Supplementary Information

Materials and Methods:

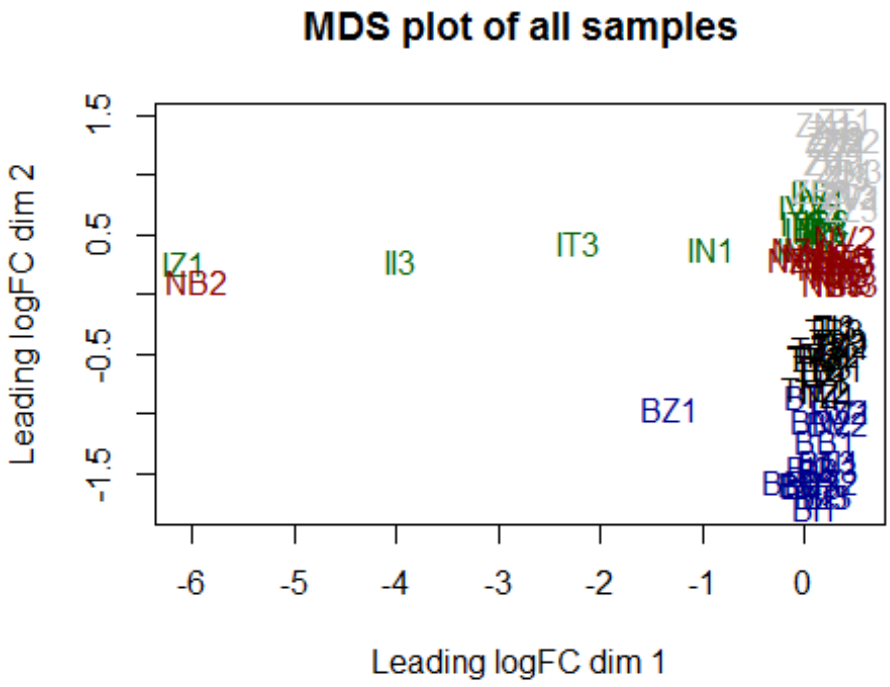

Fig. S1: Multidimensional scaling plot of all 89 samples (one sample was removed due to low quality of the RNAseq library).

Multidimensional scaling (MDS) plots were constructed for unsupervised clustering of samples. MDS plots cluster samples based on similarity, in this case, based on pairwise $\log_2$ fold changes. Two samples with larger differences in gene expression (larger absolute fold changes), are further apart in the plot. Samples that have more similar gene expression patterns (smaller absolute fold changes), cluster more closely together.

This plot demonstrates that most samples cluster by female genotype (blue: Beijing, black: Tasmania, red: Netherlands, green: Ithaca, grey: Zimbabwe). Six samples do not cluster by female genotype (I x Z-1, N x B-2, I x I-3, I x T-3, B x Z-1 and I x N-1). Further analysis demonstrated that these six samples contained

male-specific mRNAs (among which were transcripts from male accessory gland-specific genes). Based on genotype-specific SNPs, we found that these samples were probably contaminated with male mRNA (data not shown). When analyzing SNPs, evidence for male contamination was found in I x Z-3 as well (data not shown). We decided to remove I x Z-1, I x Z-3, N x B-2, I x I-3, I x T-3, B x Z- 1 and I x N-1 from the dataset.

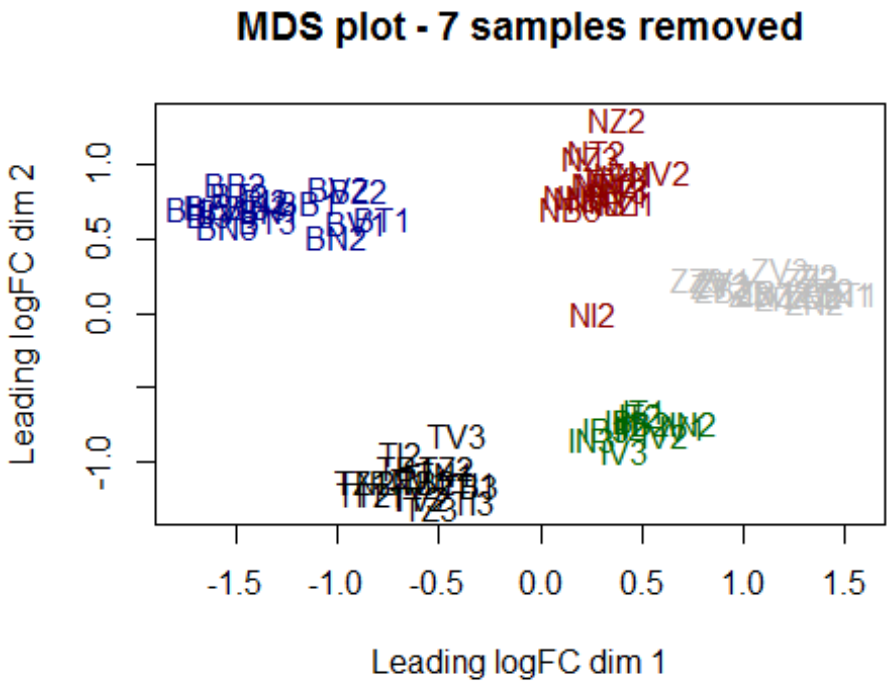

Fig. S2: Multidimensional scaling plot after removal of seven samples.

Unsupervised clustering after removal of seven outliers demonstrates that samples cluster by female genotype. One sample, N x I-2, does not cluster with other Netherlands samples. Based on genotype-specific SNPs, we found that N x I-2 likely contained a mix of Netherlands and Ithaca females (data not shown). This sample was also removed from our analysis.

