Wolbachia analysis

Here, I am reporting an updated analysis of the DrosEU phenotyping focusing on potential effects of *Wolbachia* infections on trait variation. I therefore tested for the effect of *Wolbachia* presence/absence on trait variation in different populations (hereafter called "Countries") taking into account that certain traits were measured in more than one lab. I only included countries that were polymorphic for *Wolbachia* infections, i.e. there are lines that are either infected or uninfected. Thus, I had to exclude "Russia" and "Finland", since all lines in these countries were *Wolbachia*-positive. To avoid biases, I excluded countries with less than two lines for each infection type (infected [wol+] and uninfected [wol-]) and only retained lines that were analyzed in at least n-1 labs (where n is the number of labs analyzing a given phenotype) for each trait. I had to be liberal here since I would have otherwise lost all data from most traits.

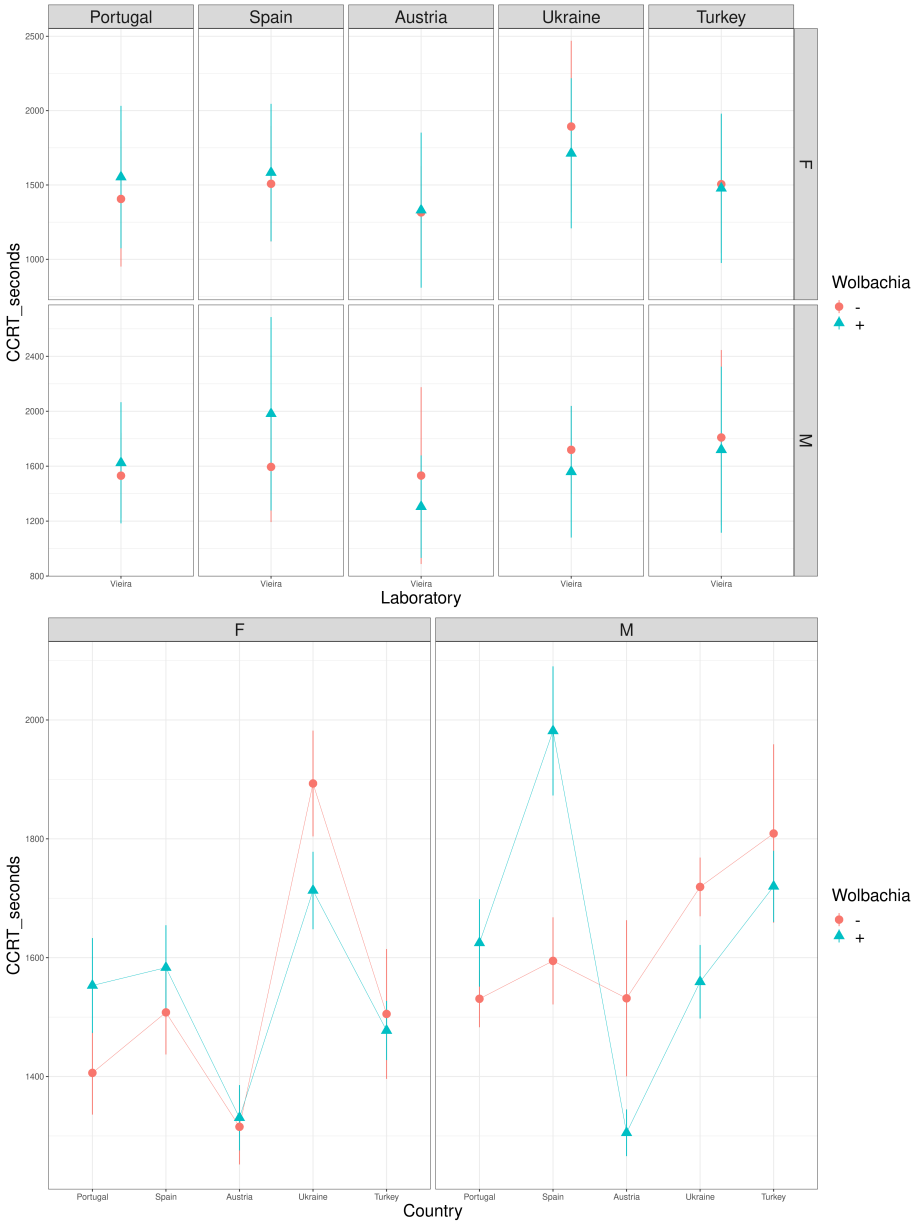
In addition to the traits analyzed in the main analysis, I also investigated developmental assymetry for the phenotype WingArea. I therefore calculated the absolute difference between left and right centroid and included this as a trait.

Following the general statistical approach of the main analyzes, I employed general linear mixed models of the form `Trait ~ Wolbachia * Sex * Country + PC.ratio+(1|Lab) + (1|Line:Country) + (1|Batch)` *Trait* is the dependent variable, *Wolbachia* is an independent variable with two levels (+,-), *Sex* is an independent variable with two levels (F,M) which was only fitted whenever both sexes were analyzed, *Country* is a nominal fixed factor describing the populations with varying number of levels (depending on the filtering explained above), which was only fitted when more than two populations were included in the analysis. Whenever a trait was investigated in more than one lab, I fitted the diet (PC.ratio) as a continuous factor and added the nominal factor *Lab* as a random factor. In addition, I included the random factors Line (nested in Country) and Batch as described in the main analysis. I further included all possible interactions among the factors *Wolbachia*, *Sex* and *Country*, whenever possible.

I used the *car* package in R to perform Type-III ANOVAs for each model. Importantly, I adjusted contrasts to fit Type - III ANOVAs in R, see here https://rcompanion.org/rcompanion/d_04.html and https://www.r-bloggers.com/2011/03/anova-%E2%80%93-type-iiiiii-ss-explained/. Essentially, this uses sum contrasts to compare each group against the grand mean. The default settings in *R* are not correct for fitting type-III ANOVAS.

In addition, I plotted the mean trait values for each *Wolbachia* type in the context of Country, Lab and Sex whenever possible. The error bars represent Standard Deviations for plots showing the lab means and Standard Errors for the Country means Below, I am showing the plots and report significant factor below.

