

Supplementary information

Persistence of diet effects on the microbiota of *Drosophila suzukii* (Diptera: Drosophilidae)

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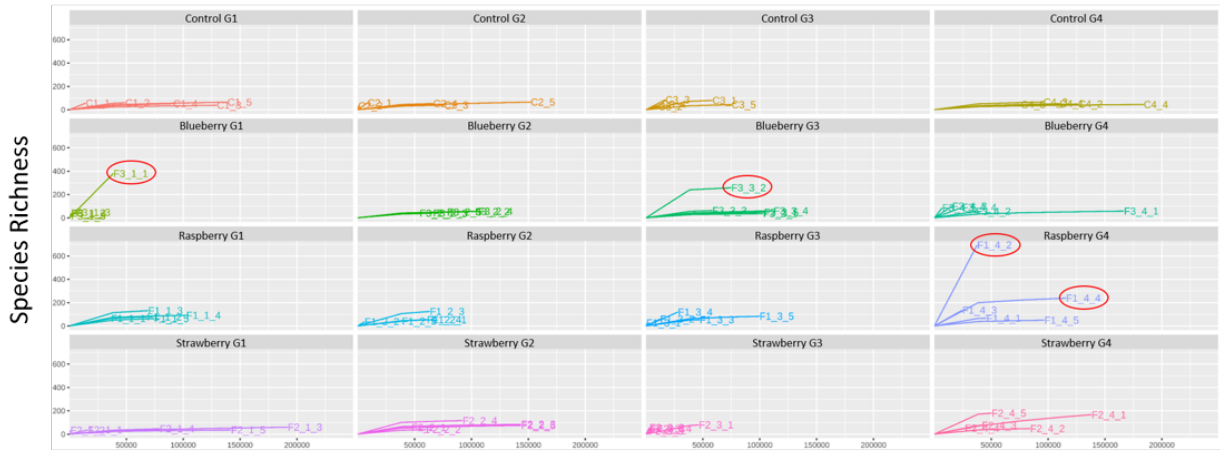
Data availability:

All data are available in an accompanying Excel spreadsheet.

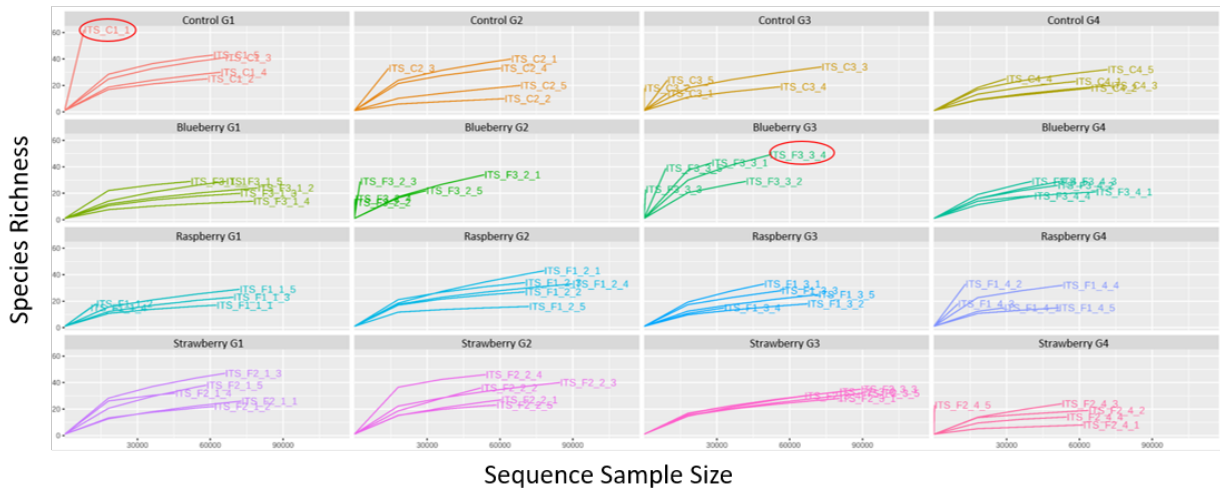
Supplementary Table S1. Staggered primers used to obtain 16S (V4 region) and ITS1 Illumina sequences. The Illumina adapter sequences are omitted. Primers are based on Caporaso *et al.* (2011) for 16S, and Martin and Rygewicz (2005) for ITS1.