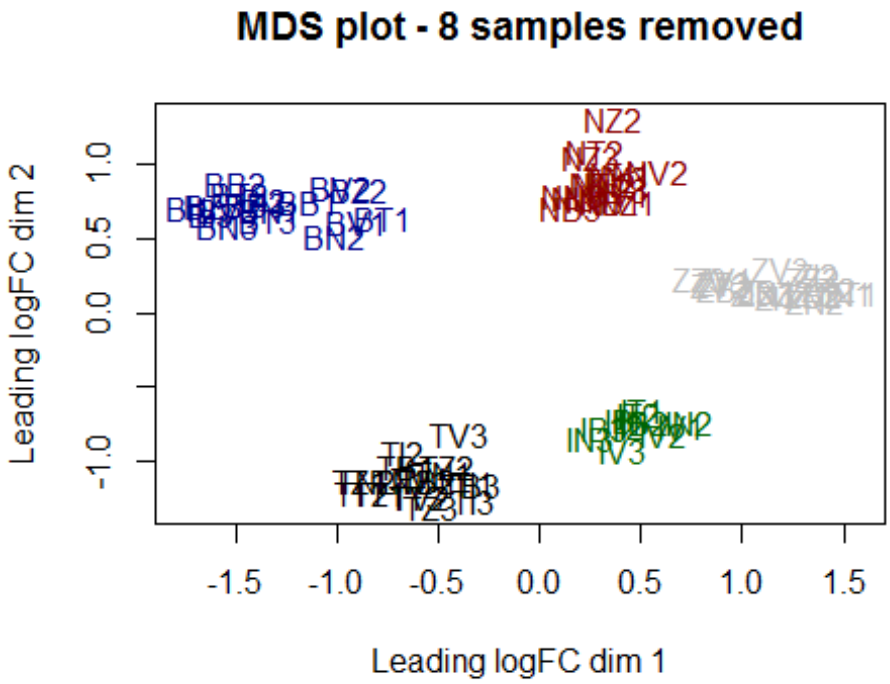

Fig. S3: Multidimensional scaling plot after removal of eight samples.

Unsupervised clustering after the removal of eight samples demonstrates clustering of samples by female genotype.

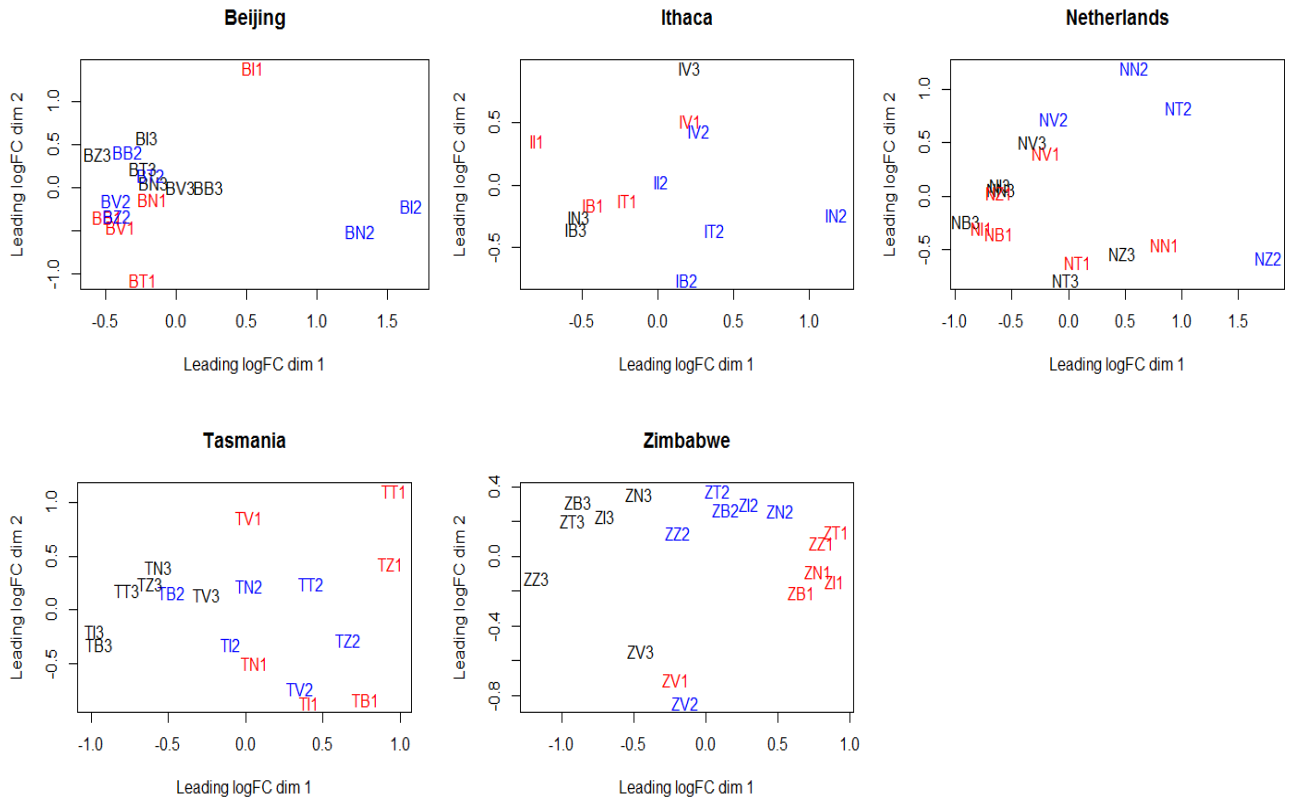

Fig. S4: Multidimensional scaling plots per female genotype.

