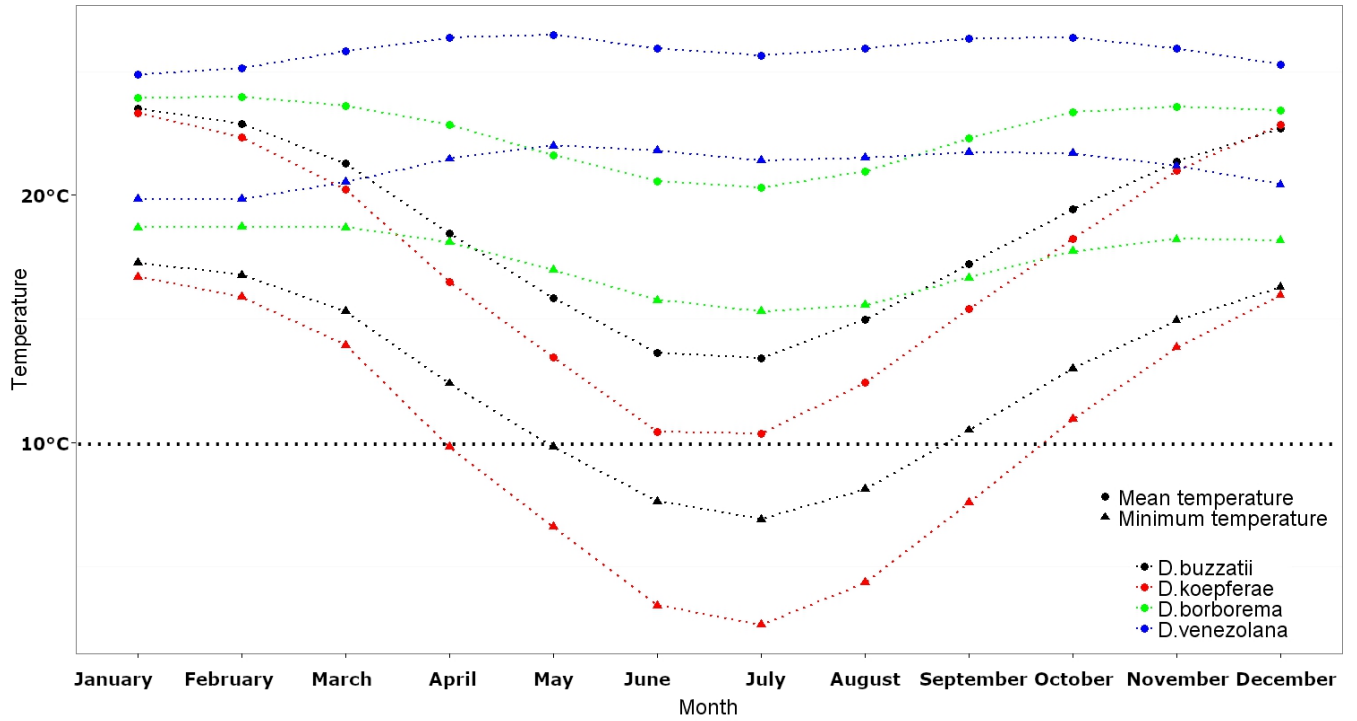
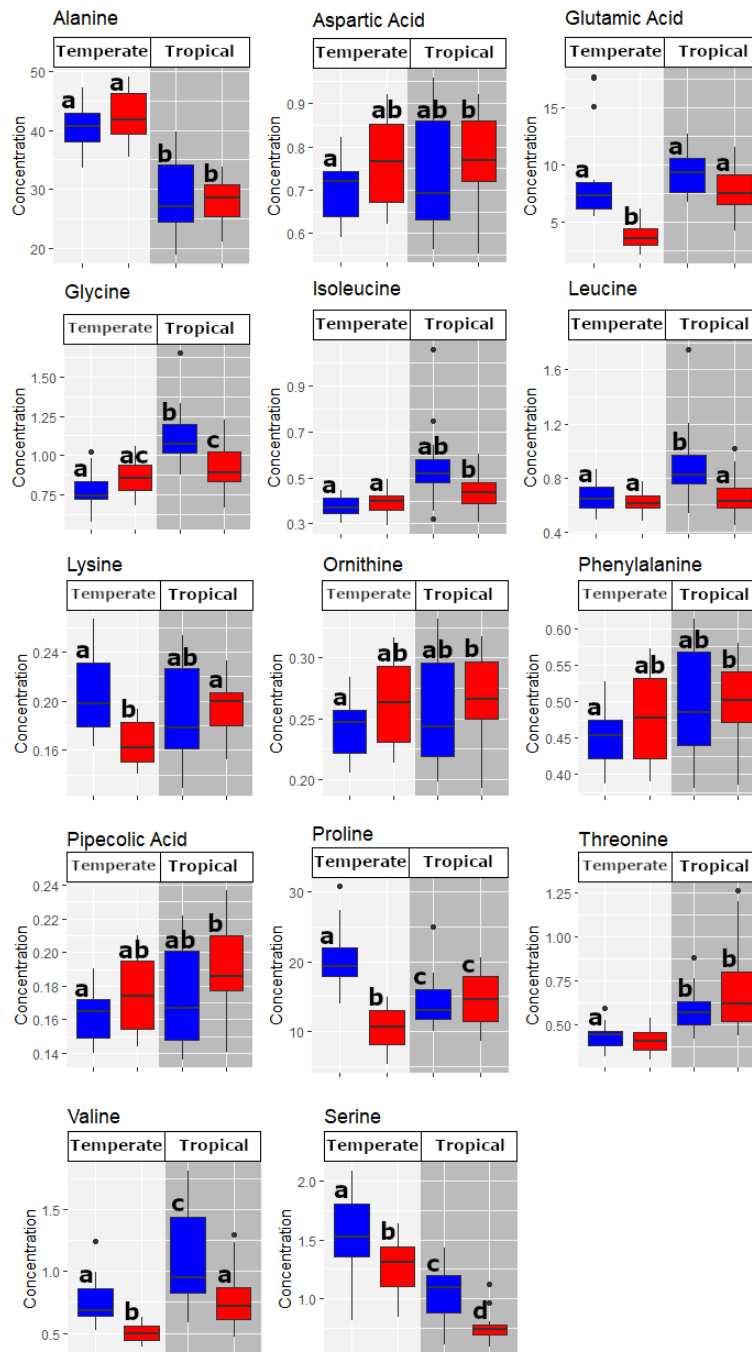