Primer name	Primer sequence (5'–3')
16S Forward	
515FP1	GTGCCAGCMGCCGCGGTAA
515FP2	TGTGCCAGCMGCCGCGGTAA
515FP3	ACGTGCCAGCMGCCGCGGTAA
515FP4	CTAGTGCCAGCMGCCGCGGTAA
16S Reverse	
806RP1	GGACTACHVGGGTWTCTAAT
806RP2	GGACTACHVGGGTWTCTAAT
806RP3	ACGGACTACHVGGGTWTCTAAT
806RP4	CTAGGACTACHVGGGTWTCTAAT
ITS1 Forward	
ITS1FP1	CTTGGTCATTTAGAGGAAGTAA
ITS1FP2	TCTTGGTCATTTAGAGGAAGTAA
ITS1FP3	ACCTTGGTCATTTAGAGGAAGTAA
ITS1FP4	CTACTTGGTCATTTAGAGGAAGTAA
ITS1 Reverse	
58A2RP1	CTGCGTTCTTCATCGAT
58A2RP2	TCTGCGTTCTTCATCGAT
58A2RP3	ACCTGCGTTCTTCATCGAT
58A2RP4	CTACTGCGTTCTTCATCGAT

A.

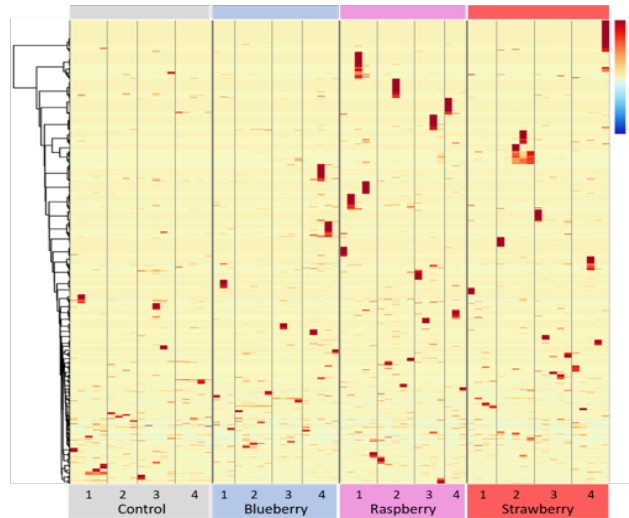


B.

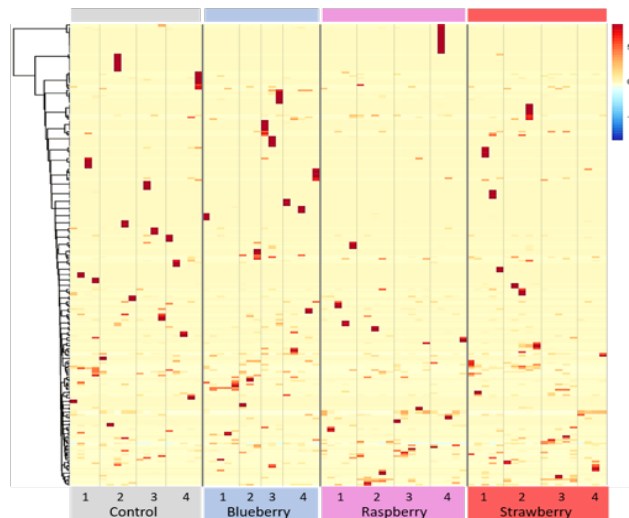


Supplementary Figure S1. Rarefaction curves displaying the bacterial (A) and fungal (B) species richness (OTUs) per sample in relation to the sequence size. Samples with unusual high species richness (red circles) were excluded from the analyses.

