The bacterial communities of *Drosophila suzukii* collected from undamaged cherries

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ABSTRACT

Drosophila suzukii is an introduced pest insect that feeds on undamaged, attached fruit. This diet is distinct from the fallen, discomposing fruits utilized by most other species of *Drosophila*. Since the bacterial microbiota of *Drosophila*, and of many other animals, is affected by diet, we hypothesized that the bacteria associated with *D. suzukii* are distinct from that of other *Drosophila*. Using 16S rDNA PCR and Illumina sequencing, we characterized the bacterial communities of larval and adult *D. suzukii* collected from undamaged, attached cherries in California, USA. We find that the bacterial communities associated with these samples of *D. suzukii* contain a high frequency of *Tatumella*. *Gluconobacter* and *Acetobacter*, two taxa with known associations with *Drosophila*, were also found, although at lower frequency than *Tatumella* in four of the five samples examined. Sampling *D. suzukii* from different locations and/or while feeding on different fruits is needed to determine the generality of the results determined by these samples. Nevertheless this is, to our knowledge, the first study characterizing the bacterial communities of this ecologically unique and economically important species of *Drosophila*.

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INTRODUCTION

D. suzukii is an introduced pest insect that has recently become established in both North America and Europe (*Rota-Stabelli, Blaxter & Anfora, 2013*). The economic impact of *D. suzukii* in fruit growing regions may be substantial (*Bolda, Goodhue & Zalom, 2010*). Unlike most species of *Drosophila*, *D. suzukii* has a serrated ovipositor that allows it to lay its eggs in undamaged fruit (*Rota-Stabelli, Blaxter & Anfora, 2013*). This is distinct from most other *Drosophila*, including the closest relatives of *D. suzukii*, which lack a serrated ovipositor and therefore lay eggs in fallen and damaged fruit (*Ashburner, Golic & Hawley, 2004*; *Mitsui, Takahashi & Kimura, 2006*; *Rota-Stabelli, Blaxter & Anfora, 2013*). Therefore, the diet of *D. suzukii* is different from that of most other species of *Drosophila*.

The microbial communities associated with natural *Drosophila* populations are well characterized (for a review see *Broderick & Lemaitre, 2012*). Most studies have focused on the bacterial communities of *Drosophila* that feed upon fallen fruit (*Cox & Gilmore, 2007*; *Corby-Harris et al., 2007*; *Staubach et al., 2013*; *Wong, Chaston & Douglas, 2013*), while others have looked at additional host diets, such as mushrooms, cacti, and flowers (*Chandler et al., 2011*). The yeast communities of various *Drosophila* species have also been investigated (*Chandler, Eisen & Kopp, 2012*), and the yeasts associated with *D. suzukii* feeding upon undamaged fruits have been characterized (*Hamby et al., 2012*). However, to our knowledge, the bacterial communities of *D. suzukii* have not been examined.

In *Drosophila*, both laboratory and natural studies have found that diet plays an important role in shaping bacterial communities (*Chandler et al., 2011*; *Staubach et al., 2013*; *Sharon et al., 2010*). Since *D. suzukii* consume a distinct diet compared to other *Drosophila*, we hypothesized that this may play a role in shaping their bacterial communities. We therefore characterized the bacterial communities of adult and larval *D. suzukii* collected from undamaged cherries.

MATERIALS AND METHODS

On June 28th 2012 at Wolfskill Experimental Orchard near the town of Winters, California, USA, adult Drosophilids were aspirated directly from attached cherries (cherry variety DPRU0327/PRUNUS/AVIUM/F 98 CAROON/C 1 52). No insecticides or fungicides were applied in this orchard during this growing season. No specific permits were required for the described field studies and site managers provided informed consent before collections took place. Collected Drosophilids were stored alive in autoclaved glass vials for transport to the University of California, Davis (UCD) where they were positively identified as *Drosophila suzukii* (24 males and 1 female). Intestines were dissected from the males under sterile conditions and randomly divided into three sets of eight intestines each. Total time between collection and dissection did not exceed four hours. Whole cherries that lacked any visible damage were collected from the same tree and placed in sterile plastic bags for transport to UCD. The cherries were macerated in the bags and the largest visible larvae were picked from the bags, externally washed in 70% ethanol, rinsed in sterile water, and divided into three sets of ten individuals each. Additional larvae were collected from the same cherries, washed and rinsed as described above, and then individually placed in yeast extract-peptone-dextrose (YEPD) plates (1% yeast extract, 2% peptone, and 2% glucose/dextrose). The larvae were allowed to migrate for 30–60 s and the resulting colonies were used in a complementary study (*Dunitz et al., 2014*). The larvae were then individually placed in plastic vials containing Bloomington *Drosophila* media and all eclosing adults were positively identified as *D. suzukii* (4 males and 6 females).