**Figure S1:** Phylogeny of South American *Drosophila* species belonging to the *buzzatii* complex. Divergence times among species range from 8.37 to 3.39 Mya, with *Drosophila venezolana* being the most diverged of the group (Oliveira et al., 2012).



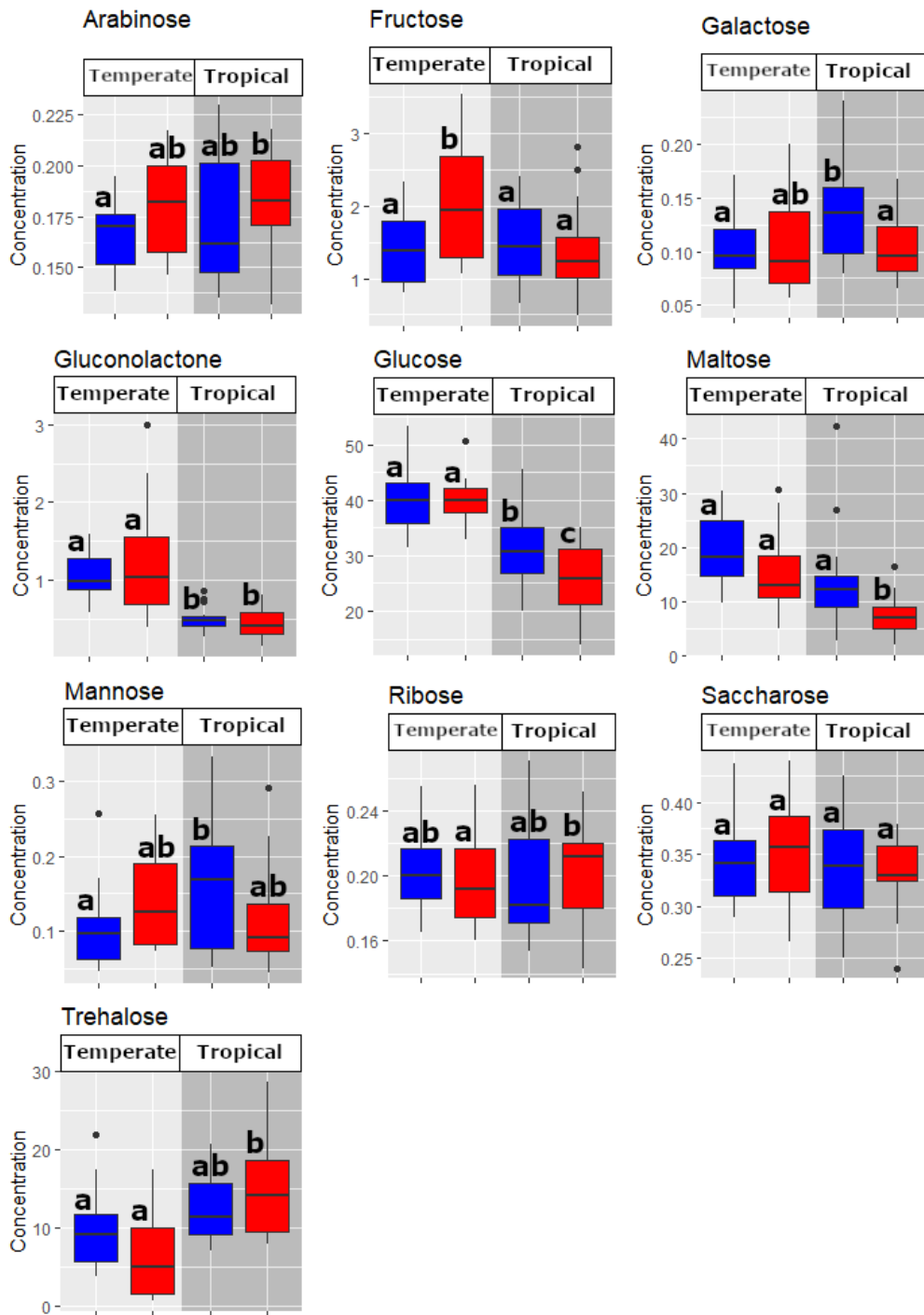
**Figure S2:** Climatic data of the localities where species were collected obtained WorldClim ([www.worldclim.org](http://www.worldclim.org)). Temperate species: *D. koepferae* and *D. buzzatii*; tropical species: *D. borborema* and *D. venezolana*. Dashed line at 10 °C corresponds to the temperature at which flies were exposed in this study.

