeness

Genome completeness was assessed using BUSCO gene set analysis version 2.0 which includes a set of 2799 genes specific to Diptera (Simao et al. 2015). The *D. serrata* assembly contained 96.2% of the BUSCO genes with 94.1% being complete single-copy (defined as complete when the gene’s length is within two standard deviations of the BUSCO group’s mean length) and 2.5% detected as fragmented. Only 1.3% of the BUSCO genes were not found in the *D. serrata* assembly (Table 2). Completeness of the *D. serrata* genome was similar to the reference *D. melanogaster* genome (version r6.05), which contained 98.7% complete BUSCO genes. As a further point of comparison we computed BUSCO metrics for a recent PacBio-only assembly of the *D. melanogaster* ISO1 strain genome using all 790 contigs rather than the 132 that were constructed from > 50 reads only ([http://www.cbcb.umd.edu/software/PBcR/MHAP/](http://www.cbcb.umd.edu/software/PBcR/MHAP/)[quivered full assembly]), and we also analysed the only other member of the montium subgroup with a publically available genome assembly, *D. kikkawai* ([https://www.hgsc.bcm.edu/arthropods/drosophila-modencode-project;](https://www.hgsc.bcm.edu/arthropods/drosophila-modencode-project) NCBI PRJNA62319). Although these assemblies tended to contain marginally lower numbers of missing BUSCOs, metrics were generally very similar (Table 2), indicating high level of completeness for the *D. serrata* assembly.
**Fragmentation and Mis-assemblies**

Although our assembly consisted of 1,360 contigs with a N50 of 0.94M, which was an N50 at the upper end of what might be expected for a short read assembly, it is much lower than a recent PacBio only assembly of the *D. melanogaster* genome (Berlin et al. 2015). There are several reasons why this might be the case. First, we report metrics on all contigs in the assembly rather than excluding those that incorporated fewer than 50 reads as was the case for the *D. melanogaster* assembly (Berlin et al. 2015) (132 contigs with an N50 of 13.6M). Excluding such contigs resulted in a *D. serrata* assembly of only 273 contigs with a total genome length of 175 Mbp (vs. 198 Mbp) and an N50 of 1.4 Mbp. In this reduced assembly, half of the genome was represented in only 25 contigs, which is closer to the performance seen for *D. melanogaster*. While contigs with less than 50 read support were generally short (median: 23.5 kbp, range: 6.3-110 kbp) and may be excluded in some cases on the basis of quality, when we examined the *D. serrata* annotation data we saw that many of these contigs contained predicted genes that had RNA-seq support, including 14 complete single-copy BUSCOs. We have therefore retained all contigs in our assembly.

Second, although our N50 filtered subread length of 12,200 kbp is on par with the *D. melanogaster* P5C3 filtered subread lengths (12.2-14.2 kbp) (Kim et al. 2014), we had approximately half the coverage of the *D. melanogaster* assembly (65x vs 130x), which may have reduced our ability to span repetitive regions of the *D. serrata* genome. To examine this further, we reran the PBcR pipeline with *D. melanogaster* data from (Kim et al. 2014) but downsampled it to 65x. We did not see genome contiguity drop to the levels seen for *D. serrata* (data not shown) and note that similar findings were observed by Chakraborty et al. (2016; Figure 5). It therefore seems likely that the *D. serrata* genome, which is longer than that of *D. melanogaster*, may also be more complex due to longer repetitive regions. Therefore, adequate repeat-spanning coverage would presumably require additional very long reads to achieve the same assembly contiguity seen for *D. melanogaster*. A third factor possibly contributing to a higher degree of fragmentation in our assembly is residual heterozygosity which may have been higher in our *D. serrata* line, which had at least 11 generations of inbreeding, than the ISO1 *D. melanogaster* line.
We used several methods to assess the quality of the genome in regards to mis-assemblies. Firstly, because physical maps indicate very strong chromosome arm-level conservation of gene content between *D. serrata* and *D. melanogaster* (Stocker et al. 2012), we examined possible mis-assemblies between chromosomal arms by aligning the six largest contigs (total length ~ 37 Mbp) to the *D. melanogaster* genome using MUMmer (Kurtz et al. 2004). If there were no chromosome arm misplacements, then it was expected that each contig would align to a single *D. melanogaster* chromosome arm, albeit fragmented due to changes in gene order. This was largely the case (Figure 1), where each contig aligned to a single *D. melanogaster* chromosome arm but with minor sections of alignment to other chromosome arms towards the contig edges where repetitive elements were more likely to be found. The one major exception to this general pattern of conservation was found in the longest contig in the assembly, contig 3208, that aligned mainly to *D. melanogaster* 3R but contained an approximately 600 kbp segment that aligned to *D. melanogaster* 3L. To test whether this was likely to be a mis-assembly, we searched the contig for previously published SNP markers that have been placed on the *D. serrata* linkage map. The marker m25 (Stocker et al 2012), which maps to 3L, was located in the suspected mis-assembled region (contig 3208, position 3,537,591) indicating that a mis-assembly rather than a genomic translocation rearrangement between 3R and 3L was most likely.

