

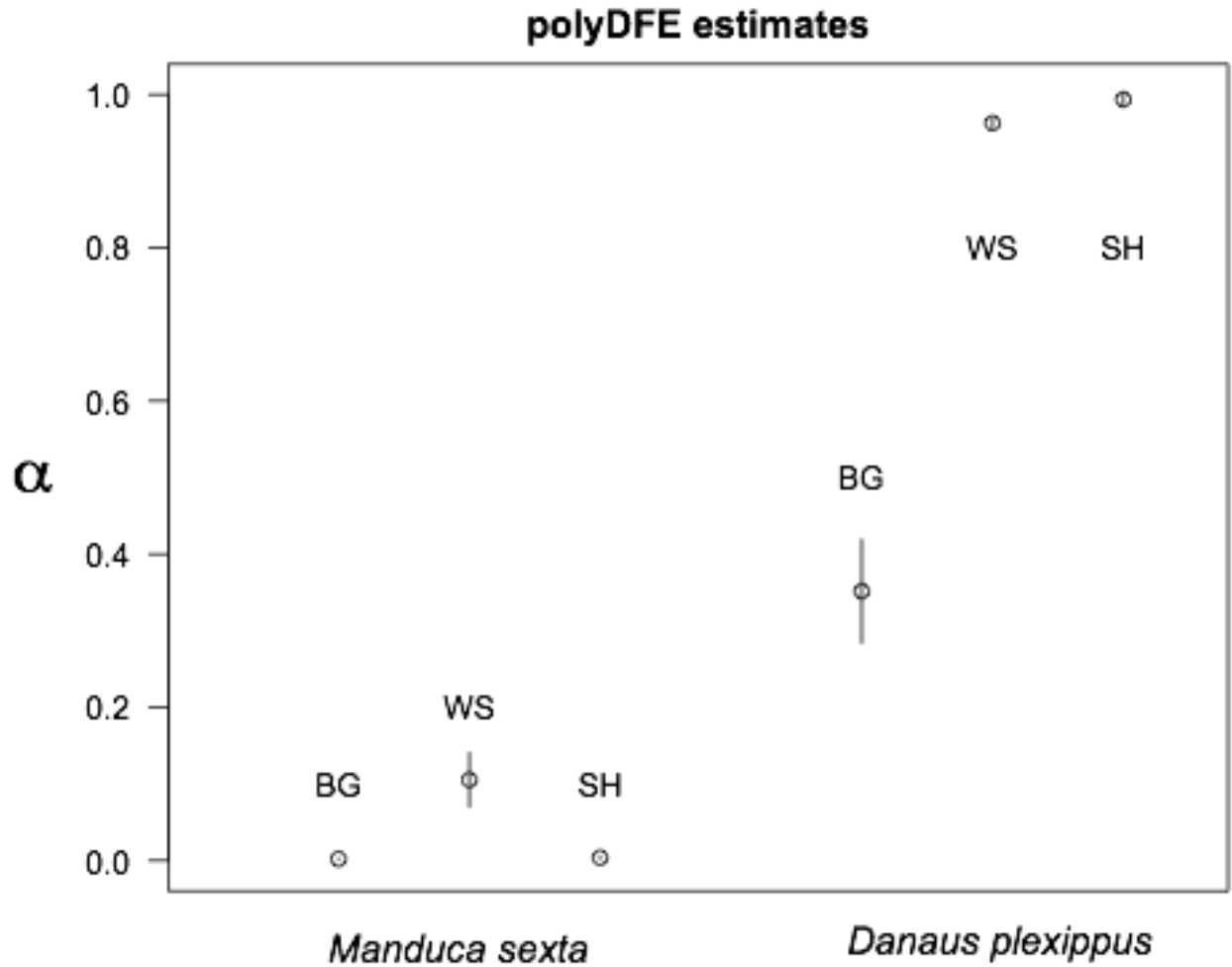
**Supplemental Tables and Figures**

Sample	Bowtie 2 alignment	Stampy alignment	Mean coverage depth
S32	94.12%	NA	18.13
S33	93.90%	NA	19.82
S34	93.84%	NA	18.90
S35	94.02%	NA	16.93
S36	94.04%	NA	19.13
S37	94.06%	NA	20.08
S38	94.08%	NA	22.44
S39	94.01%	NA	26.16
S40	93.79%	NA	19.72
S42	94.12%	NA	25.67
S44	94.07%	NA	22.10
S45	94.16%	NA	20.49
Q6	78.59%	85.44%	11.2