## Amino Acids



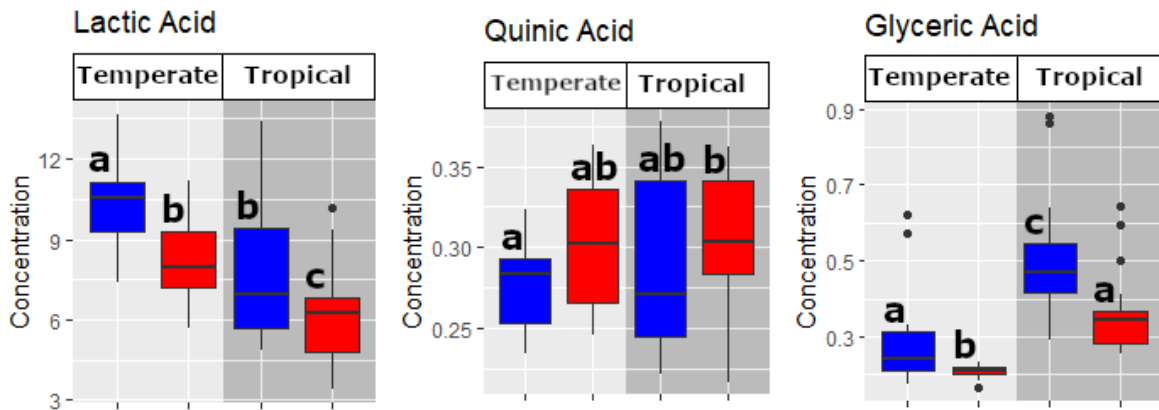
**Figure S3:** Concentration of free amino acids. Boxplots sharing the same letter are not significantly different ( $P < 0.05$ ; Tukey test). Blue boxes depict immature females and red boxes mature ones.

## Carbohydrates



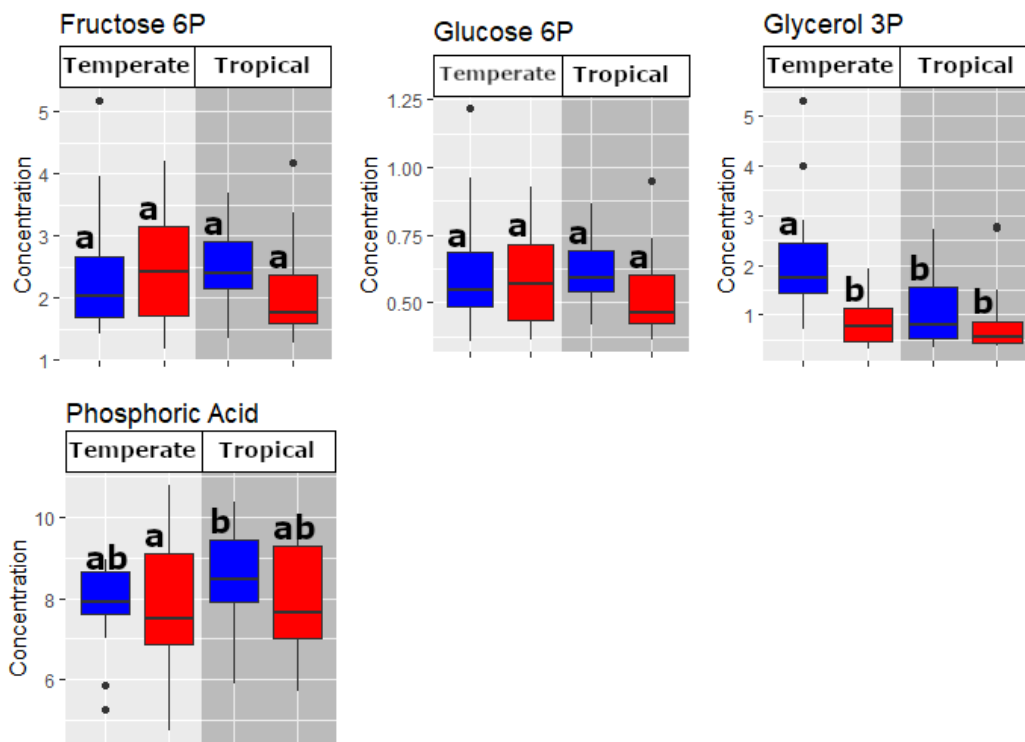
**Figure S4:** Concentration changes of carbohydrates. Boxplots sharing the same letter are not significantly different ( $P < 0.05$ ; Tukey test). Blue boxes depict immature females and red boxes mature ones.