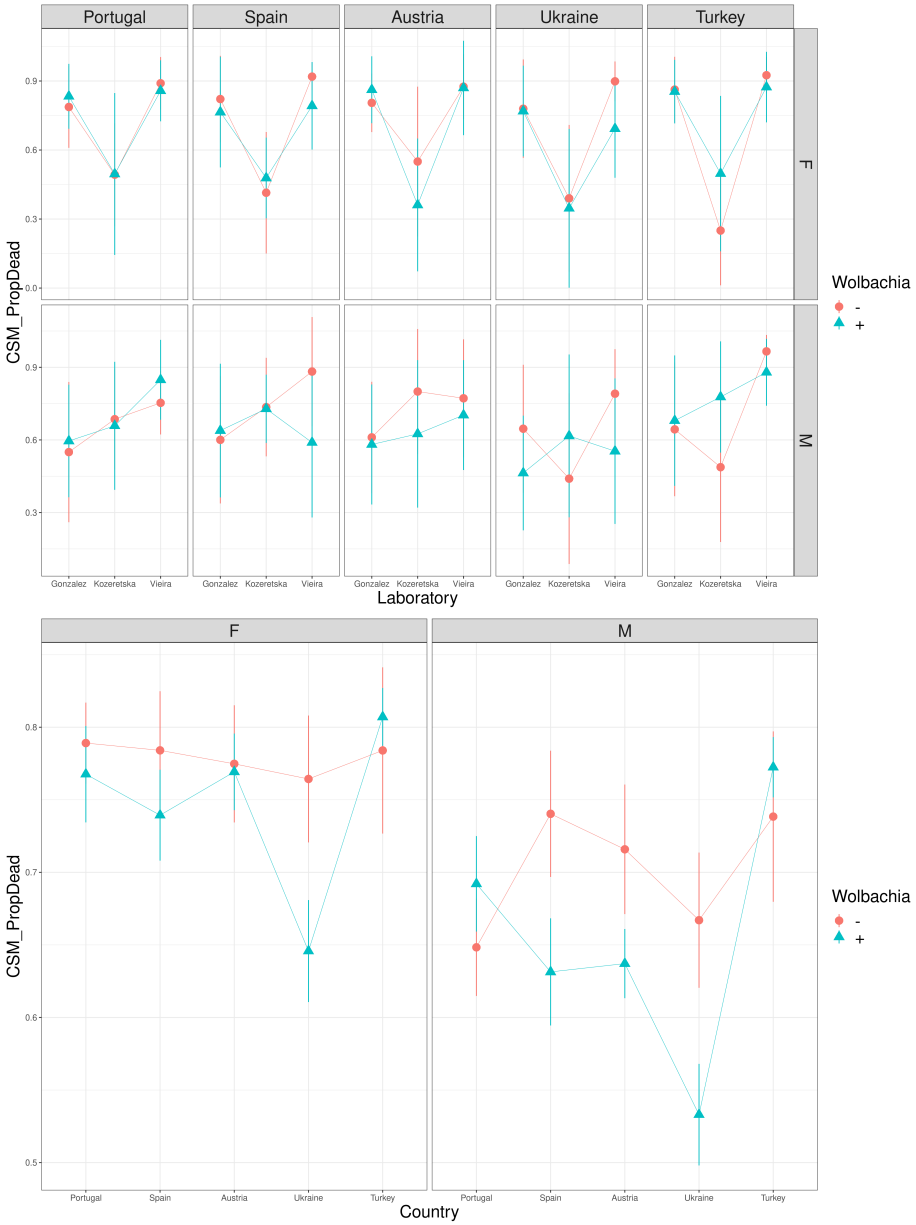
Chill Coma



Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	3781.1494	1	<2.2e-16	***
Wolbachia	0.0049	1	0.9444345	
Country	24.7465	4	5.657e-05	***

Factor	Chisq	Df	Pr(>Chisq)	
Sex	9.9067	1	0.0016468	**
Wolbachia:Country	8.8236	4	0.0656628	.
Wolbachia:Sex	0.0047	1	0.9454932	
Country:Sex	22.3480	4	0.0001709	***
Wolbachia:Country:Sex	6.5071	4	0.1643463	

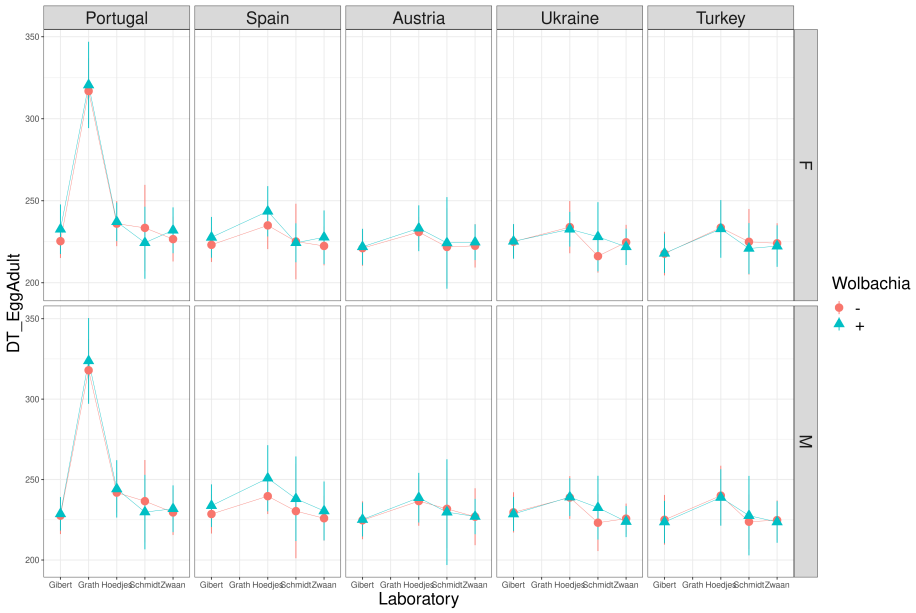
Cold Shock Mortality

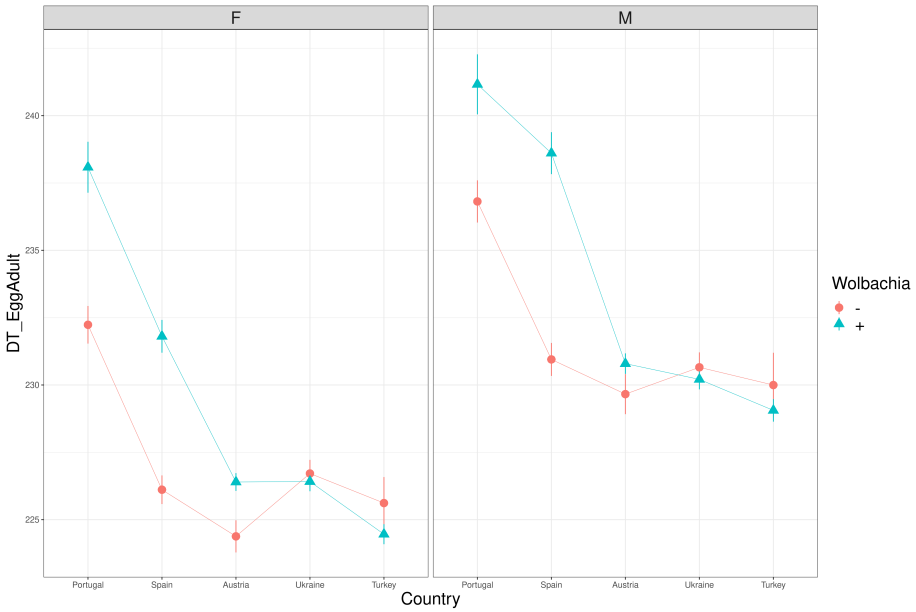


Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	127.6540	1	<2.2e-16	***
Wolbachia	2.2825	1	0.13084	
Country	7.2735	4	0.12212	

Factor	Chisq	Df	Pr(>Chisq)	
Sex	38.5557	1	5.321e-10	***
PC.ratio	5.0372	1	0.02481	*
Wolbachia:Country	5.4970	4	0.23999	
Wolbachia:Sex	0.3152	1	0.57448	
Country:Sex	3.1691	4	0.52993	
Wolbachia:Country:Sex	3.7428	4	0.44193	

Development Time (ETA)