DNA extractions were performed on these larvae and the adult intestines as previously described (*Chandler et al., 2011*). Bacterial DNA was amplified by a two-step PCR targeting the 16S rDNA gene (V4 region) with primers 515F and 806R, designed to include Illumina adaptor and barcode sequences. Sequencing was performed on an Illumina MiSeq at the UC Davis Genomics Core Facility generating 150 basepair paired-end reads. Samples were

Table 1 Proportion of the most abundant OTUs in each sample of _D. suzukii_. OTUs are identified by their closest hit in the SILVA SSU Reference Database Release 111. Number of sequences is after all quality-control steps. L, larva; A, adult.

	L1	L2	A1	A2	A3
Tatumella punctata	0.991	0.989	0.309	0.990	0.800
Gluconobacter cerinus	0.001	<0.001	0.658	0.002	0.123
Acetobacter cerevisiae	<0.001	<0.001	0.021	<0.001	0.028
Dyella sp.	0	0	0	0	0.015
Gluconobacter oxydans	<0.001	<0.001	0.001	0	0.009
Orbus sp.	<0.001	0	0	0	0.008
All other taxa	0.001	0.001	0.008	0.001	0.011
Total number of sequences in sample	50,701	65,346	55,426	44,545	40,256

multiplexed with dual barcode combinations and demultiplexed with a custom script. After demultiplexing, the six samples had between 71,131 and 1,388 raw paired-end sequences for a total of 279,046 paired-end sequences. Paired sequences were combined using FLASH (_Magoč & Salzberg, 2011_) with parameters of a minimum overlap of 20 base pairs and a maximum overlap of 120 base pairs. These parameters were chosen to accommodate the 150 base pair paired-end reads used here (Jeff Froula, pers. comm., 2013). Other parameters were left as default.

Merged sequences were quality checked using QIIME (_Caporaso & Kuczynski et al., 2010b_) and default settings (_Bokulich et al., 2013_). Using UCLUST (_Edgar, 2010_), the 267,204 quality-checked sequences were clustered into _de novo_ OTUs at the 97% similarity threshold producing 3,518 OTUs. The most abundant sequence in each OTU was chosen as a representative sequence. The representative sequences for all OTUs are available in Data S1. These representative sequences were screened for chimeras using the PyNAST aligner (_Caporaso et al., 2010a_) and ChimeraSlayer (_Haas et al., 2011_). Any OTU containing only 1 sequence was removed thus removing 2,878 OTUs (and therefore 2,878 sequences).

Taxonomic assignments were generated by querying the representative sequences against the truncated SILVA SSU Reference Database Release 111 (_Quast et al., 2013_) using the Blastn algorithm (_Altschul et al., 1990_) (Data S2). Any OTU with a best hit to mitochondria, chloroplast, or _Wolbachia_ was removed from further analysis. Two OTUs with low query coverage (<63 basepairs) within the SILVA database were removed. Since we are primarily interested in the bacterial microbiota, four Archaeal OTUs were also removed. One of the larval libraries contains less than 300 sequences (all others contain greater than 35,000; Table 1) and was removed from subsequent analyses, which also removed four OTUs that were unique to this library (totaling eight sequences). The final dataset consists of 617 OTUs containing 256,274 total sequences. The proportions of the six most abundant OTUs in each sample are given in Table 1. Further details, including information on the more rare OTUs, the singleton OTUs, and the removed larval library, are found in Data S3.
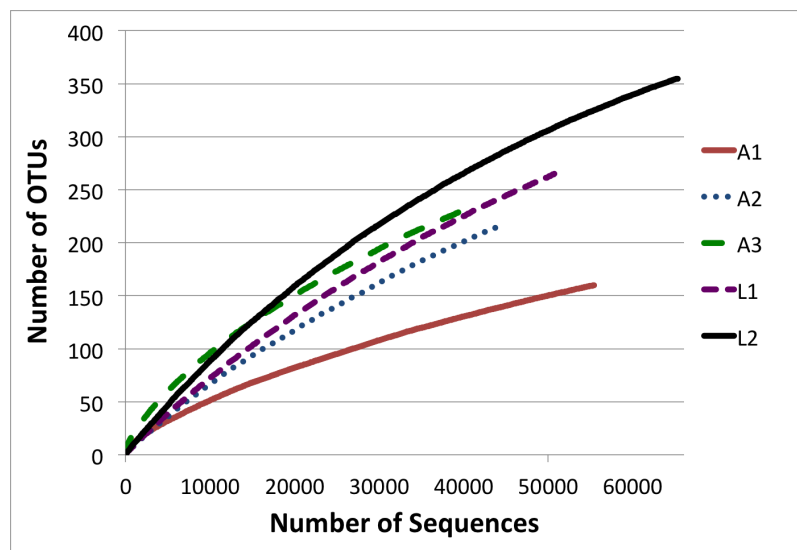
Figure 1 Rarefaction analysis of observed richness of the *D. suzukii* bacterial communities.