A. Bacteria

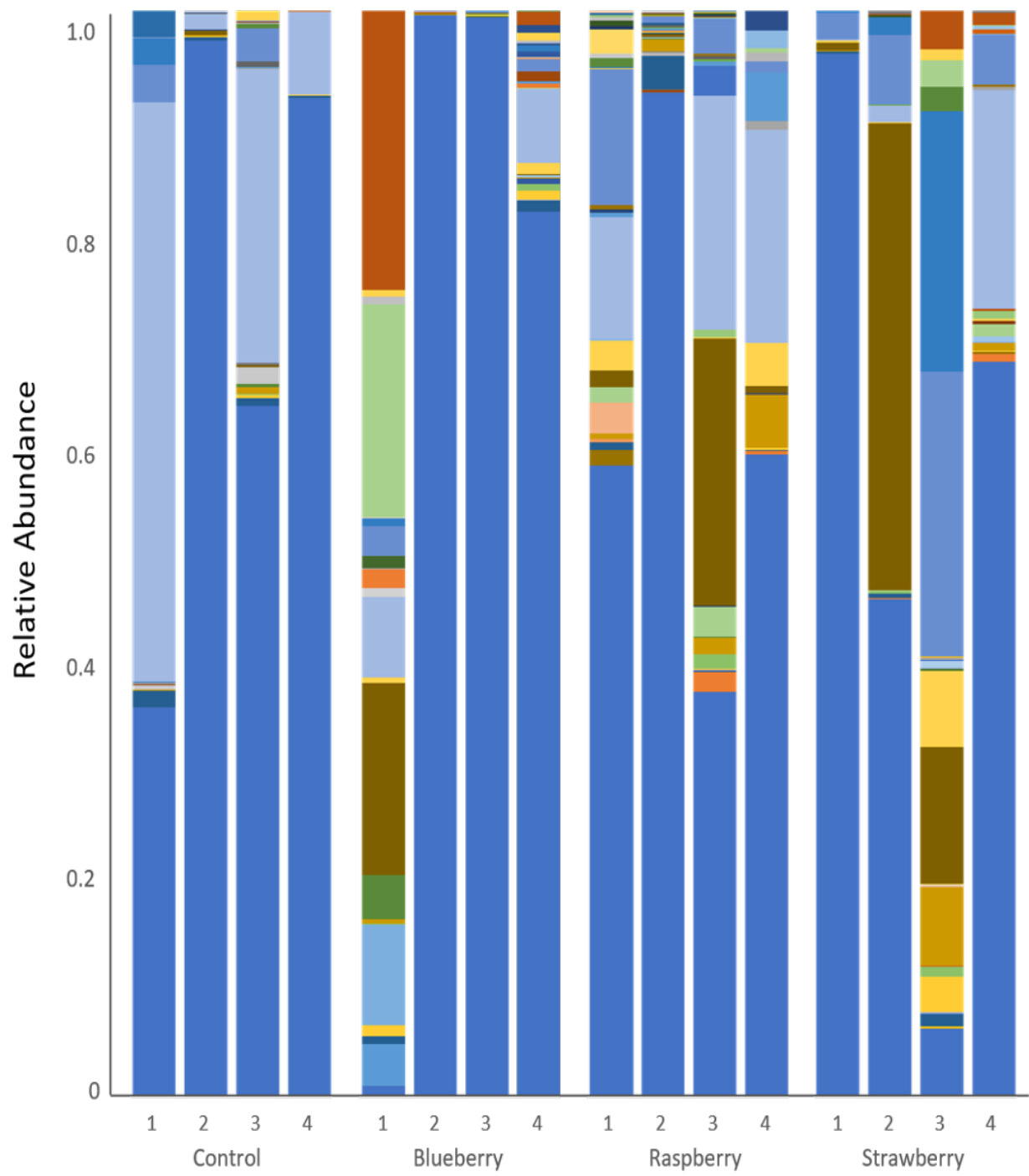


B. Fungi



Supplementary Figure S2. Heatmap showing the OTU-level bacterial (A) and fungal (B) community compositions of *D. suzukii* reared in various diets. Heatmap colour represents the scaled relative abundance of each OTU across all samples. Flies were reared on their specific diet for the first three generations and returned to control diets for the fourth generation. The data is organised by diet with each number (1-4) indicating the generation. Distances between data points in a cluster were obtained using standard Euclidean distance and the distance between clusters was calculated as an analysis of variance following Ward algorithm.