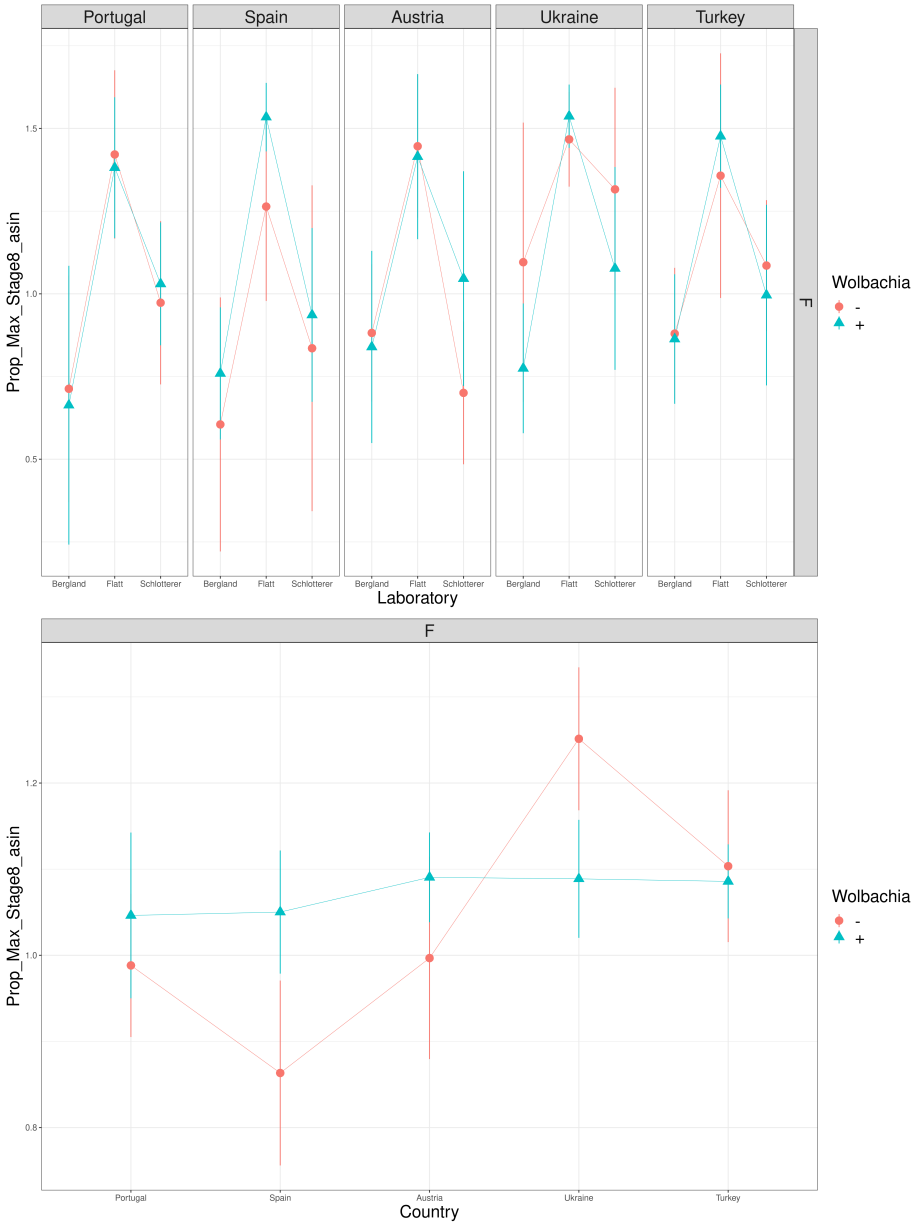




Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	15.8424	1	6.884e-05	***
Wolbachia	2.3727	1	0.123476	
Country	16.4695	4	0.002450	**

Factor	Chisq	Df	Pr(>Chisq)	
Sex	317.2622	1	<2.2e-16	***
PC.ratio	0.0613	1	0.804380	
Wolbachia:Country	6.5635	4	0.160832	
Wolbachia:Sex	0.0137	1	0.906705	
Country:Sex	14.7101	4	0.005342	**
Wolbachia:Country:Sex	2.2248	4	0.694487	

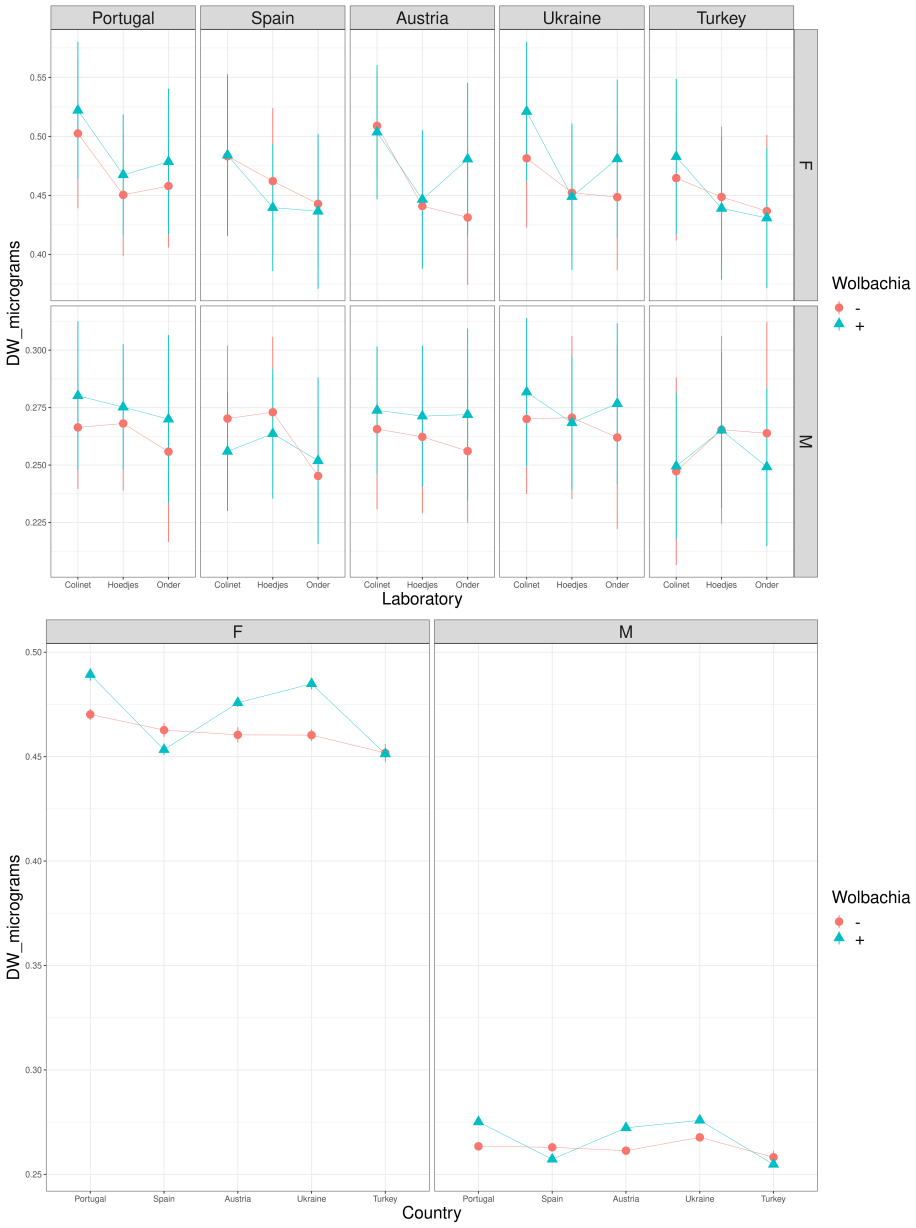
Diapause



Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	4.5751	1	0.03244	*
Wolbachia	0.0235	1	0.87819	
Country	10.5265	4	0.03243	*