## Organic Acids



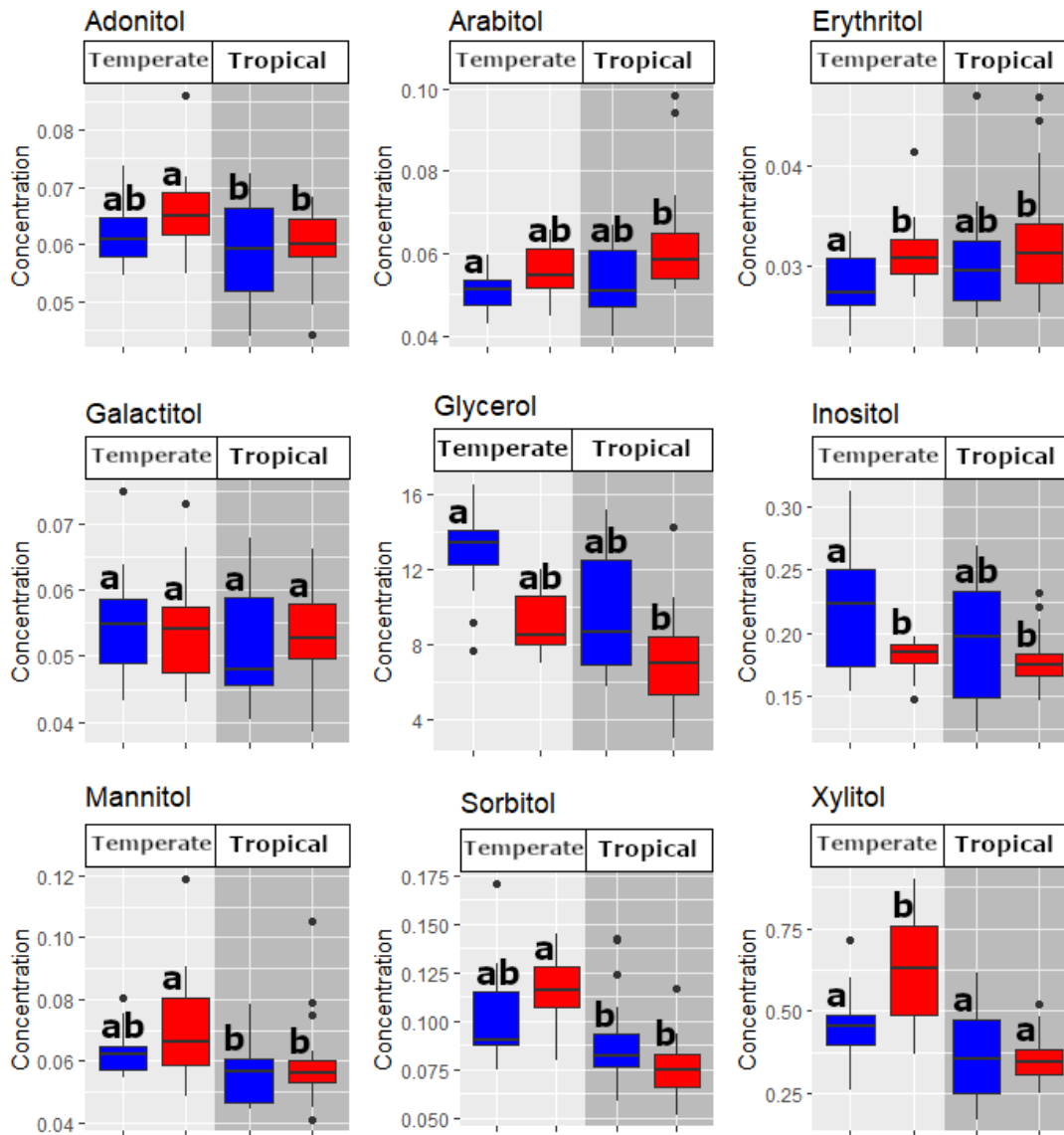
**Figure S5:** Concentration changes of organic acids. Boxplots sharing the same letter are not significantly different ( $P < 0.05$ ; Tukey test). Blue boxes depict immature females and red boxes mature ones.

## Phosphorylated-compounds



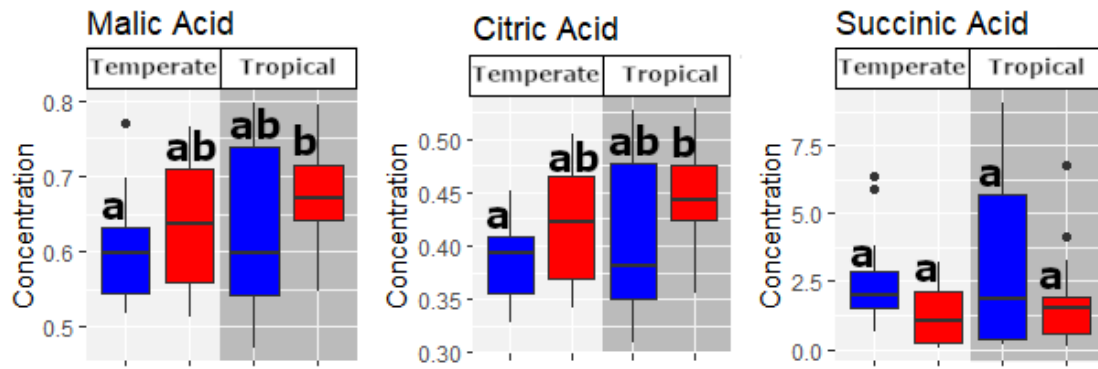
**Figure S6:** Concentration changes of phosphorylated compounds. Boxplots sharing the same letter are not significantly different ( $P < 0.05$ ; Tukey test). Blue boxes depict immature females and red boxes mature ones.