To further examine assembly quality, we compared our assembly to the entire physical genome map of *D. serrata* (Stocker et al. 2012), where *in situ* hybridization was used to physically locate 78 genes. We were able to assess possible mis-assemblies when a contig contained multiple physically mapped genes (11 contigs ranging in size from ~1 Mbp to ~6 Mbp). Using this approach, we observed no apparent chromosome arm-level assignment errors beyond that seen for contig 3208 (Figure 2). When contigs contained three or more physically mapped genes, gene order could be examined. We saw three cases of apparent gene order reversal (two on 2R and one on 3L). Interestingly, two of these regions map to the positions of known chromosomal inversions (Mavragani-Tsipidou et al. 1990), which is perhaps not unexpected given that different inbred lines were used for the physical map and genome sequencing. After considering these probable inversions, gene order and
location appears to be largely correct for these 11 contigs at least. For contig 3208, each section that aligned to 3R could be placed on the physical map only after splitting the contig into 3 pieces based on the previously identified mis-assembly above.

The conservation of chromosome arm-level gene content was a common feature of the remaining contigs as well. For example, while only 354 contigs contained significant tBLASTx hits to at least one *D. melanogaster* gene (genome version 6.05), these contigs spanned 167 Mbp, and the vast majority had greater than 90% tBLASTx hits to a single *D. melanogaster* chromosomal arm (mean = 96.35%, median = 100%) (Figure 3). Furthermore, only 34 contigs displayed less than 90% similarity to *D. melanogaster* and a linear regression where contig size predicted percent similarity indicated no significant relationship ($F_{(1,32)} = 0.4003$, $p = 0.5314$), suggesting that very large contigs were no more likely to be mis-assembled than short contigs.

**Annotation**

To facilitate annotation of the *D. serrata* genome we sequenced mRNA from male and female adult flies. The *in silico* gene predictors SNAP (Johnson et al. 2008) and Augustus (Stanke and Morgenstern 2005), found 22,718 and 15,984 genes respectively. Of these *in silico* predicted genes, a total of 14,271 protein coding genes were sufficiently supported by RNA-seq and/or protein sequence data to be annotated by Maker2 (16,202 transcripts) (Holt and Yandell 2011). While the number of genes we annotated in *D. serrata* is similar to the 13,929 protein coding genes that have currently been annotated in *D. melanogaster* (genome version 6.05), we annotated far fewer total transcripts (31,482 identified in *D. melanogaster*) (Attrill et al. 2016), this is likely due to the larger number of tissue types and life stages for which *D. melanogaster* gene expression has been characterized with RNA-seq. Maker scores annotations using the annotation edit distance (AED), a zero-to-one score where a value of zero indicates that the *in silico* annotation and the empirical evidence are in perfect agreement and a value of one indicates that the *in silico* annotation has no support from empirical data (Eilbeck et al. 2009). The AED for the *D. serrata* genome had a mean score of 0.18 and median of 0.13 suggesting that most annotations were of
high quality with strong empirical support. Considering that in *Drosophila*
appreciable numbers of genes peak in expression during early life stages such as
embryogenesis (Arbeitman et al. 2002), our use of adult fly RNA-seq data may mean
that some such genes are yet to be annotated. Furthermore, as we used mRNA-seq we
have not yet annotated non-coding genes of which there are 3,503 in the *D.
melanogaster* genome (Attrill et al. 2016). Future RNA-seq datasets will be used to
update the existing gene models.