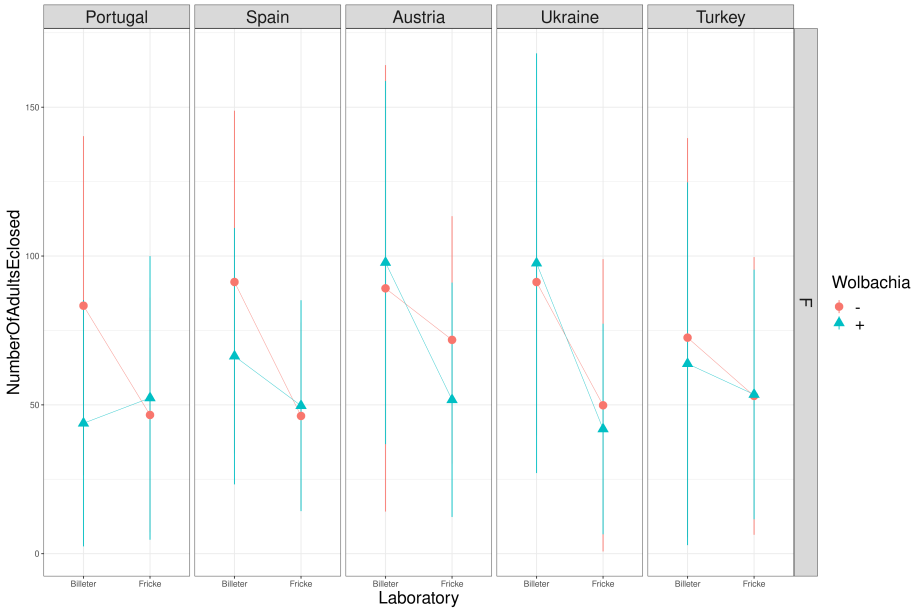
Factor	Chisq	Df	Pr(>Chisq)
PC.ratio	0.0836	1	0.77245
Wolbachia:Country	4.1100	4	0.39133

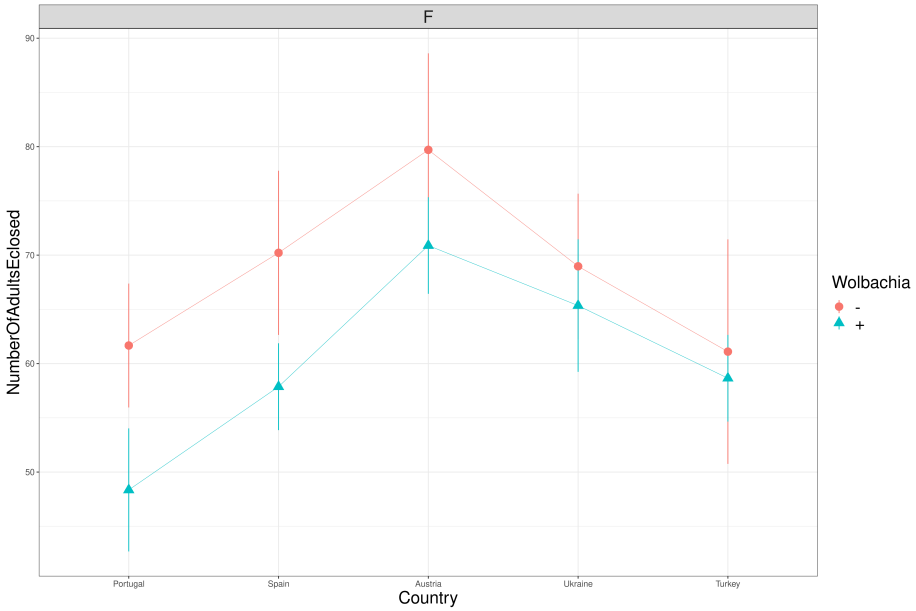
Dry weight



Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	14778.8956	1	<2.2e-16	***
Wolbachia	2.3452	1	0.125671	
Country	10.3645	4	0.034716	*
Sex	41065.4297	1	<2.2e-16	***
PC.ratio	313.9711	1	<2.2e-16	***
Wolbachia:Country	2.7430	4	0.601714	
Wolbachia:Sex	8.1435	1	0.004322	**
Country:Sex	26.9587	4	2.026e-05	***
Wolbachia:Country:Sex	12.9142	4	0.011703	*

Fecundity

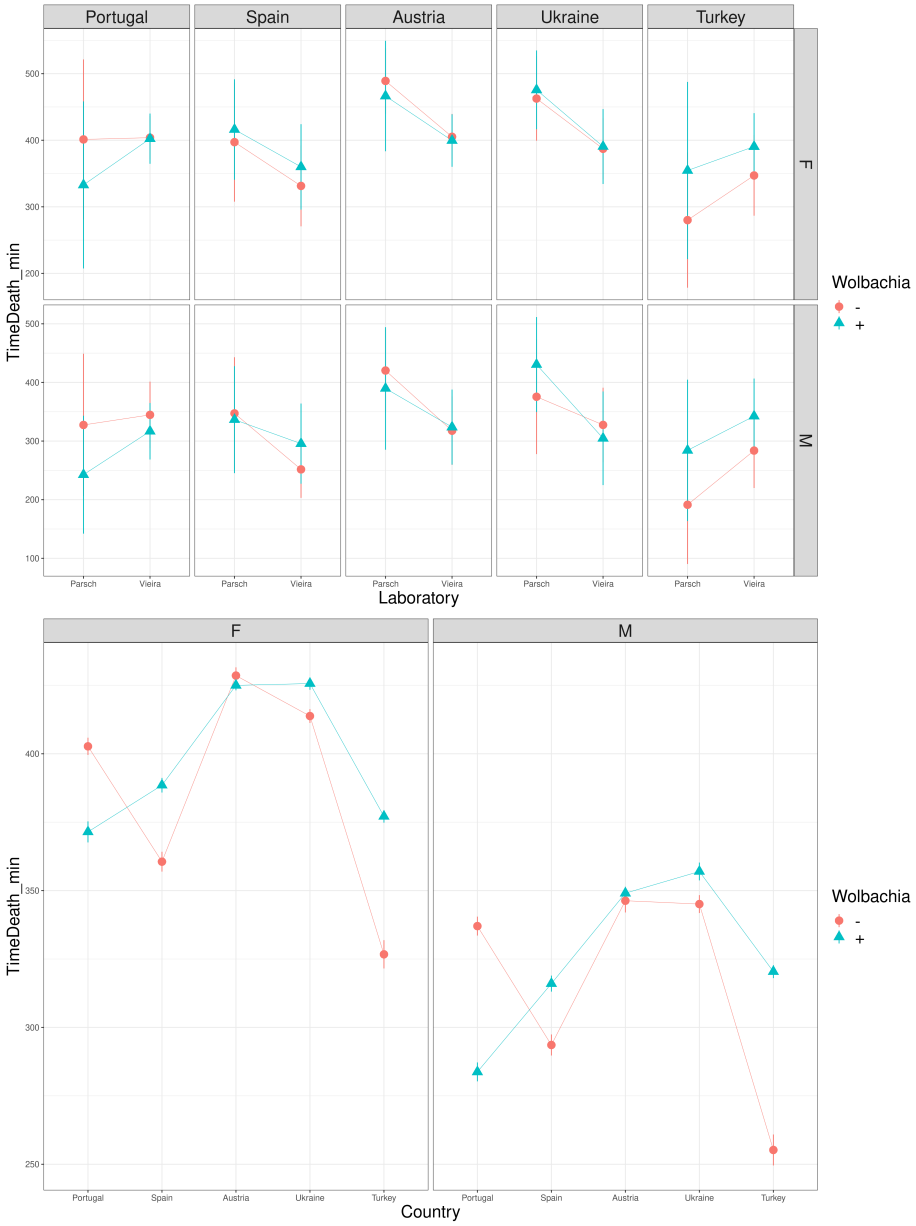




Factor	Chisq	Df	Pr(>Chisq)	
Wolbachia	1.8068	1	0.17890	
(Intercept)	5.6540	1	0.01742	*
Country	3.0578	4	0.54821	