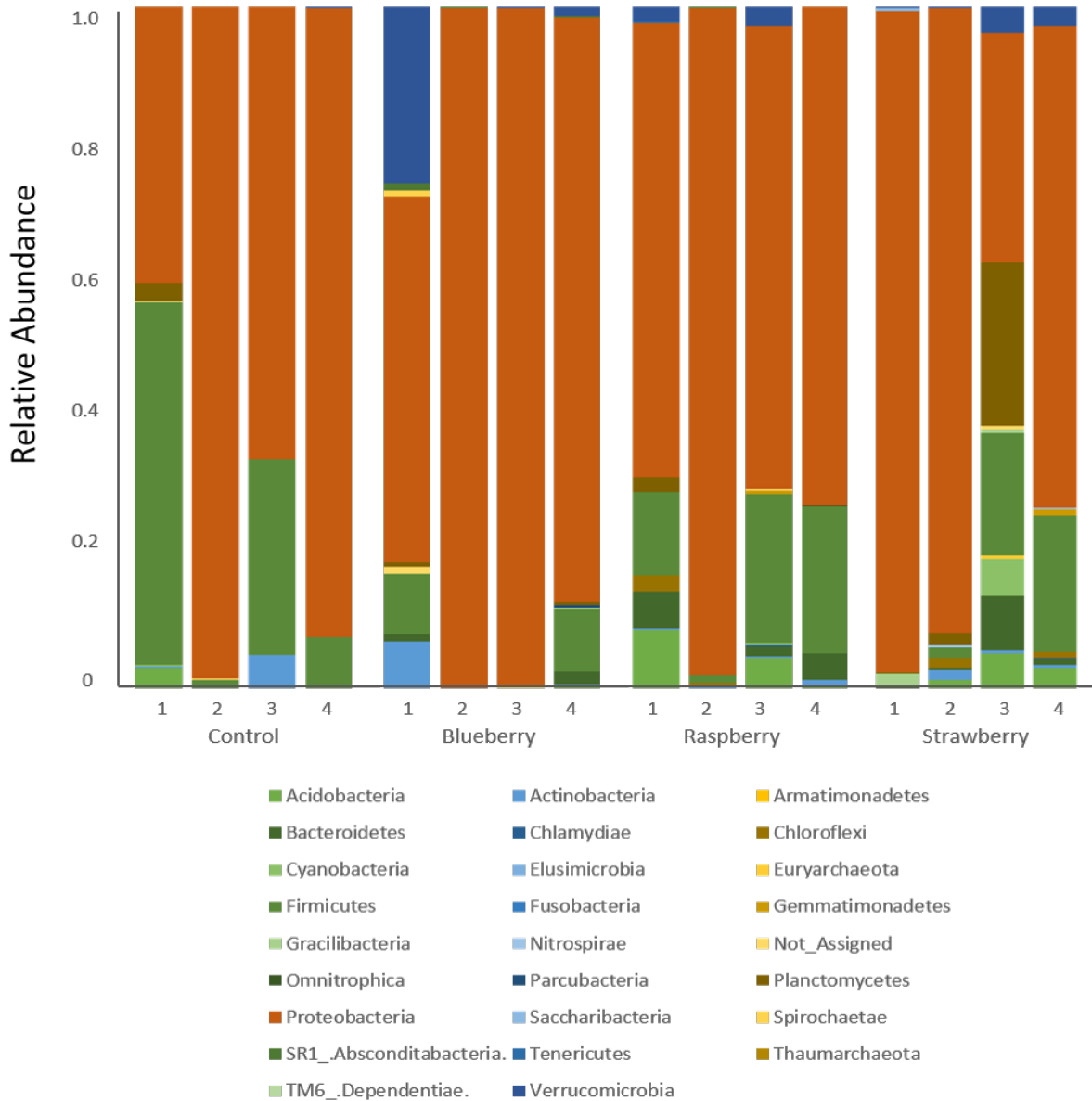
A.



B.