We observed differences in gene, exon and intron lengths between *D. serrata* and *D.
melanogaster*. In *D. serrata* there were on average 3.9 exons per protein coding gene
and the gene, exon, and intron lengths were 4,655 bp, 451 bp, and 699 bp
respectively. Apart from average exon number which does not differ between the two
species, these values are lower than those for *D. melanogaster* protein coding genes
(genome version 6.05): where the mean gene, exon, and intron lengths are 6,962 bp,
539 bp, and 1,704 bp respectively (Attrill et al. 2016). The lower average intron
length observed in *D. serrata* may be a consequence of annotating far fewer alternate
splice variants. In total, coding sequence comprised 33.6% of the genome when
including introns and 15.4% of the genome when considering only exons. Lower
percentage intron content has been associated with overall longer genomes in the
Drosophilidae (Gregory and Johnston 2008) which is consistent with our observations
here.

Many of the annotated genes in *D. serrata* were found to be putative orthologs of *D.
melanogaster* genes (Supplementary Table S1). In total 10,995 (77%) were found to
be orthologs via best reciprocal BLAST (Huynen and Bork 1998; Moreno-Hagelsieb
and Latimer 2008; Tatusov et al. 1997) using tBLASTx with default settings
(Camacho et al. 2009) and version 6.05 of the *D. melanogaster* genome (Drosophila
12 Genomes et al. 2007; McQuilton et al. 2012). The median e-value of each
reciprocal comparison was zero, indicating that most orthologs are very similar to one
another. Furthermore, when comparing *D. serrata* genes to *D. melanogaster*, the
largest e-value was 1.6 with only 85 orthologs having an e-value greater than 1e⁻¹⁰.
Similarly, when comparing *D. melanogaster* genes to *D. serrata*, the largest e-value
was 0.18 with only 78 orthologs having an e-value greater than 1e⁻¹⁰. The correlation
between e-values for the reciprocal BLAST was 0.88.
**Conclusion**

We have assembled a draft genome for a species with no existing genome using only 3GS data. Our study indicates the feasibility of long read-only genome assembly for non-model species with modest sized genomes when using an inbred line. While either greater 3GS coverage or a hybrid merged assembly (Chakraborty et al. 2016) may be required to provide greater genome contiguity, it is clear the genome has a high degree of completeness in terms of gene content and that mis-assemblies at chromosome arm level are rare. The genome and its initial annotation provide a useful resource of future population genomic and trait mapping studies in this species.

**ACKNOWLEDGEMENTS**

We thank S. Koren for advice regarding the PBcR pipeline. Funding for this research was provided by The University of Queensland.

**REFERENCES**

Alkan, C., S. Sajjadian, and E.E. Eichler, 2011 Limitations of next-generation genome sequence assembly. *Nat Methods* 8 (1):61-65.

Andrews, S., 2010 FastQC: A quality control tool for high throughput sequence data. *Reference Source*.

Arbeitman, M.N., E.E. Furlong, F. Imam, E. Johnson, B.H. Null *et al.*, 2002 Gene expression during the life cycle of Drosophila melanogaster. *Science* 297 (5590):2270-2275.