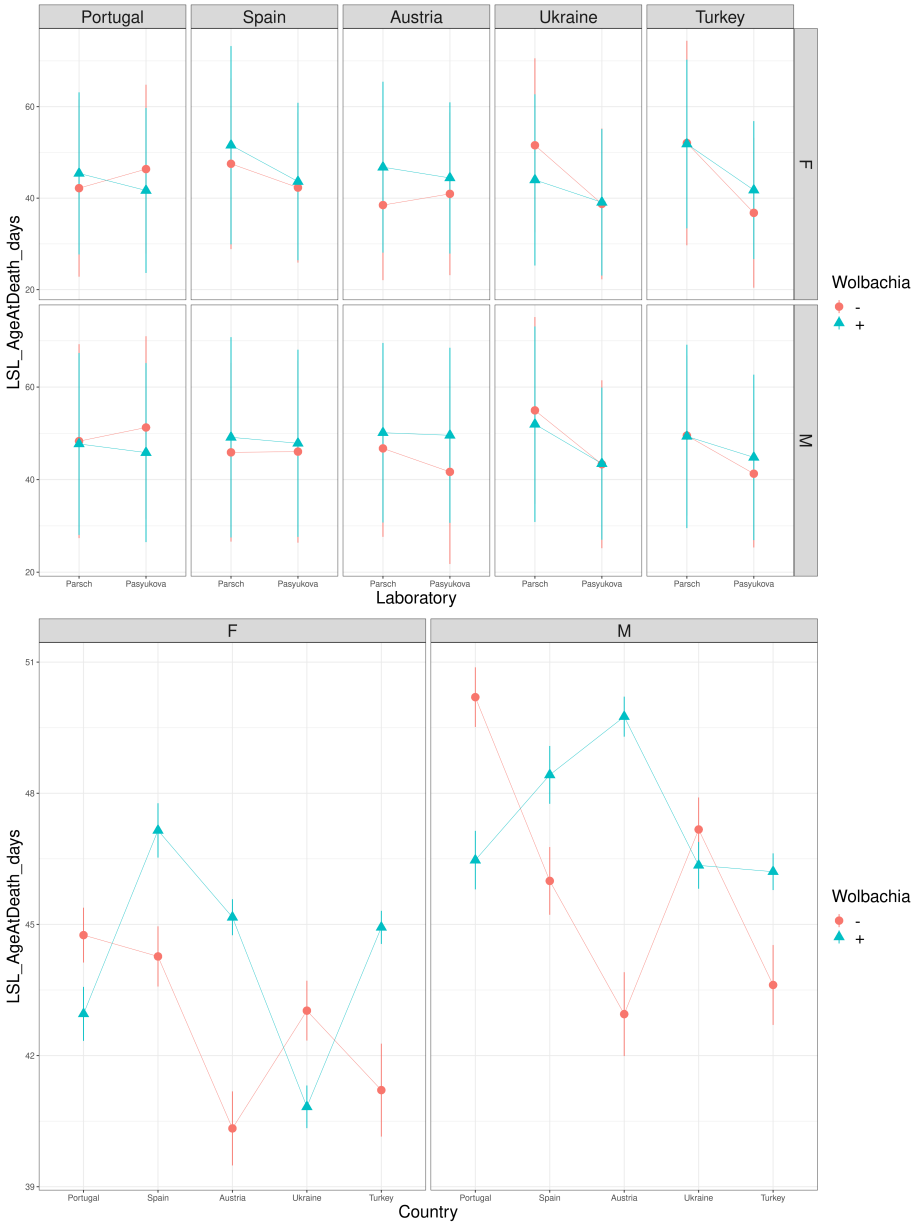
Factor	Chisq	Df	Pr(>Chisq)
PC.ratio	2.2413	1	0.13436
Wolbachia:Country	0.3586	4	0.98573

Heat Shock



Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	483.7408	1	<2.2e-16	***
Wolbachia	0.0528	1	0.8182592	
Country	102.0529	4	<2.2e-16	***
Sex	3092.3527	1	<2.2e-16	***
PC.ratio	12.1750	1	0.0004844	***
Wolbachia:Country	3.3733	4	0.4974090	
Wolbachia:Sex	0.2726	1	0.6015815	
Country:Sex	16.9285	4	0.0019958	**
Wolbachia:Country:Sex	23.2757	4	0.0001115	***