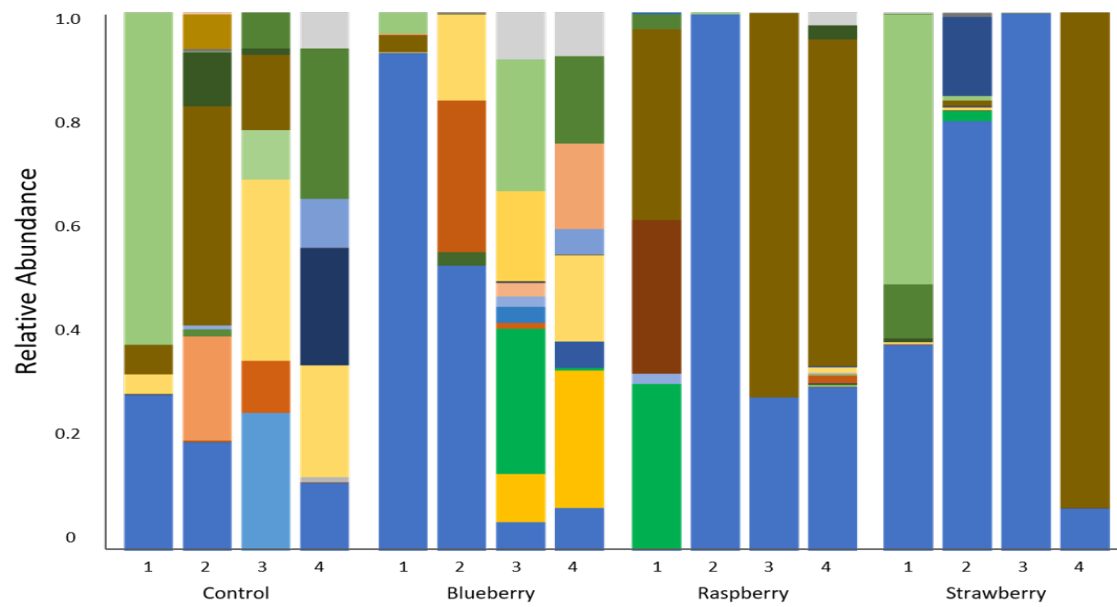


Supplementary Figure S3. Bacterial microbiome of *D. suzukii* reared on various diets. Stack bar graph showing the relative abundance at the Family level. Flies were reared on their specific diets for three generation and switched to the control diet for the fourth generation, numbers (1–4) indicate the generation. Bars have been lengthened to improve visualisation of less abundant taxa (A) and the legend contains all family names (B). Complementary to Figure 1A in main text.



Supplementary Figure S4. Bacterial microbiome of *Drosophila suzukii* reared on various diets.

Stack bar graph showing the relative abundance at the phyla level. Flies were reared on their specific diets for three generation and switched to the control diet for the fourth generation, numbers (1–4) indicate the generation. The legend contains all phyla names and is complementary to Figure 1B in main text.