Unsupervised clustering per female genotype identified additional outlier samples: three samples containing Beijing females clustered away from other Beijing samples. (B x I-1, B x I-2 and B x N-2). We further investigated these samples using MA plots (fig. 5).

In these MDS plots, samples were colored by replicate (replicate 1: red, replicate 2: blue, replicate 3: black). Samples tended to separate by replicate, indicating the presence of batch effects.

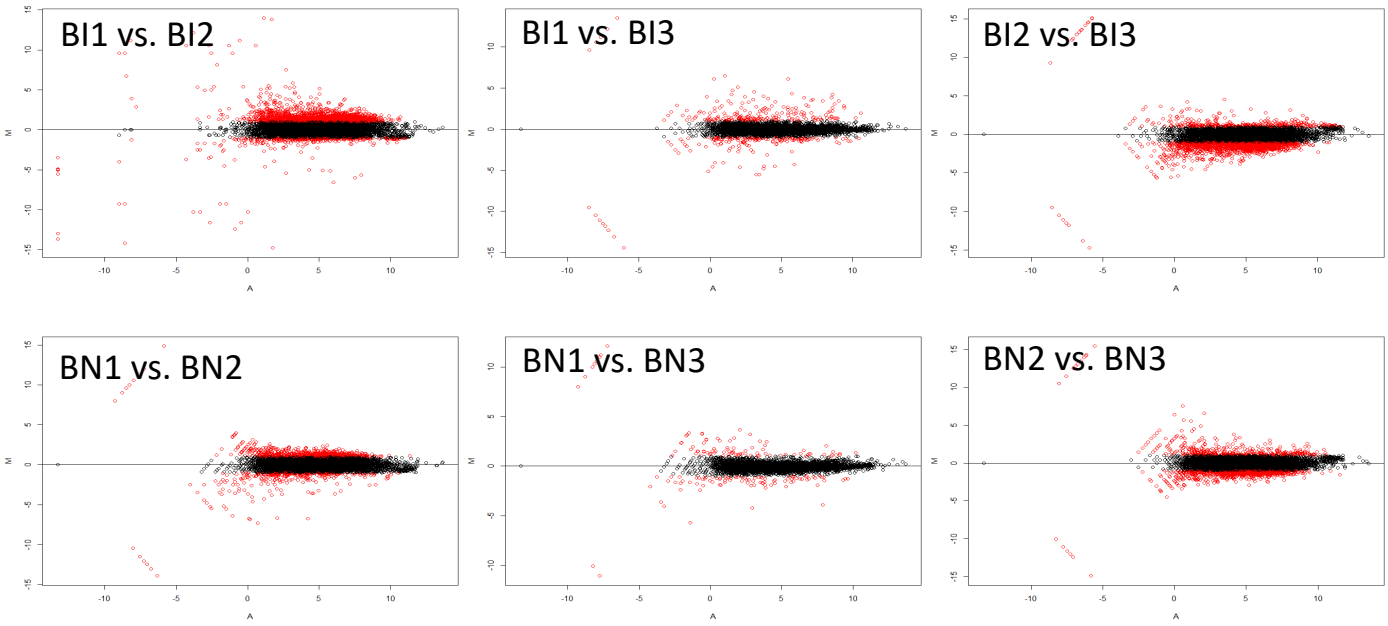

Fig. S5: MA plots were constructed to compare fold change differences between biological replicates for B x I and B x N combinations.

In these plots, each dot represents a gene. The y-axis (M) represents the $\log_2$ fold change between two replicates. The x-axis (A) represents the average expression level of each gene in both replicates. Genes with a low average expression level usually have a larger inter-replicate variability (larger fold changes). When comparing biological replicates, we expect to see small fold changes (less than 2-fold) for genes with a high average expression. In the figures above, red dots represent genes with a ≥ 2-fold change.

We found that the expression of over 2000 genes differed 2-fold or more in B x I-2, relative to B x I-1 and B x I-3. On the other hand, the expression of only 418 genes differed 2-fold or more in B x I-1 relative to B x I-3.

Similarly, the expression of over 1000 genes differed 2-fold or more in B x N-2, relative to B x N-1 and B x N-3, while the expression of only 212 genes differed 2-fold or more in B x N-1 relative to B x N-3.

Based on this information, we decided to remove B x I-2 and B x N-2 from the dataset.