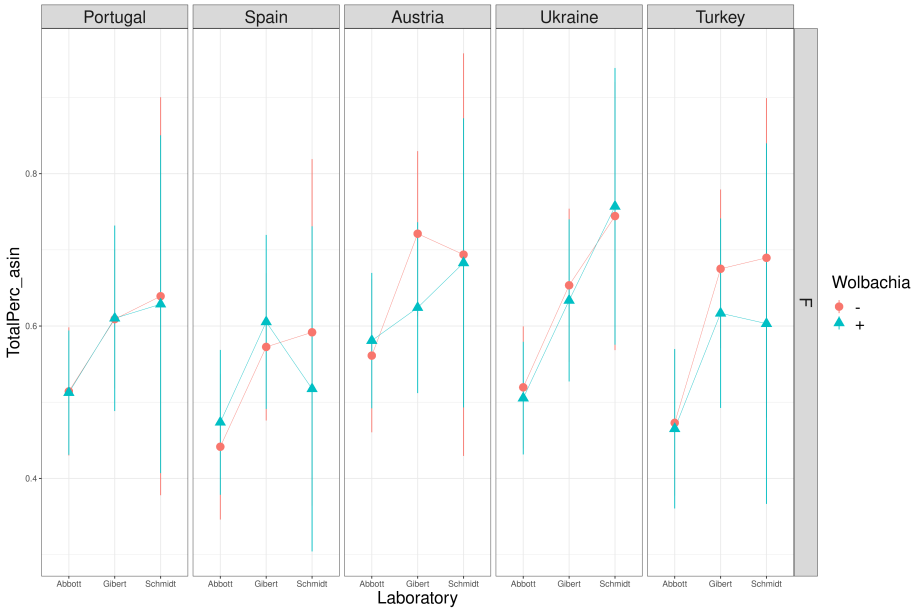
Lifespan

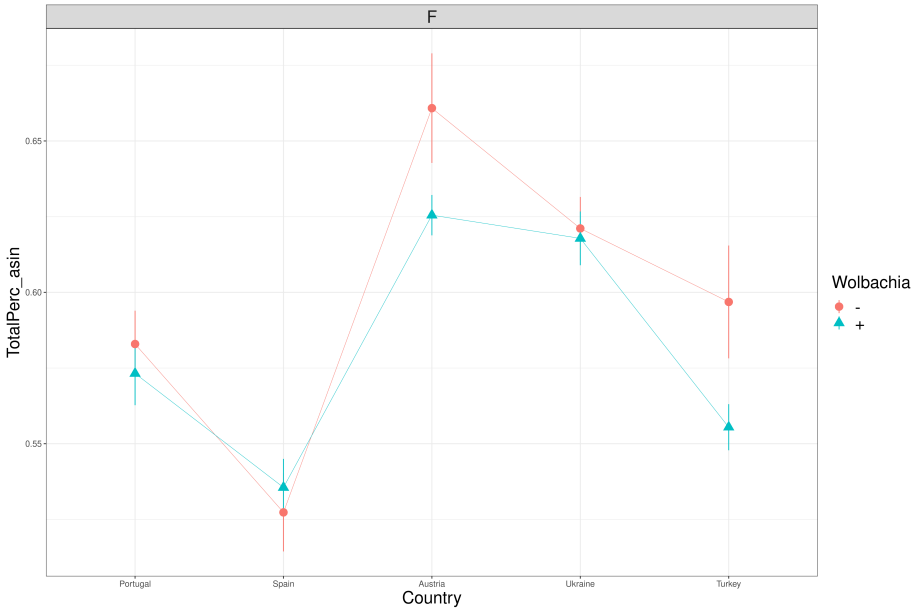


Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	8.4862	1	0.0035784	**
Wolbachia	0.8254	1	0.3636129	
Country	1.6020	4	0.8084274	

Factor	Chisq	Df	Pr(>Chisq)	
Sex	126.2562	1	<2.2e-16	***
PC.ratio	0.7508	1	0.3862209	
Wolbachia:Country	5.4552	4	0.2436999	
Wolbachia:Sex	0.0033	1	0.9540255	
Country:Sex	22.0584	4	0.0001951	***
Wolbachia:Country:Sex	7.2346	4	0.1239979	

Pigmentation

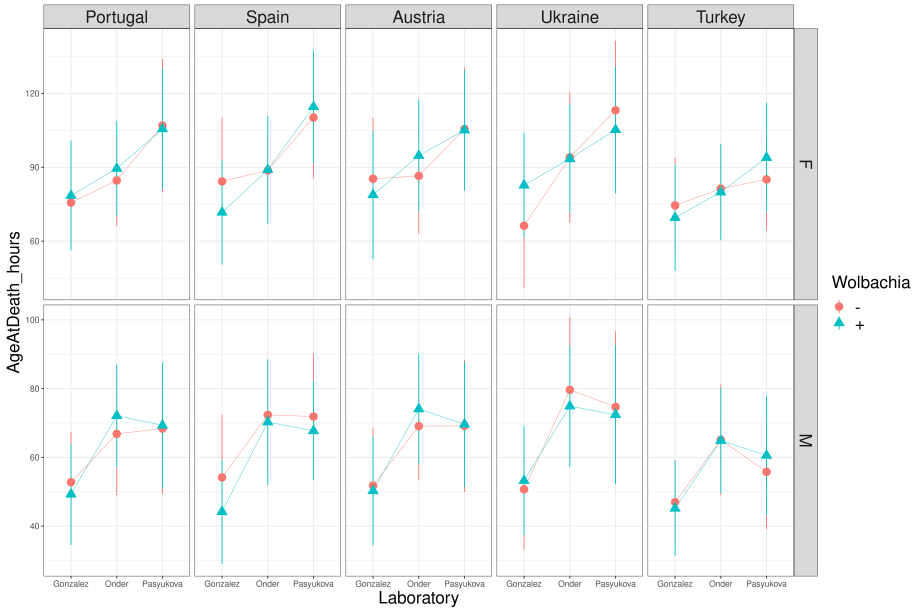


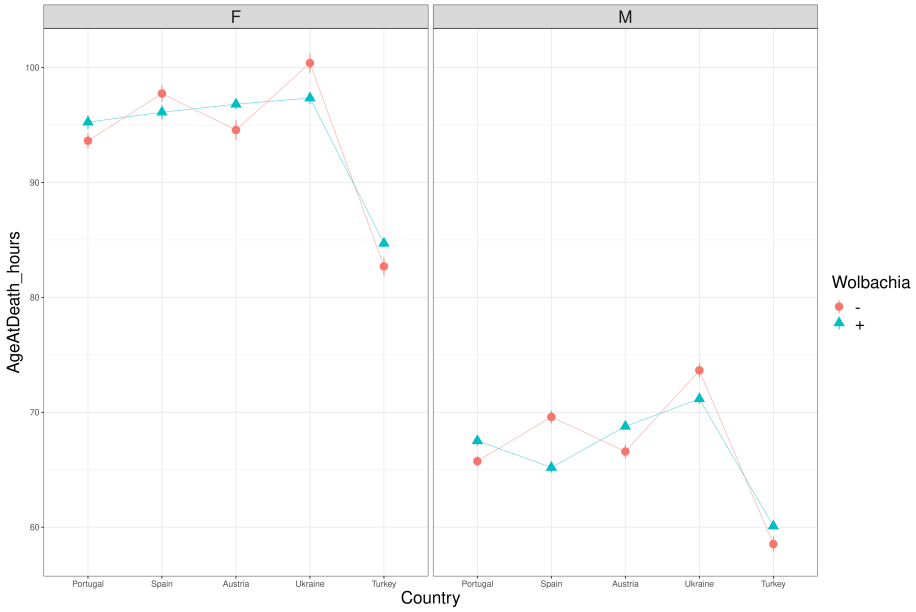


Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	18.3491	1	1.839e-05	***
Wolbachia	0.4498	1	0.50242	
Country	12.6608	4	0.01306	*

Factor	Chisq	Df	Pr(>Chisq)
PC.ratio	0.6794	1	0.40979
Wolbachia:Country	0.8059	4	0.93766