Table 2 Alpha diversity calculations for each sample of *D. suzukii.*

	L1	L2	A1	A2	A3
Observed OTUs	204.12	240.86	120.61	182.24	213.08
Observed OTUs-SD	5.53	8.78	5.35	4.61	4.59
Chao	475.54	505.76	272.45	448.24	464.91
Chao-SD	55.26	52.50	47.86	46.28	49.74
Shannon diversity	0.13	0.16	1.18	0.14	1.22
Shannon-SD	0.0038	0.0061	0.0048	0.0032	0.0044

Notes.
SD, Standard deviation; L, larva; A, adult.

Alpha diversity was determined in QIIME by rarefying each sample to 35,000 sequences and taking the average of 100 iterations of rarefication (Table 2). Rarefaction curves of the observed OTUs were made in mothur using 100 iterations of the UCLUST generated OTUs (*Schloss et al., 2009*) (Fig. 1). Beta diversity was determined using weighted UniFrac (*Lozupone & Knight, 2005*) after aligning the representative sequencing using PyNAST (*Caporaso et al., 2010a*), building a phylogenetic tree using FastTree (*Price, Dehal & Arkin, 2010*), and rarifying each sample to 35,000 sequences in QIIME (Fig. 2).

Demultiplexed sequenced reads are available through NCBI's Sequence Read Archive (SRA) under project number SRX391503.

RESULTS AND DISCUSSION

We characterized the bacterial communities of adult and larval *Drosophila suzukii* collected from undamaged, attached cherries. Three adult samples, each containing eight dissected male intestines, and two samples of larvae, each containing ten whole, externally sterilized individuals of unknown sex, are included in this analysis. 16S rDNA PCR and
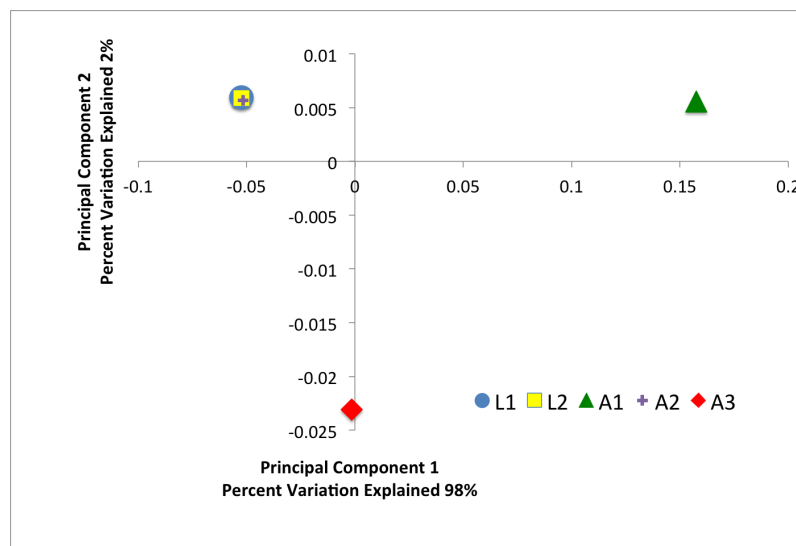
Figure 2 Weighted UniFrac principle coordinate analysis of the *D. suzukii* bacterial communities.

Illumina sequencing generated over 40,000 reads per sample (Table 1). Operational taxonomic units (OTUs) were formed by clustering sequences at the 97% similarity cutoff. Taxonomic assignments were generated by querying the representative sequence of each OTU against the truncated SILVA SSU Reference Database Release 111 using the Blastn algorithm (Data S2).