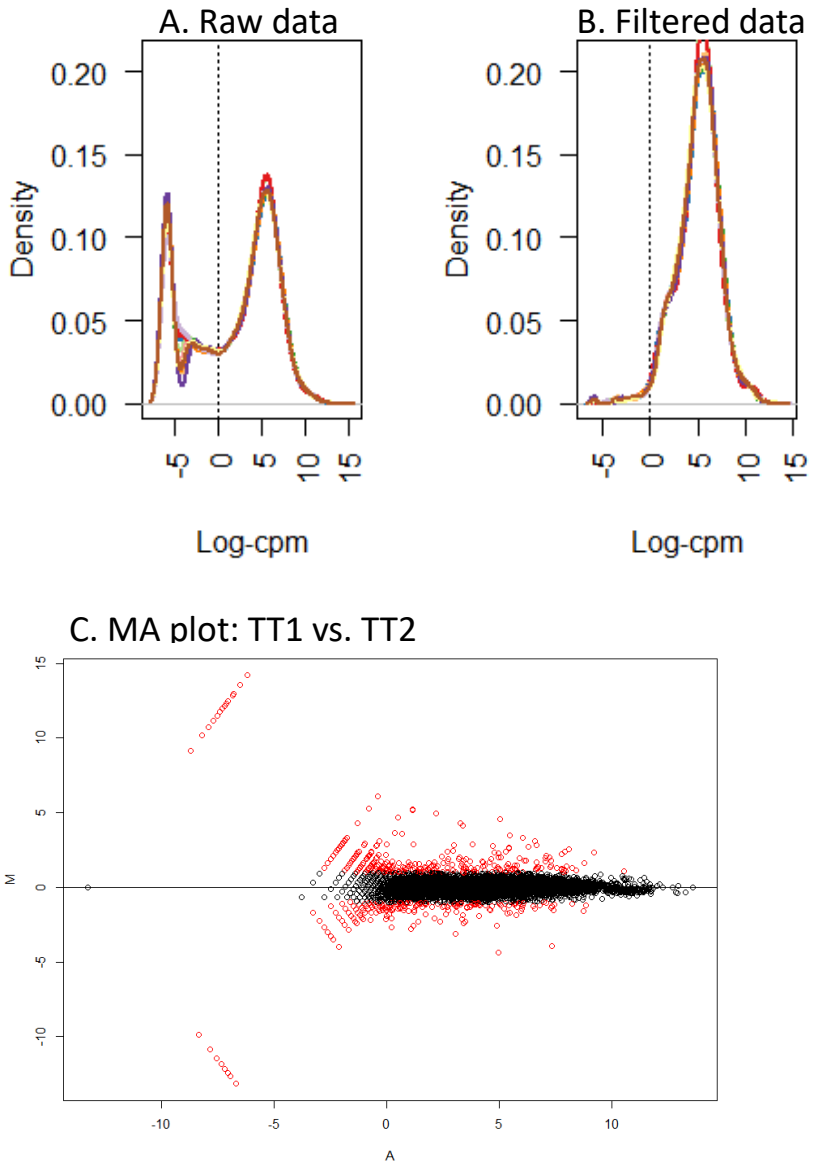

Fig. S6: Diagnostic plots used to set a filtering cutoff for lowly expressed genes.

We decided to remove lowly expressed genes from our dataset, because genes with low cpm values (count per million) generally have a larger inter-replicate variability. The higher noise/signal ratio makes statistical inference more difficult. In addition, limiting the number of genes in the dataset also limits the number of tests that need to be performed during differential expression analysis (see edgeR user guide at www.bioconductor.org, and Law et al. 2016).

To set a filtering cutoff for lowly expressed genes, we examined the distribution of cpm values across all genes and samples in our dataset. In plots A and B, each line represents one of our 79 samples. Plot A shows that a large set of genes (high density on y-axis) is expressed at low cpm values (x-axis). After

filtering, using a cutoff of cpm> 3, the majority of genes in the dataset is expressed at higher cpm values. The code to generate these plots was used from Law et al. 2016.

Plot C is a representative MA plot which shows that at cpm= 3 (A= $\log_2(3)$= 1.6), inter-replicate variability in gene expression decreases.