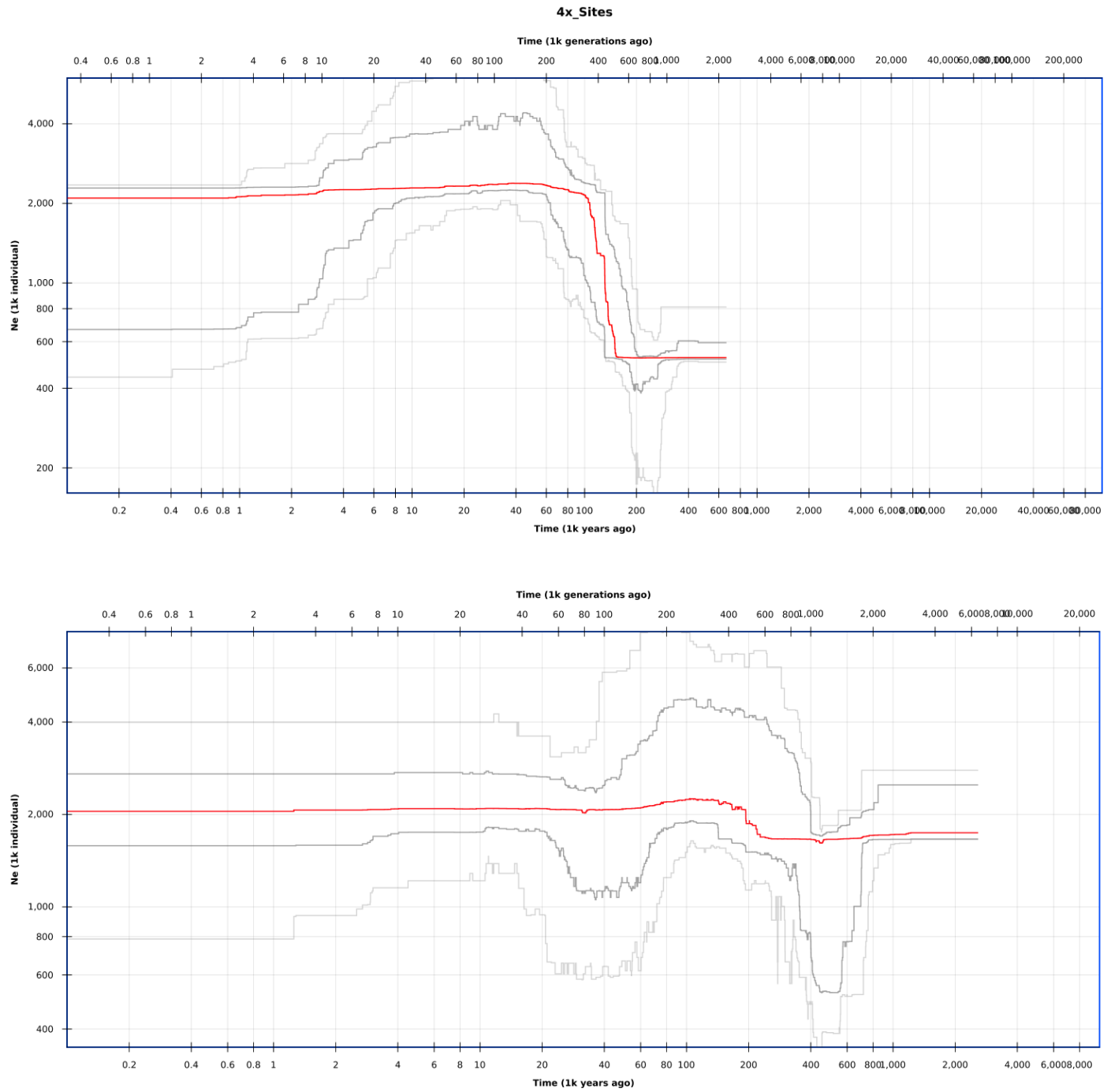
**Table S1.** Basic alignment summary statistics of newly sequenced *Manduca* samples. Those starting with S are from *M. sexta*, Q from *M. quinquemaculata*. Alignments with bowtie2 were carried out using the parameter defaults for very-sensitive-local alignment. In order to improve alignment of heterospecific reads, stampy was used, with default parameters excepting the substitution rate parameter, which was increased to 0.1. Coverage depth was calculated with deeptools. Raw sequences can be found on NCBI with the following accession: SRP144217.

Sample	Head	Thorax	Midgut	Testes	Accessory gland
M1	7,038,350	6,339,933	3,999,656	5,958,899	7,021,055
M2	6,835,543	5,418,564	7,074,743	10,350,175	7,453,113
M3	6,471,153	6,469,699	10,497,431	6,935,772	7,984,240

**Table S2.** Raw read counts per tissue for each sample generated for expression analyses. Tissues were taken from a lab colony of monarch butterflies at the University of Kansas for RNA extraction and sequencing. Reads were taken through standard expression analysis pipeline with Trinity to generate FPKM values for each gene within a tissue. Tissue specificity metrics were calculated with custom R scripts.



**Figure S1.** Estimates of  $\alpha$  from polyDFE. Points are the mean parameter estimate from 100 bootstrap replicates of the input site frequency spectra for three classes of genes: the genome background (BG), whole sperm proteome (WS), and sperm homologs (SH) in each species. Bars represent twice the standard error of the estimate. Note that for some estimates (e.g. *Manduca* genome background, likelihood nearly always converged on the same estimate, leading to low variance). While more adaptive evolution is detected in the sperm proteome of *Manduca* than the genome background, the magnitude of the difference is dwarfed by the difference in monarchs.



**Figure S2.** Inferred population sizes and histories for both monarchs (**top**) and Carolina sphinx moths (**bottom**) based on coalescent history of 4-fold degenerate sites with the program stairway plot using the estimated mutation rate from *Heliconius melpomene*. Red lines represent mean of estimates, dark and light grey bars represent 90% and 95% confidence intervals.