Attrill, H., K. Falls, J.L. Goodman, G.H. Millburn, G. Antonazzo *et al.*, 2016 FlyBase: establishing a Gene Group resource for Drosophila melanogaster. *Nucleic Acids Res* 44 (D1):D786-792.

Berlin, K., S. Koren, C.-S. Chin, J.P. Drake, J.M. Landolin *et al.*, 2015 Assembling large genomes with single-molecule sequencing and locality-sensitive hashing. *Nat Biotechnol*.

Blows, M.W., S.F. Chenoweth, and E. Hine, 2004 Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. *Am Nat* 163 (3):329-340.

Blows, M.W., and A.A. Hoffman, 1993 The genetics of central and marginal populations of Drosophila serrata. I. Genetic variation for stress resistance and species borders. *Evolution*:1255-1270.

Brake, I., and G. Bächli, 2008 *Drosophilidae (Diptera)*: Brill.
Butler, J., I. MacCallum, M. Kleber, I.A. Shlyakhter, M.K. Belmonte et al., 2008 ALLPATHS: de novo assembly of whole-genome shotgun microreads. *Genome Res* 18 (5):810-820.

Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos et al., 2009 BLAST+: architecture and applications. *BMC Bioinformatics* 10 (1):421.

Campbell, M.S., C. Holt, B. Moore, and M. Yandell, 2014 Genome Annotation and Curation Using MAKER and MAKER-P. *Current Protocols in Bioinformatics*:4.11. 11-14.11. 39.

Chaisson, M.J., D. Brinza, and P.A. Pevzner, 2009 De novo fragment assembly with short mate-paired reads: Does the read length matter? *Genome Research* 19 (2):336-346.

Chakraborty, M., J.G. Baldwin-Brown, A.D. Long, and J.J. Emerson, 2016 Contiguous and accurate de novo assembly of metazoan genomes with modest long read coverage. *Nucleic Acids Res* 44 (19):e147.

Chen, Z.X., D. Sturgill, J. Qu, H. Jiang, S. Park et al., 2014 Comparative validation of the D. melanogaster modENCODE transcriptome annotation. *Genome Res* 24 (7):1209-1223.

Chenoweth, S.F., N.C. Appleton, S.L. Allen, and H.D. Rundle, 2015 Genomic Evidence that Sexual Selection Impedes Adaptation to a Novel Environment. *Current Biology* 25 (14):1860-1866.

Chenoweth, S.F., and M.W. Blows, 2003 Signal trait sexual dimorphism and mutual sexual selection in *Drosophila serrata*. *Evolution* 57 (10):2326-2334.

Chenoweth, S.F., D. Petfield, P. Doughty, and M.W. Blows, 2007 Male choice generates stabilizing sexual selection on a female fecundity correlate. *J Evol Biol* 20 (5):1745-1750.

Chenoweth, S.F., H.D. Rundle, and M.W. Blows, 2008 Genetic constraints and the evolution of display trait sexual dimorphism by natural and sexual selection. *Am Nat* 171 (1):22-34.

Chenoweth, S.F., H.D. Rundle, and M.W. Blows, 2010a The contribution of selection and genetic constraints to phenotypic divergence. *Am Nat* 175 (2):186-196.

Chenoweth, S.F., H.D. Rundle, and M.W. Blows, 2010b Experimental evidence for the evolution of indirect genetic effects: changes in the interaction effect coefficient, psi (ψ), due to sexual selection. *Evolution* 64 (6):1849-1856.

Chin, C.S., D.H. Alexander, P. Marks, A.A. Klammer, J. Drake et al., 2013 Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10 (6):563-569.

Chin, C.S., J. Sorenson, J.B. Harris, W.P. Robins, R.C. Charles et al., 2011 The origin of the Haitian cholera outbreak strain. *N Engl J Med* 364 (1):33-42.

Chung, H., D.W. Loehlin, H.D. Dufour, K. Vaccarro, J.G. Millar et al., 2014 A Single Gene Affects Both Ecological Divergence and Mate Choice in Drosophila. *Science* 343 (6175):1148-1151.