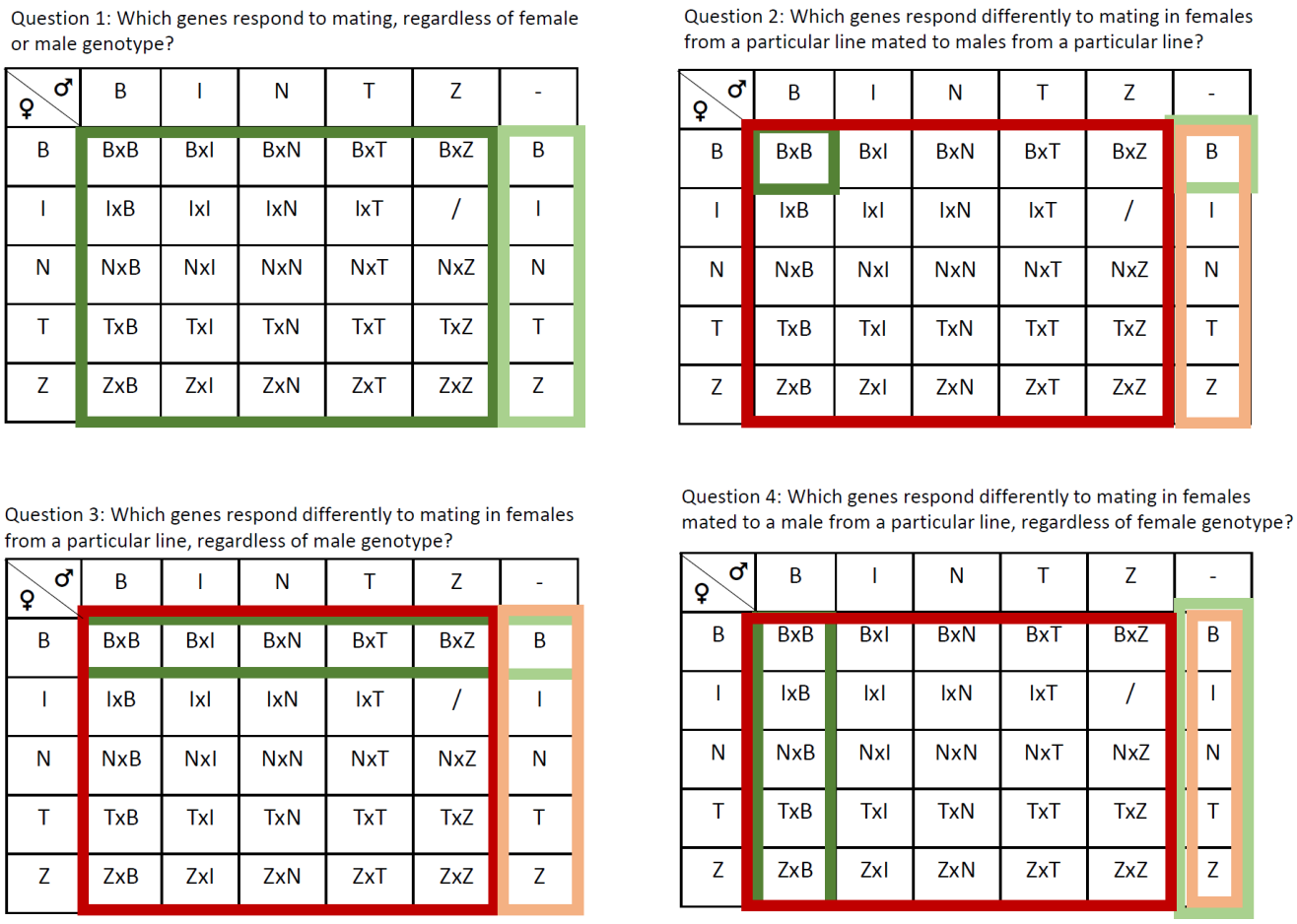

Fig. S7: Overview of the four questions that were asked regarding the RNAseq dataset, and the general approach used to answer these questions.

To answer four different questions regarding the post-mating transcriptional response, four differential expression analyses were conducted, each with its own linear model. To calculate the transcriptional response to mating, we compared gene expression between mated females (dark green or red boxes) and resp. virgin females (light green or red boxes). For questions 2, 3 and 4, we compared the transcriptional response to mating in the genotype of interest (green) with the average response to mating across all genotypes (red). Questions 3 and 4 were investigated for the Beijing genotype, as shown in the figure, but were also repeated for the remaining 4 genotypes. Question 2 was investigated for females from the Beijing line mated to a male from the Beijing line, as shown in the figure, but was repeated for the remaining 23 combinations. Samples for the I x Z combination were excluded from our analysis.

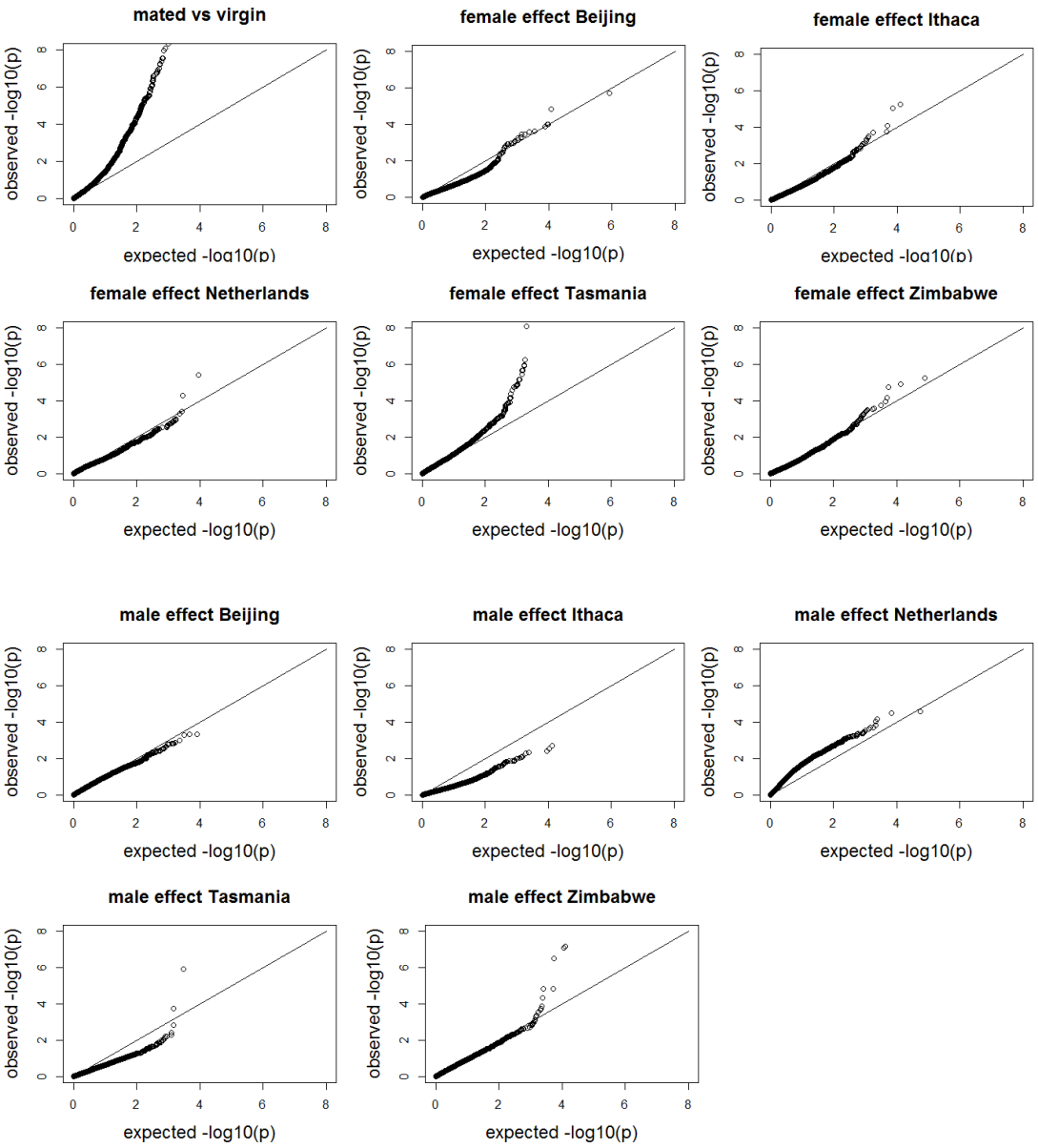

Fig. S8: Quantile quantile-plots of p-values from differential expression tests conducted using edgeR. Shown here are qq-plots for question 1 (Which genes respond to mating regardless of female or male genotype?), question 3 (Which genes respond to mating in a female genotype-dependent manner?) and question 4 (Which genes respond to mating in a male genotype-dependent manner?).