## Polyols



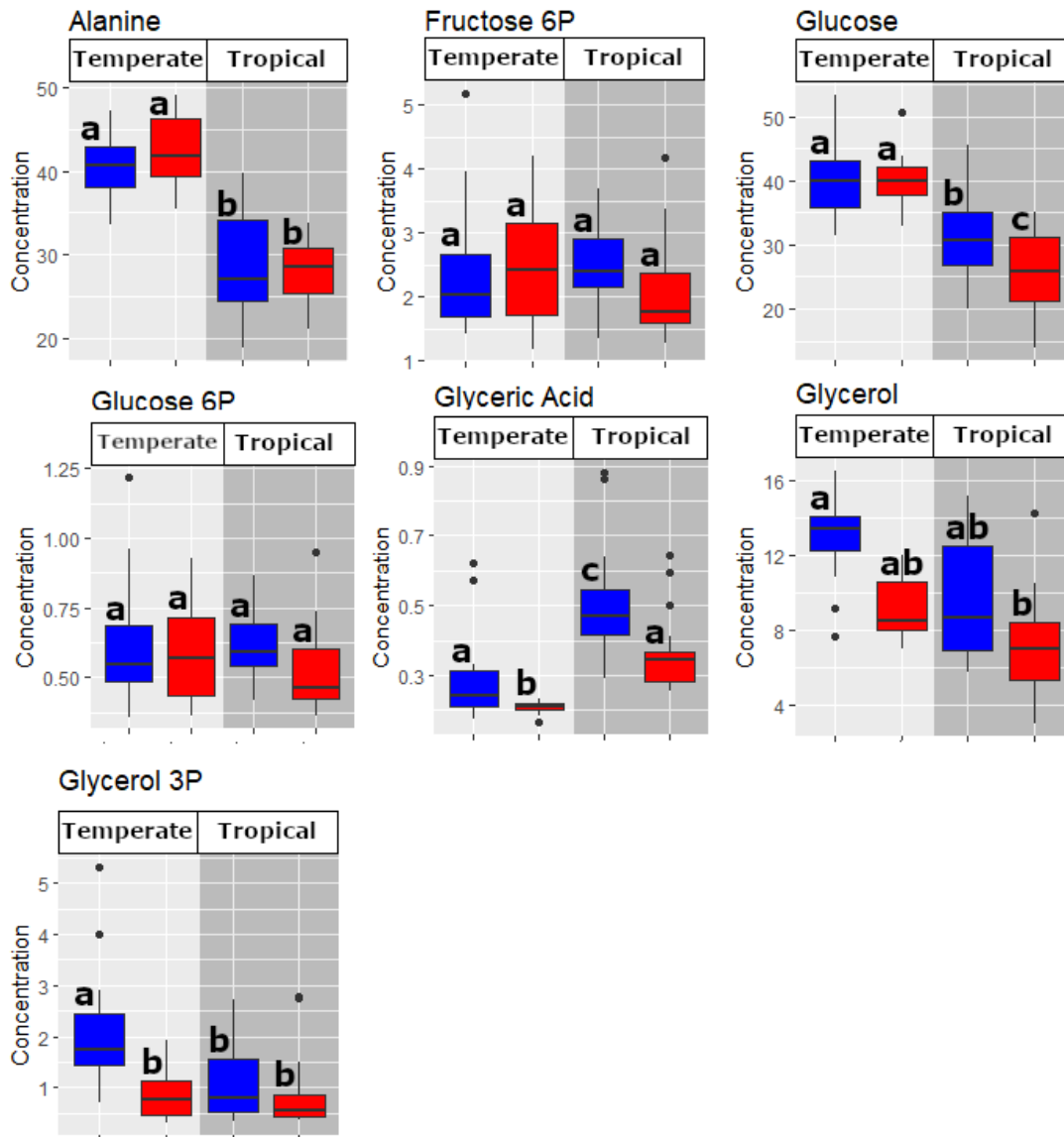
**Figure S7:** Concentration changes of polyols. Boxplots sharing the same letter are not significantly different ( $P < 0.05$ ; Tukey test). Blue boxes depict immature females and red boxes mature ones.

## TCA intermediates



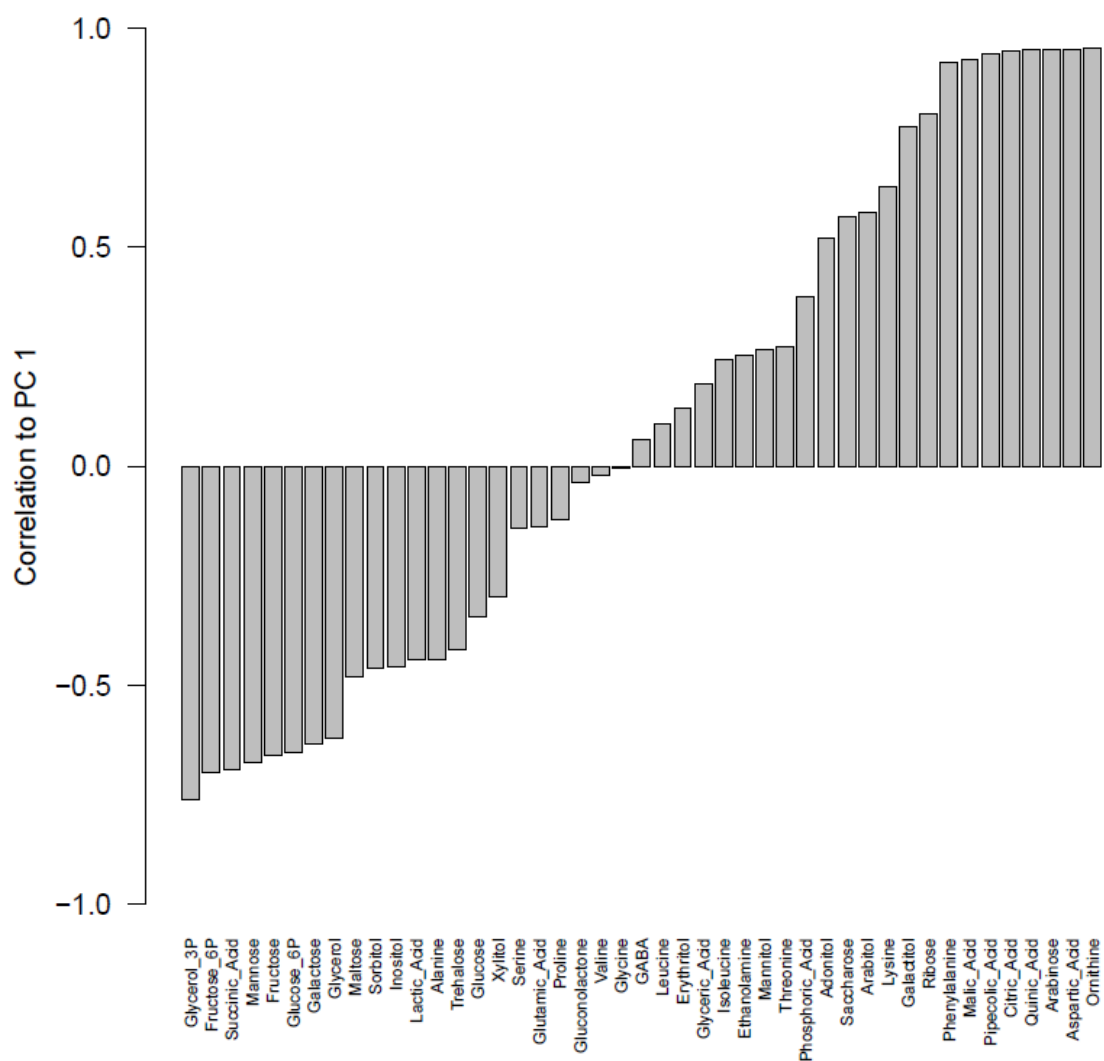
**Figure S8:** Concentration changes of TCA intermediates. Boxplots sharing the same letter are not significantly different ( $P < 0.05$ ; Tukey test). Blue boxes depict immature females and red boxes mature ones.