We find that the microbiota of both of the larval samples and adult sample A2 are composed of at least 99% *Tatumella*, and the remaining two adult samples contain 31% and 80% *Tatumella* (Table 1) (the larval sample that was excluded from formal analysis due to its extremely small library size was composed of 83% *Tatumella* [Data S3]). *Tatumella* is an Enterobacteriaceae that has been linked to both human and plant infections (*Costa, Mendes & Ribeiro, 2008*; *Marin-Cevada et al., 2010*). *Tatumella punctata*, the nearest hit to the largest *Tatumella* OTU identified in this study, was originally isolated from oranges (*Kageyama et al., 1992*). Although this genus is not considered a common *Drosophila* associate (*Broderick & Lemaitre, 2012*), it was recovered from *D. melanogaster* at an apple farm in New York, USA (*Wong, Chaston & Douglas, 2013*). Recently, several species previously classified as *Pantoea* have been transferred into *Tatumella* (*Brady et al., 2010*). Given that these species of *Pantoea* have been reported in *Drosophila* (*Wong, Chaston & Douglas, 2013*), perhaps *Tatumella* is a more common *Drosophila* associate than currently recognized. Nevertheless, *Tatumella* is the dominant bacteria associated with these samples of *D. suzukii*, while being absent, or at minimal levels, with other species of *Drosophila*. Sampling *D. suzukii* from different locations and/or while feeding on different fruits is needed to determine the ubiquity of the *D. suzukii*/*Tatumella* association.

The next most abundant taxa are species of Acetobacteraceae, specifically *Gluconobacter* and *Acetobacter* (Table 1). These are found in all five samples, but are primarily associated with adult samples A1 and A3. The Acetobacteraceae are commonly found associated with natural *Drosophila* populations (*Chandler et al., 2011*; *Staubach et al., 2013*; *Wong, Chaston*

& Douglas, 2013; *Corby-Harris et al., 2007*; *Cox & Gilmore, 2007*). A minor, but notable, component to the bacterial community of adult sample A3 is a Gammaproteobacteria in the *Orbus* genus (0.8% of total community in A3). *Orbus* was the most common genus in a global survey of *Drosophilid* species (*Chandler et al., 2011*), but has not been recovered in most other studies of *Drosophila*-associated bacteria (*Broderick & Lemaitre, 2012*). The reasons for this are unclear, although it has been found at low frequencies in naturally collected fruit-feeding *D. melanogaster* and *D. simulans* (*Staubach et al., 2013*).

It is well established that alpha diversity measurements in 16S-based studies are affected by amplicon length, primer selection, alignment method, and quality control procedures (*Schloss, 2010*; *Bokulich et al., 2013*; *Youssef et al., 2009*). Furthermore, differences in sample collection and preparation can affect perceived bacterial diversity. For example, studies that examine whole bodies (*Staubach et al., 2013*; *Wong, Chaston & Douglas, 2013*) may have artificially high diversity compared to those using dissected intestines (such as was done here for the adult samples). Indeed, in laboratory raised flies, dissected intestines have slightly lower observed and Chao richness than whole bodies (*Chandler et al., 2011*). Furthermore, since transit time through the *Drosophila* intestine can be as low as 50 min (*Wong et al., 2008*), undue time between collection and sample preparation can affect diversity measurements as the individuals purge their intestinal contents. Because of these caveats, it is difficult to compare results of previous studies to those generated here (Fig. 1 and Table 2).

Weighted UniFrac analysis (a phylogenetically-informed beta-diversity metric that takes into account between-sample frequency differences) finds the two samples of *D. suzukii* larvae harbor similar bacterial communities, while the three samples of *D. suzukii* adults each have a distinct community (Fig. 2). The same pattern was found in a weighted UniFrac analysis that did not exclude singleton OTUs (data not shown). Furthermore, the observed OTUs, Chao richness, and Shannon diversity are very similar for both larval samples, whereas the adult samples exhibit much higher between sample variability in these three indices (Table 2). It should be noted that a consequence of our pooling method means that it cannot be determined if this variability is the result of a single individual with a highly different bacterial community or if multiple individuals, each with the same community, were pooled together by chance. Furthermore, since whole larvae were used it cannot be determined if non-intestinal bacteria, for example in the trachea or salivary glands, are obscuring potential variability of the larval intestinal microbiota.

One explanation for the differences in variability between larval and adult samples is that larvae are confined to the fruit that they were laid into, while adults can travel to other surfaces where they can acquire different bacteria. This result informs other studies of *Drosophila*, and insect-microbe studies in general, many of which characterize only a single sample from each population under investigation. The variability of adult samples described here indicates that, despite pooling multiple individuals, a single sample may not provide an accurate representation of the microbiota associated with that population.