Delcourt, M., M.W. Blows, and H.D. Rundle, 2009 Sexually antagonistic genetic variance for fitness in an ancestral and a novel environment. *Proc Biol Sci* 276 (1664):2009-2014.

Drosophila 12 Genomes, C., A.G. Clark, M.B. Eisen, D.R. Smith, C.M. Bergman et al., 2007 Evolution of genes and genomes on the Drosophila phylogeny. *Nature* 450 (7167):203-218.

Drosopoulou, E., I. Konstantopoulou, and Z.G. Scouras, 1996 The heat shock genes in the Drosophila montium subgroup: Chromosomal localization and evolutionary implications. *Chromosoma* 105 (2):104-110.
Drosopoulou, E., and Z.G. Scouras, 1995 The β-tubulin gene family evolution in the Drosophila montium subgroup of the melanogaster species group. *Journal of Molecular Evolution* 41 (3):293-298.

Drosopoulou, E., and Z.G. Scouras, 1998 The organization of the alpha-tubulin gene family in the Drosophila montium subgroup of the melanogaster species group. *Genome* 41 (4):504-509.

Drosopoulou, E., M. Tsiafouli, P. Mavragani-Tsipidou, and Z.G. Scouras, 1997 The glutamate dehydrogenase, E74 and putative actin gene loci in the Drosophila montium subgroup. Chromosomal homologies among the montium species and D. melanogaster. *Chromosoma* 106 (1):20-28.

Drosopoulou, E., K. Wiebauer, M. Yiangou, P. Mavragani-Tsipidou, H. Domdey et al., 2002 Isolation, characterization, and localization of beta-tubulin genomic clones of three Drosophila montium subgroup species. *Genome* 45 (3):604-607.

Eilbeck, K., B. Moore, C. Holt, and M. Yandell, 2009 Quantitative measures for the management and comparison of annotated genomes. *BMC Bioinformatics* 10 (1):67.

Frentiu, F.D., M. Adamski, E.A. McGraw, M.W. Blows, and S.F. Chenoweth, 2009 An expressed sequence tag (EST) library for *Drosophila serrata*, a model system for sexual selection and climatic adaptation studies. *BMC Genomics* 10:40.

Frentiu, F.D., and S.F. Chenoweth, 2008 Polyandry and paternity skew in natural and experimental populations of Drosophila serrata. *Mol Ecol* 17 (6):1589-1596.

Frentiu, F.D., and S.F. Chenoweth, 2010 Clines in cuticular hydrocarbons in two *Drosophila* species with independent population histories. *Evolution* 64 (6):1784-1794.

Gosden, T.P., and S.F. Chenoweth, 2011 On the evolution of heightened condition dependence of male sexual displays. *J Evol Biol* 24 (3):685-692.

Grabherr, M.G., B.J. Haas, M. Yassour, J.Z. Levin, D.A. Thompson et al., 2011 Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol* 29 (7):644-652.

Green, P., 1997 Against a whole-genome shotgun. *Genome Research* 7 (5):410-417.

Gregory, T.R., and J.S. Johnston, 2008 Genome size diversity in the family Drosophilidae. *Heredity (Edinb)* 101 (3):228-238.

Hallas, R., M. Schiffer, and A.A. Hoffmann, 2002 Clinal variation in *Drosophila serrata* for stress resistance and body size. *Genet Res* 79 (2):141-148.

Higgie, M., S. Chenoweth, and M.W. Blows, 2000 Natural selection and the reinforcement of mate recognition. *Science* 290 (5491):519-521.

Hine, E., S. Lachish, M. Higgie, and M.W. Blows, 2002 Positive genetic correlation between female preference and offspring fitness.

Holt, C., and M. Yandell, 2011 MAKER2: an annotation pipeline and genome-database management tool for second-generation genome projects. *BMC Bioinformatics* 12 (1):491.