## Glycolysis-related compounds

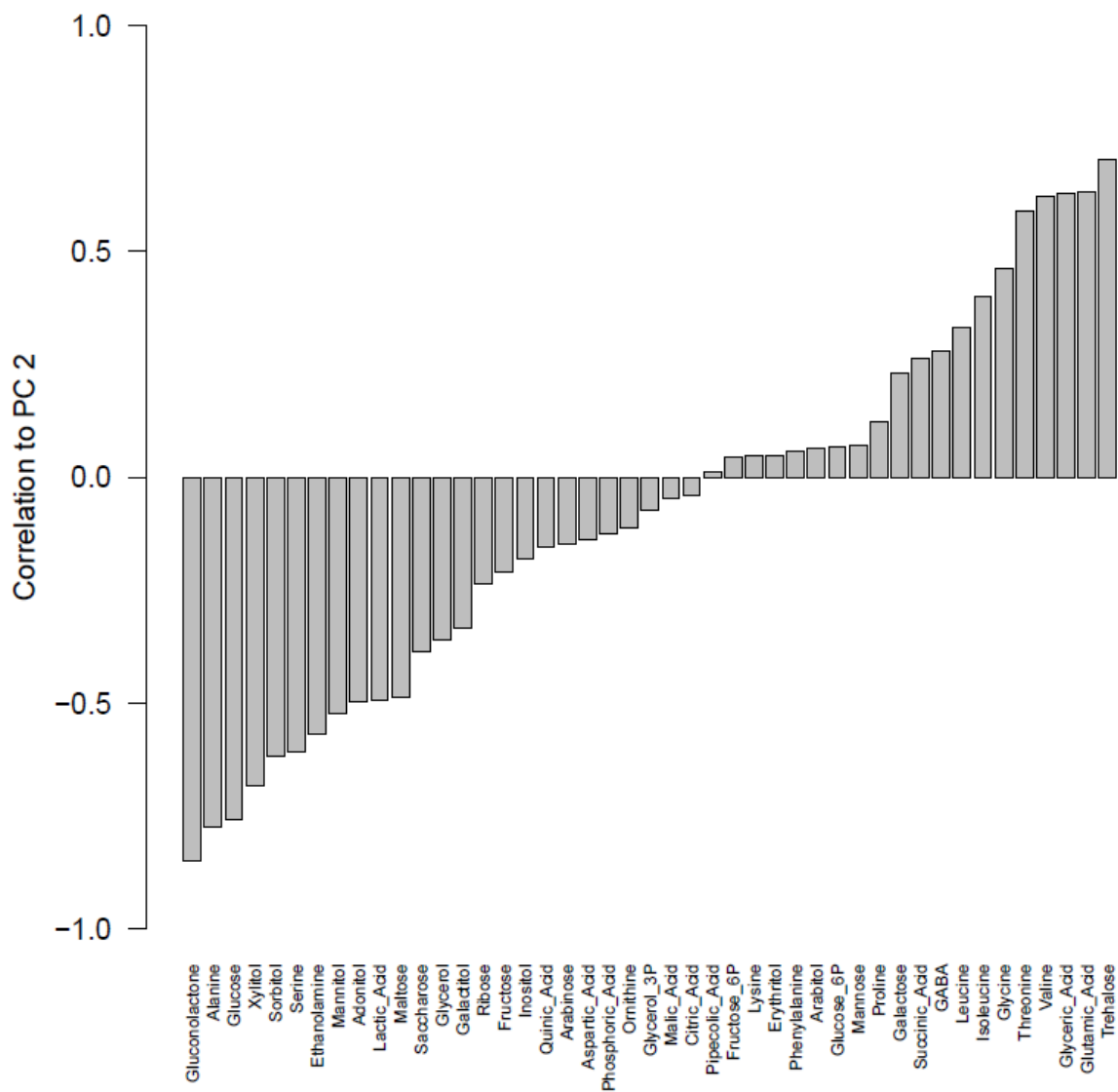


**Figure S9:** Concentration changes of glycolysis-related compounds. Boxplots sharing the same letter are not significantly different ( $P < 0.05$ ; Tukey test). Blue boxes depict immature females and red boxes mature ones.