The solid diagonal represents the distribution of p-values under the null hypothesis (no differentially expressed genes). Circles represent observed p-values for each gene in our dataset. Circles that lie above the diagonal have smaller p-values compared to what is expected under the null hypothesis.

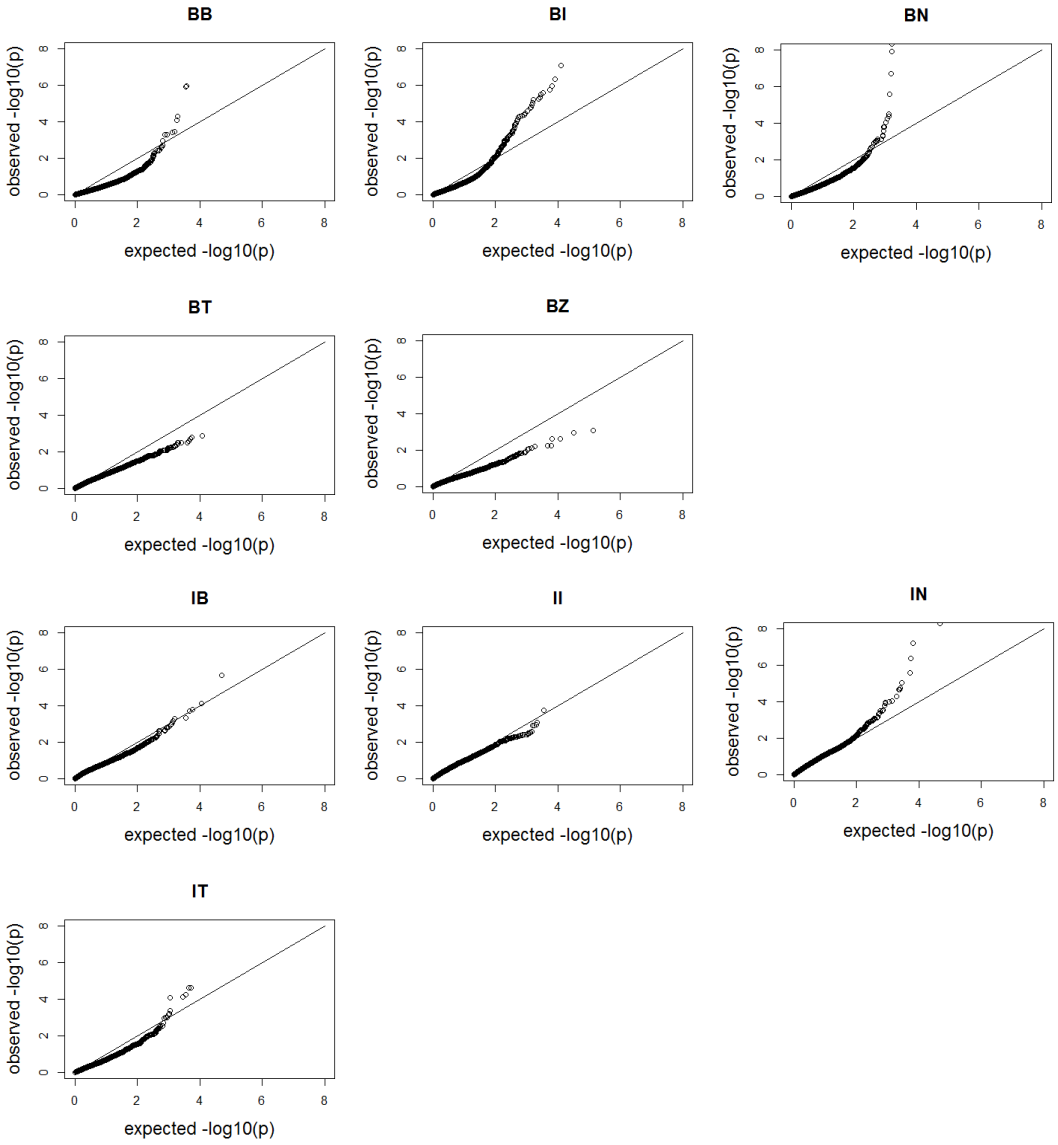

Fig. S9: Quantile quantile-plots of p-values from differential expression tests conducted using edgeR.

Shown here are qq-plots for question 2 (Which genes respond to mating in a female x male genotype interaction-dependent manner?), for the B and I female genotypes.

The solid diagonal represents the distribution of p-values under the null hypothesis (no differentially expressed genes). Circles represent observed p-values for each gene in our dataset. Circles that lie above the diagonal have smaller p-values compared to what is expected under the null hypothesis.