Huynen, M.A., and P. Bork, 1998 Measuring genome evolution. *Proc Natl Acad Sci USA* 95 (11):5849-5856.

Johnson, A.D., R.E. Handsaker, S.L. Pulit, M.M. Nizzari, C.J. O'Donnell et al., 2008 SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics* 24 (24):2938-2939.
Kellermann, V., B. van Heerwaarden, C.M. Sgro, and A.A. Hoffmann, 2009
Fundamental evolutionary limits in ecological traits drive Drosophila species
distributions. Science 325 (5945):1244-1246.
Kim, K.E., P. Peluso, P. Babayan, P.J. Yeadon, C. Yu et al., 2014 Long-read, whole-
genome shotgun sequence data for five model organisms. Sci Data 1:140045.
Koren, S., M.C. Schatz, B.P. Walenz, J. Martin, J.T. Howard et al., 2012 Hybrid error
correction and de novo assembly of single-molecule sequencing reads. Nat
Biotechnol 30 (7):693-700.
Kurtz, S., A. Phillippy, A.L. Delcher, M. Smoot, M. Shumway et al., 2004 Versatile
and open software for comparing large genomes. Genome Biol 5 (2):R12.
Latimer, C.A., B.R. Foley, and S.F. Chenoweth, 2015 Connecting thermal
performance curve variation to the genotype: a multivariate QTL approach. J
Evol Biol 28 (1):155-168.
Latimer, C.A., R.S. Wilson, and S.F. Chenoweth, 2011 Quantitative genetic variation
for thermal performance curves within and among natural populations of
Drosophila serrata. J Evol Biol 24 (5):965-975.
Lee, H., J. Gurtowski, S. Yoo, S. Marcus, W.R. McCombie et al., 2014 Error
correction and assembly complexity of single molecule sequencing reads.
BioRxiv:006395.
Li, R., H. Zhu, J. Ruan, W. Qian, X. Fang et al., 2010 De novo assembly of human
genomes with massively parallel short read sequencing. Genome Res 20
(2):265-272.
Magiafoglou, A., M. Carew, and A. Hoffmann, 2002 Shifting clinal patterns and
microsatellite variation in Drosophila serrata populations: a comparison of
populations near the southern border of the species range. Journal of
Evolutionary Biology 15 (5):763-774.
Mavragani-Tsipidou, P., N. Kyrpides, and Z.G. Scouras, 1990 Evolutionary
implications of duplications and Balbiani rings in Drosophila. A study of
Drosophila serrata. Genome 33 (4):478-485.
McGuigan, K., J.M. Collet, S.L. Allen, S.F. Chenoweth, and M.W. Blows, 2014a
Pleiotropic mutations are subject to strong stabilizing selection. Genetics 197
(3):1051-1062.
McGuigan, K., J.M. Collet, E.A. McGraw, Y.H. Ye, S.L. Allen et al., 2014b The
nature and extent of mutational pleiotropy in gene expression of male
Drosophila serrata. Genetics 196 (3):911-921.
McGuigan, K., D. Petfield, and M.W. Blows, 2011a Reducing mutation load through
sexual selection on males. Evolution 65 (10):2816-2829.
McGuigan, K., L. Rowe, and M.W. Blows, 2011b Pleiotropy, apparent stabilizing
selection and uncovering fitness optima. Trends Ecol Evol 26 (1):22-29.
McQuilton, P., S.E. St Pierre, J. Thurmond, and C. FlyBase, 2012 FlyBase 101--the
basics of navigating FlyBase. Nucleic Acids Res 40 (Database issue):D706-
714.
Miller, J.R., A.L. Delcher, S. Koren, E. Venter, B.P. Walenz et al., 2008 Aggressive
assembly of pyrosequencing reads with mates. Bioinformatics 24 (24):2818-
2824.
Moreno-Hagelsieb, G., and K. Latimer, 2008 Choosing BLAST options for better
detection of orthologs as reciprocal best hits. Bioinformatics 24 (3):319-324.
Nagarajan, N., and M. Pop, 2013 Sequence assembly demystified. Nat Rev Genet 14
(3):157-167.
Pardali, E., E. Feggou, E. Drosopoulou, I. Konstantopoulou, Z.G. Scouras et al., 1996 The Afrotropical Drosophila montium subgroup: Balbiani ring 1, polytene chromosomes, and heat shock response of Drosophila vulcana. *Genome* 39 (3):588-597.