CONCLUSIONS

In this study, we find that *Drosophila suzukii* larvae and adults harbor simple bacterial communities that are mostly dominated by *Tatumella*. As *D. suzukii* is a generalist feeder that has been introduced to many areas of North America and Europe, sampling *D. suzukii* from different locations and/or while feeding on different fruits is needed to determine the ubiquity of the *D. suzukii*/*Tatumella* association. Nevertheless, given the distinct food source of *D. suzukii* (relative to most *Drosophila* species), the potential role of *Tatumella* (or other, yet to be identified *D. suzukii*-associated bacteria) on host fitness or physiology is intriguing. In particular, the draft genome of the most abundant *Tatumella* strain associated with this population of *D. suzukii* is available (*Dunitz et al., 2014*) and analysis of this genome may reveal the metabolic potential of the microbiota to supplement the *D. suzukii* diet with nutrients that are scarce on unfallen fruit. Furthermore, by inoculating *D. suzukii* with defined bacterial communities under controlled dietary conditions, future experimental work can explicitly reveal the microbiota's role in host biology. In summary, by characterizing the bacterial microbiota of these samples of *D. suzukii*, this study is the initial step in the investigation of the interplay between diet and bacteria in this interesting and economically important host-microbe system.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- James Angus Chandler conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables.
- Pamela M. James, Guillaume Jospin and Jenna M. Lang performed the experiments, reviewed drafts of the paper.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:
    NCBI's Sequence Read Archive, Project Number SRX391503

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.474.

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215**:403–410 DOI 10.1016/S0022-2836(05)80360-2.

Ashburner M, Golic K, Hawley RS. 2004. *Drosophila: a laboratory handbook*, 2nd edition. New York: Cold Spring Harbor Laboratory Press.

Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG. 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods* **10**:57–59 DOI 10.1038/nmeth.2276.

Bolda MP, Goodhue RE, Zalom FG. 2010. Spotted Wing *Drosophila*: potential impact of a newly established pest. *Agricultural and Resource Economics Update* **13**:5–8.

Brady CL, Venter SN, Cleenwerck I, Vandemeulebroecke K, De Vos P, Coutinho TA. 2010. Transfer of *Pantoea citrea*, *Pantoea punctata* and *Pantoea terrea* to the genus *Tatumella* emend. as *Tatumella citrea* comb. nov., *Tatumella punctata* comb. nov. and *Tatumella terrea* comb. nov. and description of *Tatumella morbirosei* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **60**:484–494 DOI 10.1099/ijs.0.012070-0.

Broderick NA, Lemaitre B. 2012. Gut-associated microbes of *Drosophila melanogaster*. *Gut Microbes* **3**:307–321 DOI 10.4161/gmic.19896.

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R. 2010a. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**:266–267 DOI 10.1093/bioinformatics/btp636.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010b. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**:335–336 DOI 10.1038/nmeth.f.303.
Chandler JA, Eisen JA, Kopp A. 2012. Yeast communities of diverse *Drosophila* species: comparison of two symbiont groups in the same hosts. *Applied and Environmental Microbiology* **78**:7327–7336 DOI 10.1128/AEM.01741-12.

Chandler JA, Lang JM, Bhatnagar S, Eisen JA, Kopp A. 2011. Bacterial communities of diverse *Drosophila* species: ecological context of a host-microbe model system. *PLoS Genetics* **7**:e1002272 DOI 10.1371/journal.pgen.1002272.

Corby-Harris V, Pontaroli AC, Shimkets LJ, Bennetzen JL, Habel KE, Promislow DEL. 2007. Geographical distribution and diversity of bacteria associated with natural populations of *Drosophila melanogaster*. *Applied and Environmental Microbiology* **73**:3470–3479 DOI 10.1128/AEM.02120-06.

Costa PSGD, Mendes JM de C, Ribeiro GM. 2008. *Tatumella ptyseos* causing severe human infection: report of the first two Brazilian cases. *Brazilian Journal of Infectious Diseases* **12**:442–443 DOI 10.1016/j.ijid.2007.09.014.

Cox CR, Gilmore MS. 2007. Native microbial colonization of *Drosophila melanogaster* and its use as a model of *Enterococcus faecalis* pathogenesis. *Infection and Immunity* **75**:1565–1576 DOI 10.1128/IAI.01496-06.