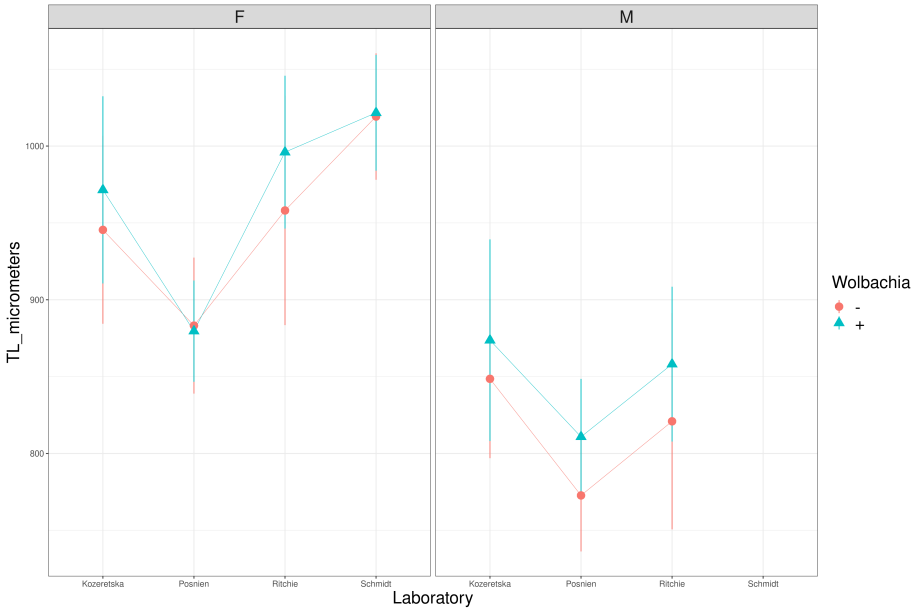
Starvation

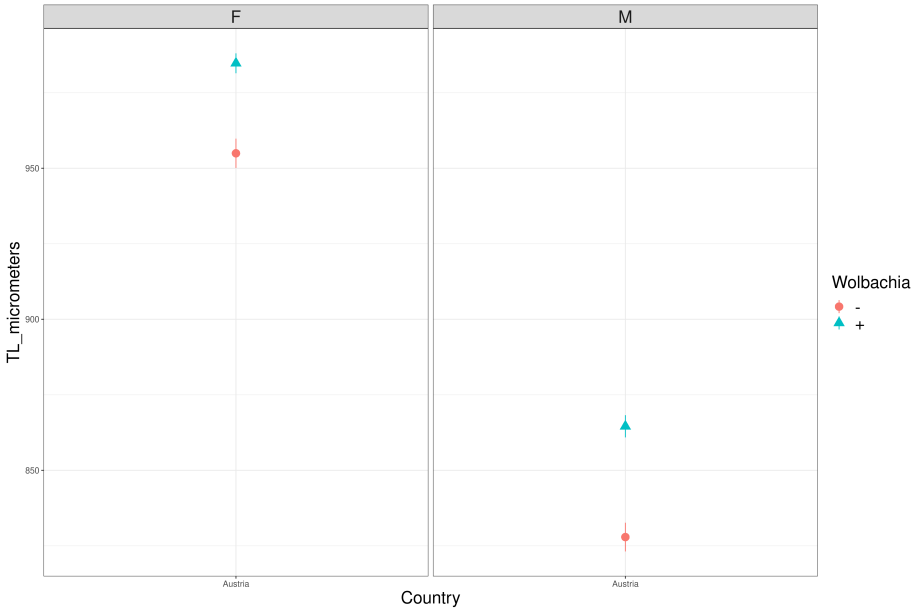




Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	13.4597	1	0.0002437	***
Wolbachia	0.0177	1	0.8942880	
Country	17.2913	4	0.0016965	**
Sex	13936.4179	1	<2.2e-16	***
PC.ratio	2.5622	1	0.1094428	
Wolbachia:Sex	6.0875	1	0.0136141	*
Wolbachia:Country	0.9866	4	0.9118229	
Country:Sex	69.6422	4	2.701e-14	***
Wolbachia:Country:Sex	9.9410	4	0.0414338	*

Thorax Length

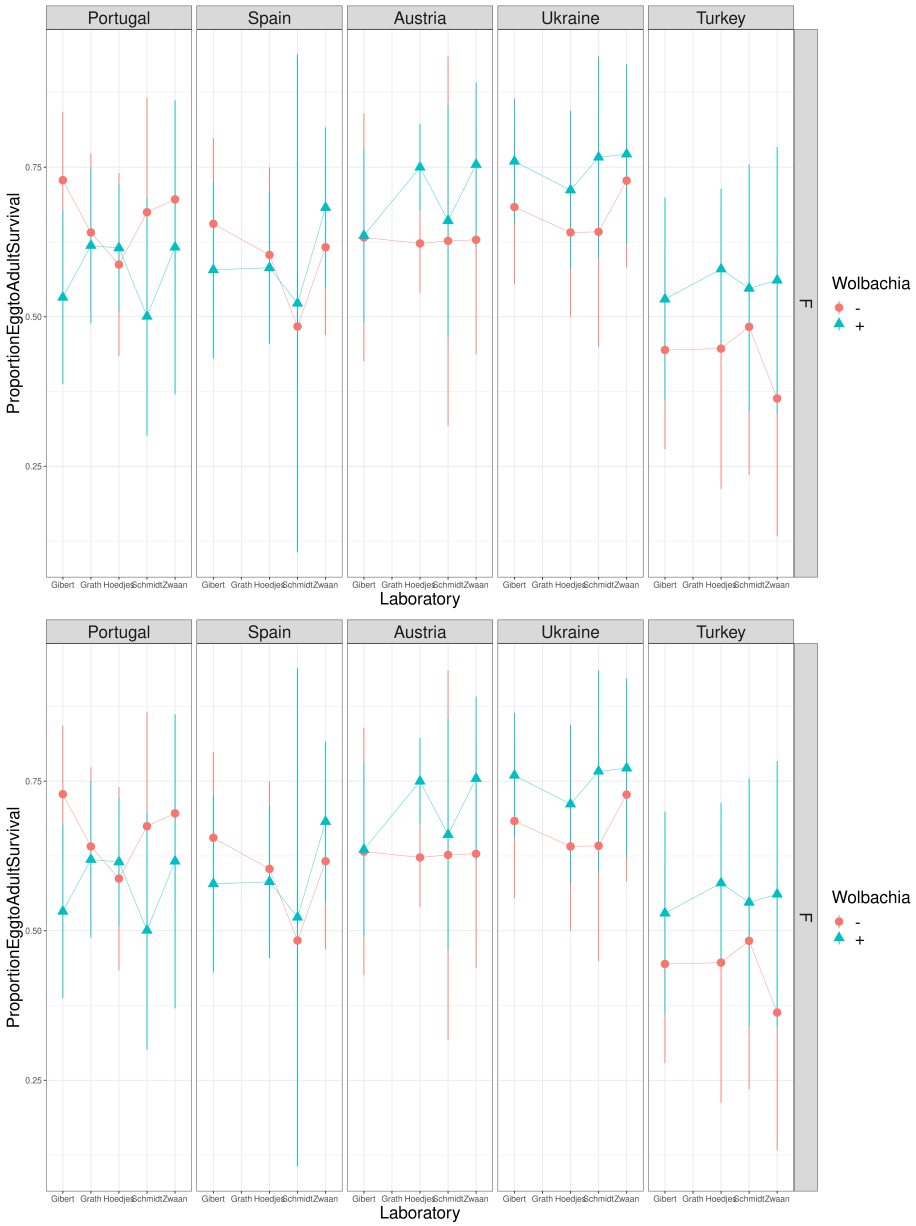




Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	148.6740	1	<2e-16	***
Wolbachia	0.1802	1	0.6712	
Sex	1143.8346	1	<2e-16	***

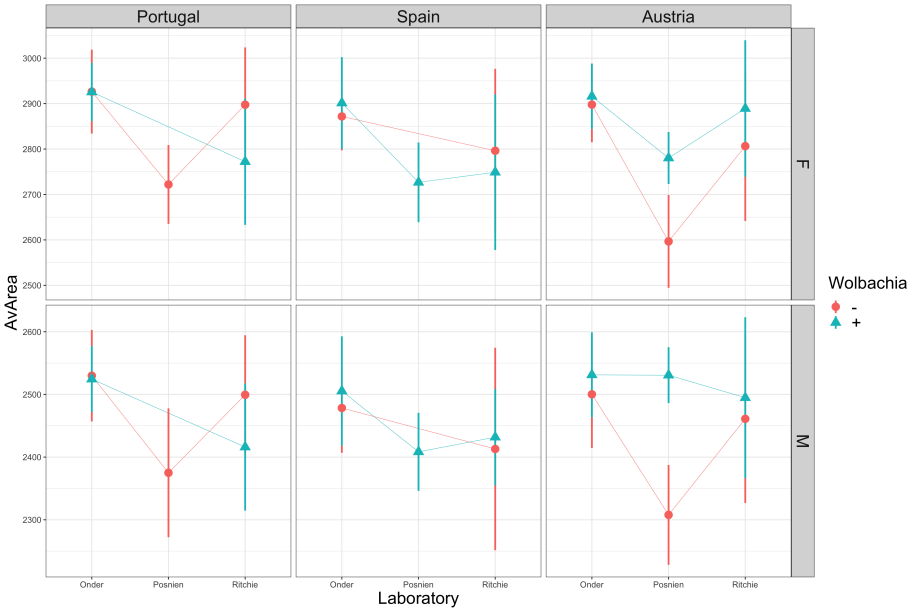
Factor	Chisq	Df	Pr(>Chisq)
PC.ratio	0.3235	1	0.5695
Wolbachia:Sex	0.1213	1	0.7276