Phillippy, A.M., M.C. Schatz, and M. Pop, 2008 Genome assembly forensics: finding the elusive mis-assembly. *Genome Biol* 9 (3):R55.

Quick, J., A.R. Quinlan, and N.J. Loman, 2014 A reference bacterial genome dataset generated on the MiniION portable single-molecule nanopore sequencer. *GigaScience* 3 (22):22.

Rasko, D.A., D.R. Webster, J.W. Sahl, A. Bashir, N. Boisen et al., 2011 Origins of the E. coli strain causing an outbreak of hemolytic-uremic syndrome in Germany. *N Engl J Med* 365 (8):709-717.

Rundle, H.D., S.F. Chenoweth, and M.W. Blows, 2009 The diversification of mate preferences by natural and sexual selection. *J Evol Biol* 22 (8):1608-1615.

Sahlin, K., R. Chikhi, and L. Arvestad, 2016 Assembly scaffolding with PE-contaminated mate-pair libraries. *Bioinformatics* 32 (13):1925-1932.

Schatz, M.C., A.L. Delcher, and S.L. Salzberg, 2010 Assembly of large genomes using second-generation sequencing. *Genome Research* 20 (9):1165-1173.

Shendure, J., and H. Ji, 2008 Next-generation DNA sequencing. *Nat Biotechnol* 26 (10):1135-1145.

Simao, F.A., R.M. Waterhouse, P. Ioannidis, E.V. Kriventseva, and E.M. Zdobnov, 2015 BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31 (19):3210-3212.

Simpson, J.T., K. Wong, S.D. Jackman, J.E. Schein, S.J. Jones et al., 2009 ABYSS: a parallel assembler for short read sequence data. *Genome Res* 19 (6):1117-1123.

Smit, A.F., R. Hubley, and P. Green, 1996 RepeatMasker Open-3.0.

Stanke, M., and B. Morgenstern, 2005 AUGUSTUS: a web server for gene prediction in eukaryotes that allows user-defined constraints. *Nucleic Acids Research* 33 (suppl 2):W465-W467.

Stocker, A.J., B. Foley, and A. Hoffmann, 2004 Inversion frequencies in Drosophila serrata along an eastern Australian transect. *Genome* 47 (6):1144-1153.

Stocker, A.J., B.B. Rusuwa, M.J. Blacket, F.D. Frentiu, M. Sullivan et al., 2012 Physical and Linkage Maps for *Drosophila serrata*, a Model Species for Studies of Clinal Adaptation and Sexual Selection. *G3 (Bethesda)* 2 (2):287-297.

Tamura, K., S. Subramanian, and S. Kumar, 2004 Temporal patterns of fruit fly (*Drosophila*) evolution revealed by mutation clocks. *Mol Biol Evol* 21 (1):36-44.

Tatusov, R.L., E.V. Koonin, and D.J. Lipman, 1997 A genomic perspective on protein families. *Science* 278 (5338):631-637.

Treangen, T.J., and S.L. Salzberg, 2012 Repetitive DNA and next-generation sequencing: computational challenges and solutions. *Nature Reviews Genetics* 13 (1):36-46.