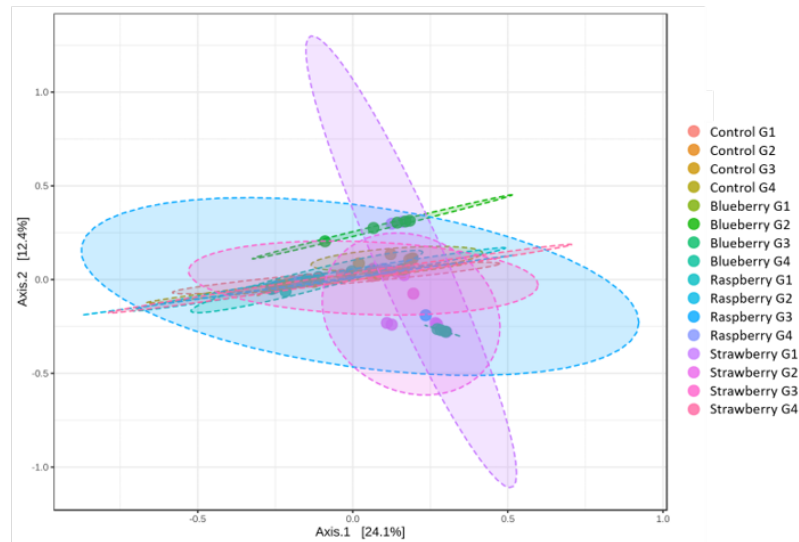


- | | | |
|--------------------------------------|---------------------|--|
| Aspergillaceae | Bionectriaceae | Bulleraceae |
| Bulleribasidiaceae | Ceratobasidiaceae | Chaetomiaceae |
| Cladosporiaceae | Clavariaceae | Clavicipitaceae |
| Coniochaetaceae | Cordycipitaceae | Cystofilobasidiales_fam_Incertae_sedis |
| Didymellaceae | Dothideaceae | Erythrobasidiaceae |
| Geoglossaceae | Glomeraceae | Helotiaceae |
| Helotiales_fam_Incertae_sedis | Herpotrichiellaceae | Hydnodontaceae |
| Hygrophoraceae | Hypocreaceae | Hypocreales_fam_Incertae_sedis |
| Hyponectriaceae | Lasiosphaeriaceae | Lentitheciaceae |
| Malasseziaceae | Melanommataceae | Meruliaceae |
| Microascaceae | Microdochiaceae | Mortierellaceae |
| Mycosphaerellaceae | Myxotrichaceae | Nectriaceae |
| Phaeosphaeriaceae | Physalacriaceae | Piskurozymaceae |
| Plectosphaerellaceae | Pleosporaceae | Saccharomycetaceae |
| Saccharomycetales_fam_Incertae_sedis | Sporormiaceae | Stachybotryaceae |
| Tremellaceae | Trichocomaceae | Tricholomataceae |
| Trichomeriaceae | Trimorphomycetaceae | Not_Assigned |

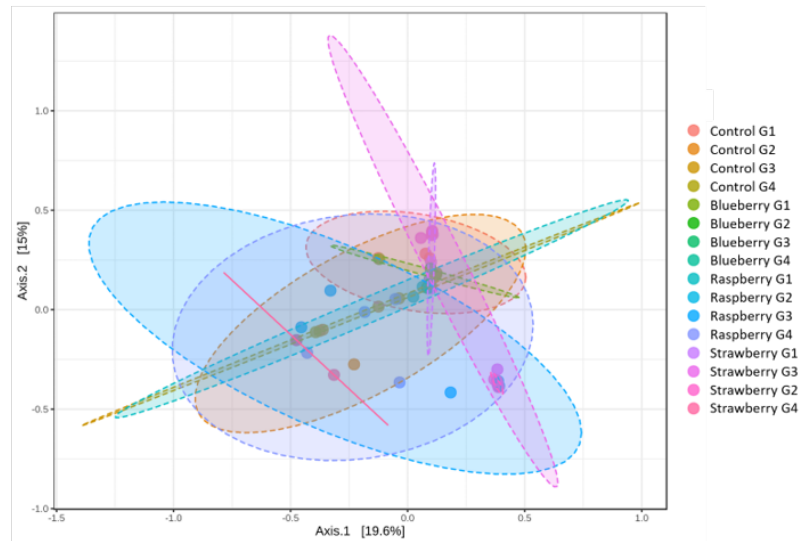
Supplementary Figure S5. Fungal microbiome of *Drosophila suzukii* reared on various diets.

Stack bar graph showing the relative abundance at the family level. Flies were reared on their specific diets for three generation and switched to the control diet for the fourth generation, numbers (1–4) indicate the generation. The legend contains all family names and is complementary to Figure 2A in main text.

A.



B.



Supplementary Figure S6. Principal coordinate analysis (PCoA) of the bacterial (A) and fungal (B) microbiome composition of flies reared on various diets. Beta-diversity based on OUTs was calculated using the Bray-Curtis index. Flies were reared on their specific diets for three generation and switched to the control diet for the fourth generation. The percentage of variation is indicated in the axes (Bacteria: PC1 – 24.1 %, PC2 – 12.4 %; Fungi: PC1 – 19.9 %, PC2 – 15%). All samples (3–5) for diet-generations combinations were plotted individually.