Dunitz MI, James PM, Jospin G, Eisen JA, Coil DA, Chandler JA. 2014. Draft genome sequence of *Tatumella* sp. strain UCD-D_suzukii (phylum *Proteobacteria*) isolated from *Drosophila suzukii* larvae. *Genome Announcements* **2**:e00349-14 DOI 10.1128/genomeA.00349-14.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**:2460–2461 DOI 10.1093/bioinformatics/btq461.

Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, Ciulla D, Tabbaa D, Highlander SK, Sodergren E, Methé B, DeSantis DZ, The Human Microbiome Consortium, Petrosino JF, Knight R, Birren BW. 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Research* **21**:494–504 DOI 10.1101/gr.112730.110.

Hamby KA, Hernández A, Boundy-Mills K, Zalom FG. 2012. Associations of yeasts with spotted-wing *Drosophila* (*Drosophila suzukii*; diptera: Drosophilidae) in cherries and raspberries. *Applied and Environmental Microbiology* **78**:4869–4873 DOI 10.1128/AEM.00841-12.

Kageyama B, Nakae M, Yagi S, Sonoyama T. 1992. *Pantoea punctata* sp. nov., *Pantoea citrea* sp. nov., and *Pantoea terrea* sp. nov. isolated from fruit and soil samples. *International Journal of Systematic Bacteriology* **42**:203–210 DOI 10.1099/00207713-42-2-203.

Lozupone C, Knight R. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology* **71**:8228–8235 DOI 10.1128/AEM.71.12.8228-8235.2005.

Magoč T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**:2957–2963 DOI 10.1093/bioinformatics/btr507.

Marin-Cevada V, Caballero-Mellado J, Bustillos-Cristales R, Munoz-Rojas J, Mascarua-Esparza MA, Castaneda-Lucio M, Lopez-Reyes L, Martinez-Aguilar L, Fuentes-Ramirez LE. 2010. *Tatumella ptyseos*, an unrevealed causative agent of pink disease in pineapple. *Journal of Phytopathology* **158**:93–99 DOI 10.1111/j.1439-0434.2009.01575.x.

Mitsui H, Takahashi KH, Kimura MT. 2006. Spatial distributions and clutch sizes of *Drosophila* species ovipositing on cherry fruits of different stages. *Population Ecology* **48**:233–237 DOI 10.1007/s10144-006-0260-5.

Price MN, Dehal PS, Arkin AP. 2010. FastTree 2—approximately maximum-likelihood trees for large alignments. *PLoS ONE* **5**:e9490 DOI 10.1371/journal.pone.0009490.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**:D590–D596 DOI 10.1093/nar/gks1219.

Rota-Stabelli O, Blaxter M, Anfora G. 2013. *Drosophila suzukii. Current Biology* **23**:R8–R9 DOI 10.1016/j.cub.2012.11.021.

Schloss PD. 2010. The effects of alignment quality, distance calculation method, sequence filtering, and region on the analysis of 16S rRNA gene-based studies. *PLoS Computational Biology* **6**:e1000844 DOI 10.1371/journal.pcbi.1000844.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**:7537–7541 DOI 10.1128/AEM.01541-09.

Sharon G, Segal D, Ringo JM, Hefetz A, Zilber-Rosenberg I, Rosenberg E. 2010. Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* **107**:20051–20056 DOI 10.1073/pnas.1009906107.

Staubach F, Baines JF, Künzel S, Bik EM, Petrov DA. 2013. Host species and environmental effects on bacterial communities associated with *Drosophila* in the laboratory and in the natural environment. *PLoS ONE* **8**:e70749 DOI 10.1371/journal.pone.0070749.

Wong AC-N, Chaston JM, Douglas AE. 2013. The inconstant gut microbiota of *Drosophila* species revealed by 16S rRNA gene analysis. *The ISME Journal* **7**:1922–1932 DOI 10.1038/ismej.2013.86.

Wong R, Piper MDW, Blanc E, Partridge L. 2008. Pitfalls of measuring feeding rate in the fruit fly *Drosophila melanogaster*. *Nature Methods* **5**:214–215 DOI 10.1038/nmeth0308-214.

Youssef N, Sheik CS, Krumholz LR, Najar FZ, Roe BA, Elshahed MS. 2009. Comparison of species richness estimates obtained using nearly complete fragments and simulated pyrosequencing-generated fragments in 16S rRNA gene-based environmental surveys. *Applied and Environmental Microbiology* **75**:5227–5236 DOI 10.1128/AEM.00592-09.