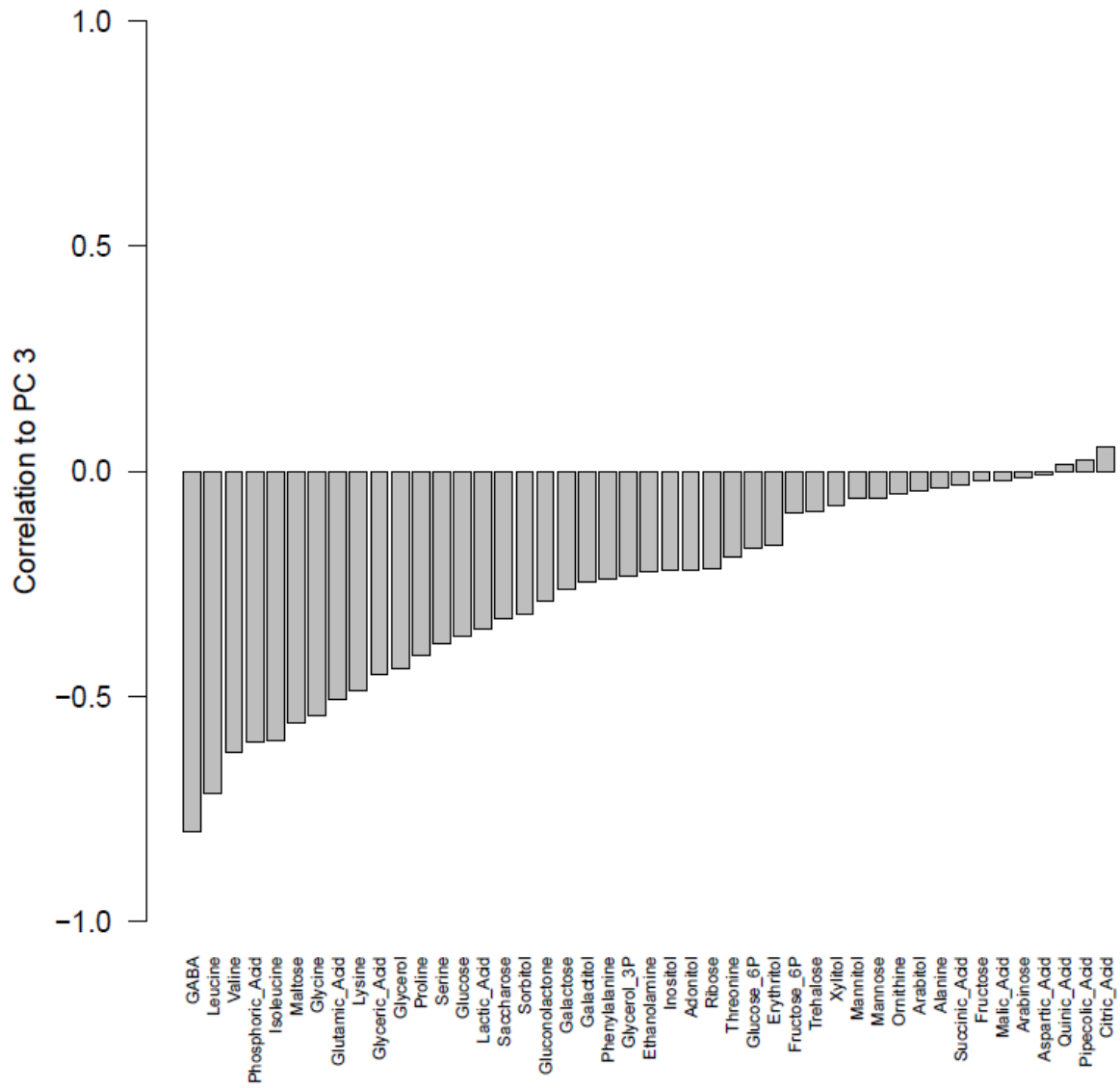




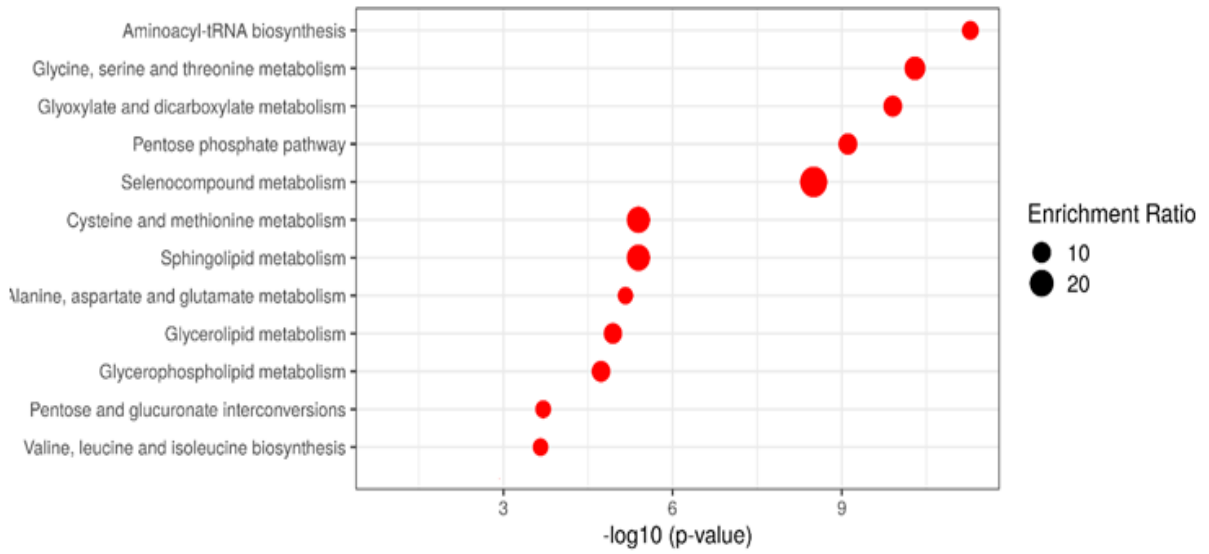
**Fig S10:** Correlation values of the different metabolites to the first principal component (PC1) of the PCA.