Viability

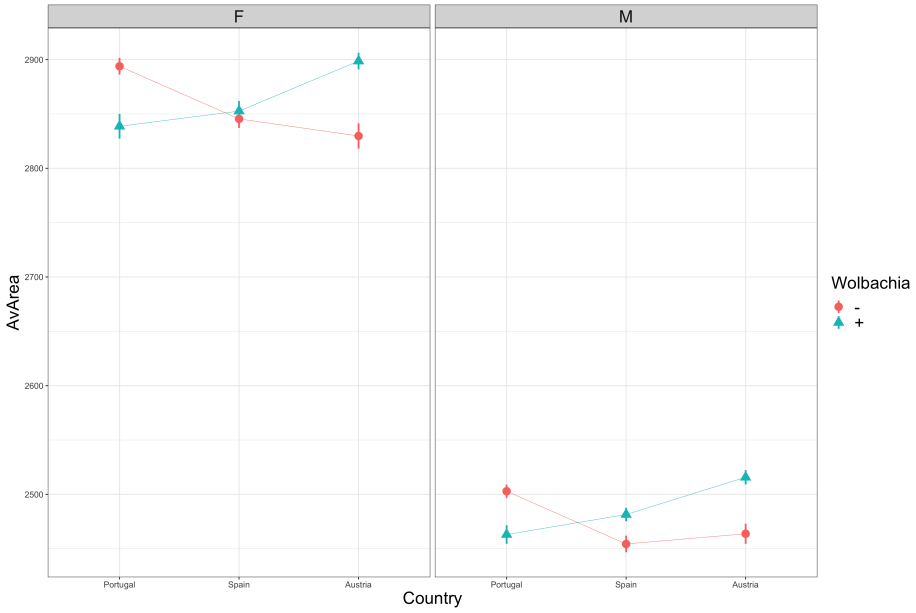


Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	522.5781	1	<2.2e-16	***
Wolbachia	3.0356	1	0.08146	.
Country	34.7633	4	5.195e-07	***
PC.ratio	2.5563	1	0.10986	
Wolbachia:Country	9.9248	4	0.04171	*

Wing area

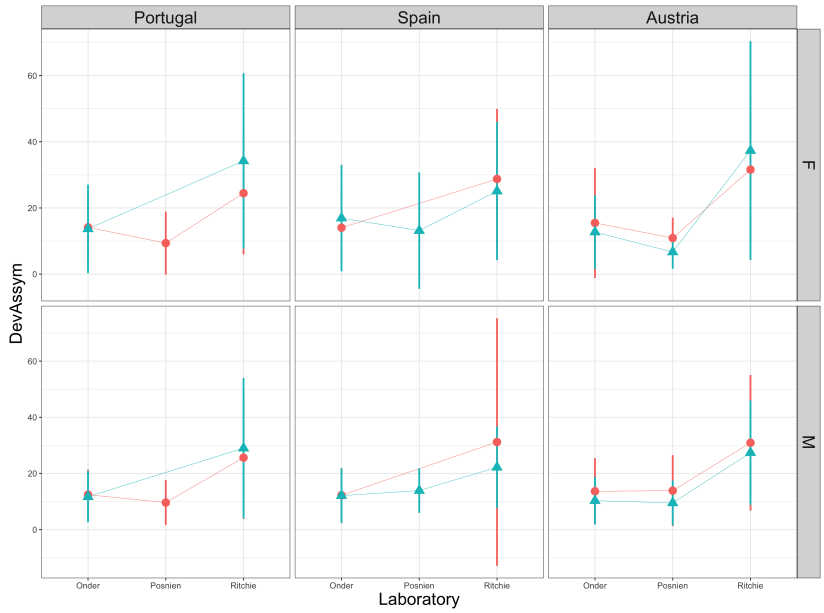


Average Size

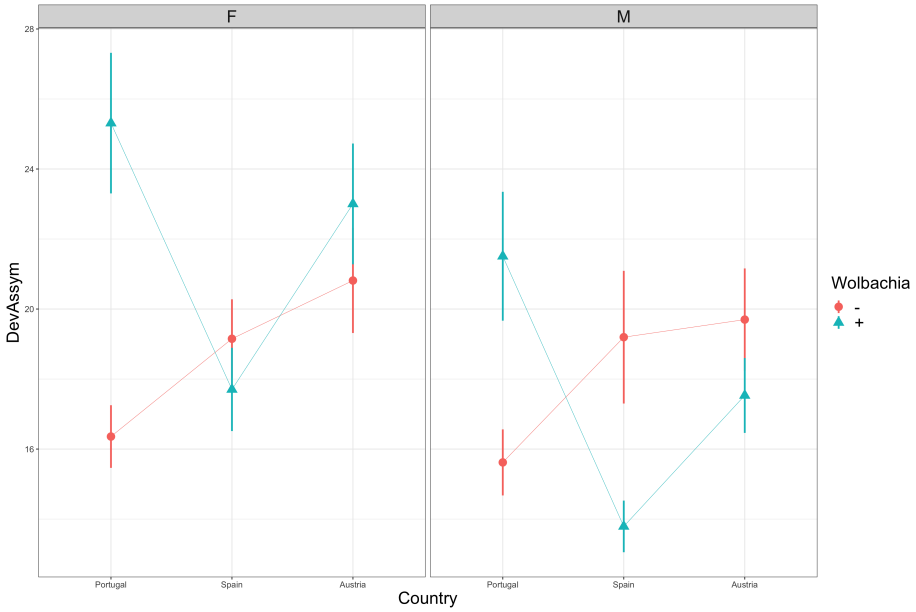


Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	148.1305	1	<2e-16	***
Wolbachia	2.4846	1	0.11497	
Country	8.2861	2	0.01587	*

Factor	Chisq	Df	Pr(>Chisq)	
Sex	9133.5741	1	<2e-16	***
PC.ratio	1.1974	1	0.27385	
Wolbachia:Country	3.4554	2	0.17769	
Wolbachia:Sex	0.3384	1	0.56078	
Country:Sex	0.9356	2	0.62639	
Wolbachia:Country:Sex	5.3901	2	0.06754	.



Developmental Assymetry



Factor	Chisq	Df	Pr(>Chisq)	
(Intercept)	0.0482	1	0.826226	
Wolbachia	1.6634	1	0.197143	
Country	0.7166	2	0.698850	
Sex	10.7692	1	0.001032	**

Factor	Chisq	Df	Pr(>Chisq)	
PC.ratio	0.5219	1	0.470052	
Wolbachia:Country	3.8649	2	0.144791	
Wolbachia:Sex	5.9411	1	0.014792	*
Country:Sex	0.4089	2	0.815085	
Wolbachia:Country:Sex	0.1563	2	0.924803	