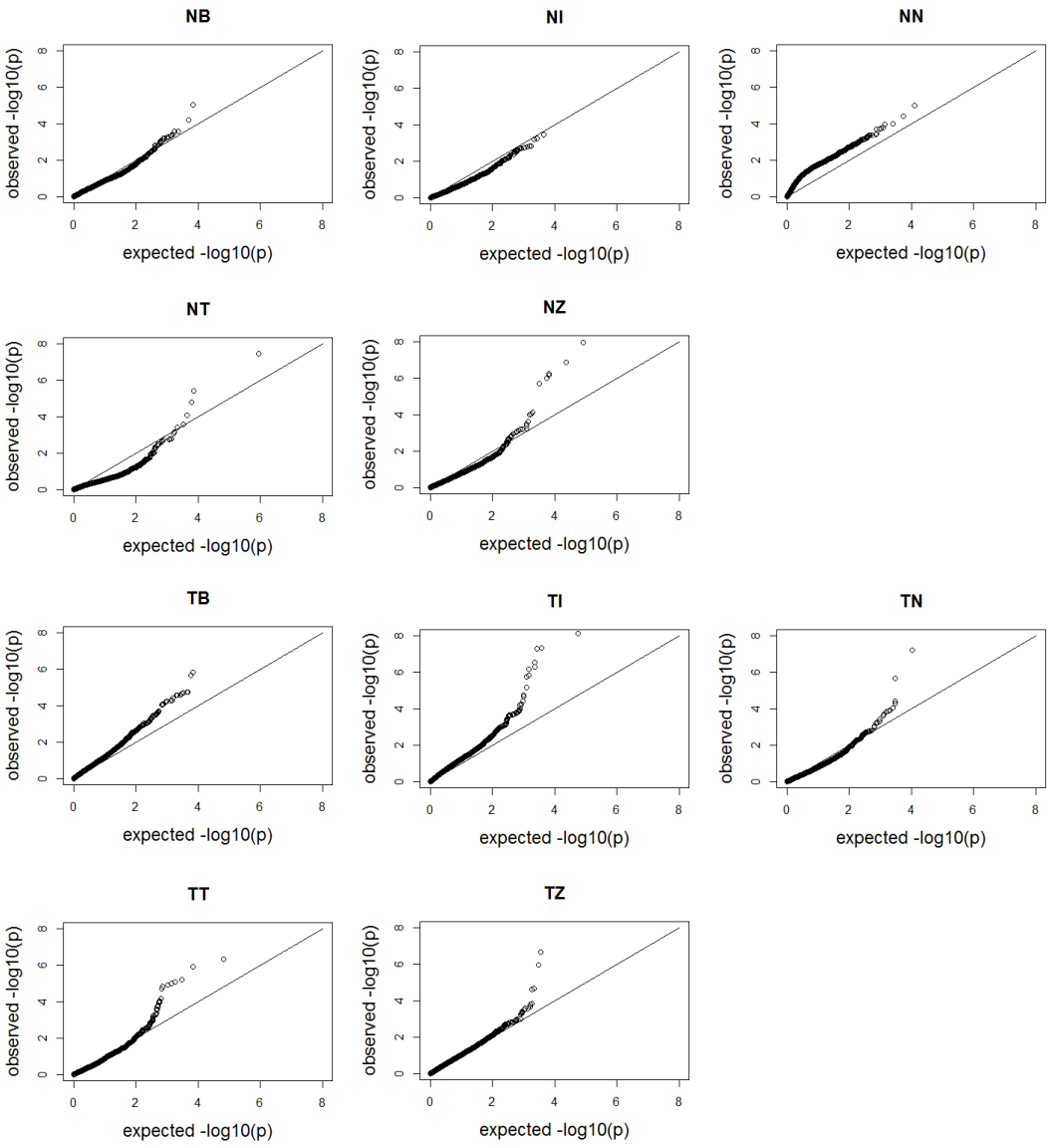

Fig. S10: Quantile quantile-plots of p-values from differential expression tests conducted using edgeR. Shown here are qq-plots for question 2 (Which genes respond to mating in a female x male genotype interaction-dependent manner?), for the N and T female genotypes.

The solid diagonal represents the distribution of p-values under the null hypothesis (no differentially expressed genes). Circles represent observed p-values for each gene in our dataset. Circles that lie above the diagonal have smaller p-values compared to what is expected under the null hypothesis.