Ukkonen, E., 1992 Approximate String-Matching with Q-Grams and Maximal Matches. *Theoretical computer science* 92 (1):191-211.

van Heesch, S., W.P. Kloosterman, N. Lansu, F.-P. Ruzius, E. Levandowsky et al., 2013 Improving mammalian genome scaffolding using large insert mate-pair next-generation sequencing. *BMC Genomics* 14 (1):1.
Wetzel, J., C. Kingsford, and M. Pop, 2011 Assessing the benefits of using mate-pairs to resolve repeats in de novo short-read prokaryotic assemblies. *BMC Bioinformatics* 12 (1):1.

Yassin, A., E.K. Delaney, A.J. Reddiex, T.D. Seher, H. Bastide *et al.*, 2016 The pdm3 locus is a hotspot for recurrent evolution of female-limited color dimorphism in *Drosophila*. *Current Biology* 26 (18):2412-2422.
Supplementary figure S1. Distribution of filtered subread lengths from 11 SMRT cells on the RS II with P6C4 chemistry.
Figure 1. Alignment of the six longest contigs from the *D. serrata* assembly, to the *D. melanogaster* genome version 6.05. Red dots indicate a MUMmer hit that aligns to the *D. melanogaster* genome in the forward orientation; blue dots indicate a MUMmer hit that aligns to the *D. melanogaster* genome in the reverse orientation.
Figure 2. Comparison between the draft genome assembly and the physical *D. serrata* genome map, image is adapted from Stocker et al. (2012). Genes in red were mapped by (Drosopoulou and Scouras 1995), (Drosopoulou et al. 1996; Drosopoulou et al. 1997; Drosopoulou et al. 2002), (Pardali et al. 1996), (Drosopoulou and Scouras 1998; Drosopoulou and Scouras 1995). Genes in blue are also included in the linkage map produced by Stocker et al. (2012). Thin red lines are inversions found by Stocker et al. (2004) and thin black lines are inversions found by Mavragani-Tsipidou et al. (1990). Contig3208 (shown in red), was split into three parts based on the mis-assembly, parts 1 and 3 aligned with *D. melanogaster* 3R and part 2 aligned with 3L (Figure 1). Markers Act88F and hsp70 were not mapped to contigs because the former appears twice and nomenclature changes meant we could not be certain exactly which gene hsp70 was referring to.
Figure 3. Comparison of *D. serrata* gene locations relative to *D. melanogaster*. On average > 95% of tBLASTx hits to *D. melanogaster* genes (version 6.05) in each contig map to a single *D. melanogaster* arm.
Table 1. *D. serrata* genome assembly statistics. Contig length percentages refer to percent total length in each size bin.

| Description            |      |
|------------------------|------|
| Number of contigs      | 1360 |
| Genome size (bp)       | 198298763 |
| Longest contig (bp)    | 7300740 |
| < 1 Kbp                | 0.0% |
| 1-10 Kbp               | 3.3% |
| 10-100 Kbp             | 78.8% |
| 100-1000 Kbp           | 15.3% |
| > 1 Mbp                | 2.6% |
| N50 (bp)               | 942627 |
| GC content             | 39.13% |
Table 2. BUSCO gene content assessment for *D. serrata* and two different *D. melanogaster* assemblies, version r6.05 from [www.flybase.org](http://www.flybase.org), and the full ISO 1 pacbio assembly of Berlin et al. (2015) consisting of 790 contigs, also constructed with the PBcR pipeline. A total of 2799 BUSCOs were searched that form a set of highly conserved Dipteran genes.

| Category                  | D. serrata | D. kikkawai | D. melanogaster | r6.05 | PacBio |
|---------------------------|------------|-------------|-----------------|-------|--------|
| Single-copy (%)           | 94.1       | 97.1        | 98.2            | 97.7  |
| Duplicated (%)            | 2.1        | 1.0         | 0.5             | 0.6   |
| Fragmented BUSCOs (%)     | 2.5        | 1.2         | 0.8             | 0.8   |
| Missing BUSCOs (%)        | 1.3        | 0.8         | 0.5             | 0.9   |

Supplementary Table S1. List of *D. serrata* to *D. melanogaster* orthologs.