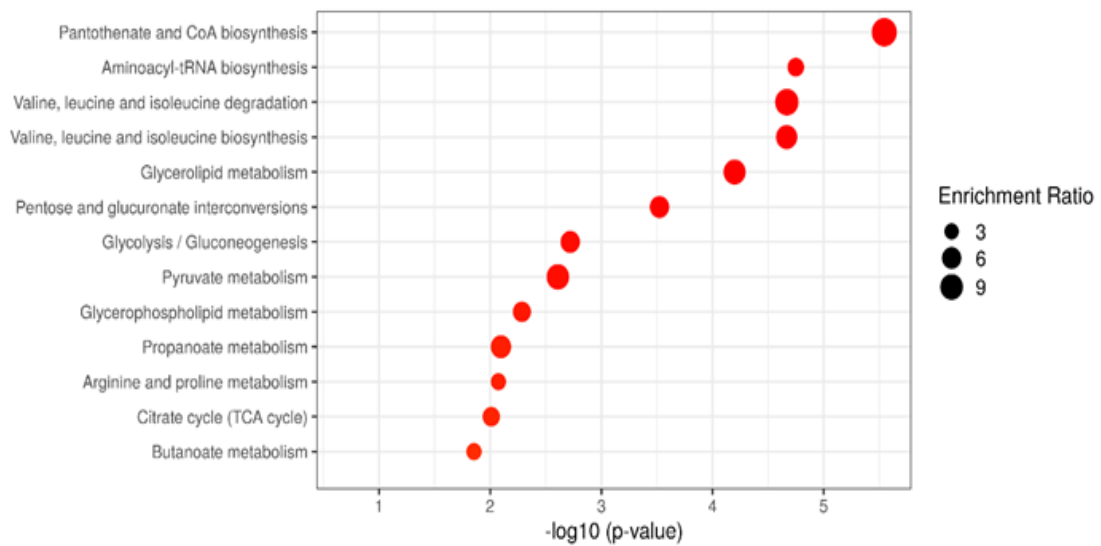
**Fig S11:** Correlation values of the different metabolites to the second principal component (PC2) of the PCA.



**Fig S12:** Correlation values of the different metabolites to the third principal component (PC3) of the PCA.



**Fig S13:** Enrichment analysis for metabolites that varied between biogeographic origins.



**Fig S14:** Pathway enrichment analysis for metabolites that varied with reproductive maturity status

## Supplementary Tables

**Supplementary Table S1:** Outcomes of GLMs for all individual metabolites assessing the effect of reproductive maturity status (immature vs. mature), biogeographic origin (tropical vs. temperate) and their interaction on concentration levels.

Metabolite	<i>p</i> -Values		
	Reproductive maturity	Species	Interaction
Adonitol	0.162	<0.001	0.231
Alanine	0.275	<0.001	<0.001
Arabinose	<0.001	<0.001	0.069
Arabitol	<b>0.008</b>	<0.001	<b>0.014</b>
Aspartic Acid	<0.001	<0.001	0.081
Citric Acid	<0.001	<0.001	0.084
Erythritol	<0.001	<b>0.019</b>	0.427
Ethanolamine	0.990	<0.001	<b>0.001</b>
Fructose	0.068	<0.001	<b>0.009</b>
Fructose-6P	0.318	<0.001	0.085
GABA	<0.001	<0.001	0.113
Galactitol	0.967	<0.001	0.471
Galactose	0.069	<0.001	<0.001
Gluconolactone	0.737	<0.001	<b>0.003</b>
Glucose	<b>0.012</b>	<0.001	<b>0.020</b>
Glucose-6P	<b>0.023</b>	<0.001	0.192
Glutamic Acid	<0.001	<0.001	<0.001
Glyceric Acid	<0.001	<0.001	<b>0.009</b>
Glycerol	<0.001	<0.001	<0.001
Glycerol-3P	<0.001	<0.001	<0.001
Glycine	0.922	<0.001	<0.001
Inositol	<b>0.016</b>	<0.001	<0.001
Isoleucine	0.083	<0.001	<0.001
Lactic Acid	<0.001	<0.001	0.054
Leucine	<0.001	<0.001	<0.001
Lysine	<0.001	<0.001	<0.001
Malic Acid	<b>0.007</b>	<0.001	0.326
Maltose	<0.001	<0.001	0.930
Mannitol	0.518	<b>0.002</b>	<0.001
Mannose	0.910	<0.001	<0.001
Ornithine	<0.001	<0.001	0.086
Phenylalanine	<b>0.028</b>	<0.001	<b>0.032</b>
Phosphoric Acid	<0.001	<0.001	0.051
Pipecolic Acid	<0.001	<0.001	0.125
Proline	<0.001	<0.001	<0.001
Quinic Acid	<0.001	<0.001	0.076
Ribose	0.959	<0.001	0.088
Saccharose	0.777	<b>0.011</b>	0.631
Serine	<0.001	<0.001	<0.001
Sorbitol	0.598	<0.001	<b>0.001</b>
Succinic Acid	0.083	<0.001	<0.001
Threonine	0.437	<0.001	<0.001
Trehalose	0.425	<0.001	<0.001
Valine	<0.001	<0.001	<b>0.001</b>
Xylitol	<b>0.010</b>	<0.001	<0.001

## Supplementary Table S2

Metabolites identified with GC-MS, grouped by biochemical families.

Organic acids	TCA intermediates	Phosphorylated compounds	Polyols	Carbohydrates	Amino acids	Glycolysis-related compounds
Lactic_Acid	Citric_Acid	Fructose_6P	Adonitol	Arabinose	Alanine	Alanine
Quinic_Acid	Malic_Acid	Glucose_6P	Arabitol	Fructose	Aspartic_Acid	Fructose_6P
Glyceric_Acid	Succinic_Acid	Glycerol_3P	Erythritol	Galactose	Glutamic_Acid	Glucose
		Phosphoric_Acid	Galactitol	Gluconolactone	Glycine	Glucose_6P
			Glycerol	Glucose	Isoleucine	Glyceric_Acid
			Inositol	Maltose	Leucine	Glycerol
			Mannitol	Mannose	Lysine	Glycerol_3P
			Sorbitol	Ribose	Ornithine	
			Xylitol	Saccharose	Phenylalanine	
				Trehalose	Pipecolic_Acid	
					Proline	
					Serine	
					Threonine	
					Valine	