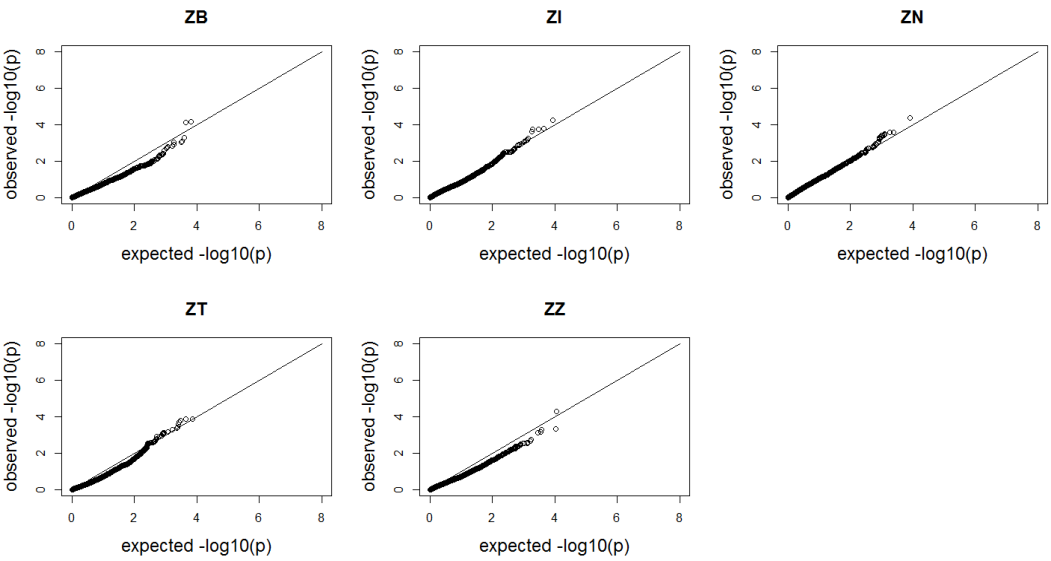

Fig. S11: Quantile quantile-plots of p-values from differential expression tests conducted using edgeR. Shown here are qq-plots for question 2 (Which genes respond to mating in a female x male genotype interaction-dependent manner?), for the Z female genotype. The solid diagonal represents the distribution of p-values under the null hypothesis (no differentially expressed genes). Circles represent observed p-values for each gene in our dataset. Circles that lie above the diagonal have smaller p-values compared to what is expected under the null hypothesis.

Table S1: Post-mating $\log_2$ fold changes of *Rp49* in each of the 24 mating combinations, based on the RNAseq dataset. Q-values are shown in parentheses. In all combinations, *Rp49* undergoes fold changes after mating that are smaller than 2-fold, and none of these fold changes are significant when comparing mated and virgin females. These data demonstrate that *Rp49* transcript levels do not change after mating, making *Rp49* a suitable gene for qPCR normalization.

Male **Female**	**B**	**I**	**N**	**T**	**Z**
B	0.13 (0.99)	-0.12 (1)	-0.07 (0.9)	-0.16 (0.9)	-0.05 (0.9)
I	0.019 (0.9)	-0.17 (0.8)	0.25 (0.7)	0.01 (1)	/
N	-0.17 (0.9)	0.09 (1)	0.58 (0.1)	0.15 (0.9)	0.07 (0.9)
T	0.63 (0.1)	0.47 (0.3)	0.30 (0.9)	0.33 (0.6)	0.31 (0.8)
Z	0.06 (0.9)	0.08 (0.9)	-0.02 (0.9)	0.07 (0.9)	-0.06 (1)

Table S2: qPCR primer sequences.

Gene name	**Forward primer**	**Reverse primer**
Rp49	CTGGTTTCCGGCAAGCTTCA	GCCATTTGTGCGACAGCTTA
AttB	CGGTTGAATCTCAGCAAGG	AAAGTTCCGCCAGGTGTGAC
Dro	CCGCCTAAAGATGTGTGCAT	ATGGGAACCCCTCATTGTGTC
Def	CTCGTGGCTATCGCTTTTGC	CCACTTGGAGAGTAGGTCGC
CG3088	CAGATCCACTTGCTTTGGCG	AGGCCACAAATGCGGAAATG
Obp49a	CATCCCCCACCATGCTTCTT	TCCTGCAAGTAGGCGTTCAG
Cyp4p2	AGGAACTGCATAGGCCAGAA	CCGCCTCACAAGCTTGACTT

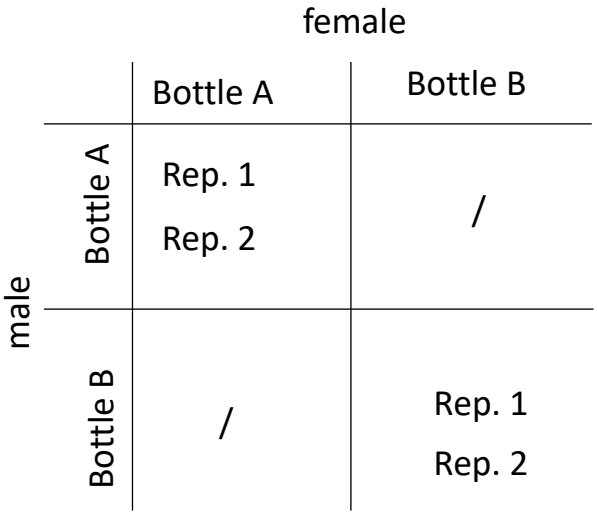

Fig. S12: Overview of the design used to collect flies for immune gene qRT-PCR.

Two bottles containing flies with the female genotype of interest were set up (female bottle A and female bottle B), together with two bottles containing flies with the male genotype of interest (male bottle A and male bottle B). From each bottle, we collected two biological replicates on two different days, giving us a total of four replicates: (1) Females from bottle A, replicate 1 were mated to males from bottle A, replicate 1. (2) Females from bottle B, replicate 1 were mated to males from bottle B, replicate 1. These matings were performed on the same day. Two days later, (3) females from bottle A, replicate 2 were mated to males from bottle A, replicate 2. (4) Females from bottle B, replicate 2 were mated to males from bottle B, replicate 2. For each of the four replicates, virgin females were sampled as well.

Keeping track of which bottle the flies were collected from allowed us to take variation in the micro-environment of each bottle into account. For example, if virgin females from bottle A were found to have higher than expected expression of antimicrobial peptides, this suggests that pathogens might have been present in bottle A. In this case, expression levels of antimicrobial peptides in mated females from bottle A might have been influenced by other factors (pathogens) besides mating.

RNA-seq analysis – alternative method:

A 5 by 5 factorial ANOVA was used as a different method to address the roles of female and male genotype on post-mating gene expression changes. In particular, an ANOVA was used to set up a combined model to test the overall contribution to variance in post-mating fold changes due to female effects, male effects and their interaction (questions 3, 4 and 2). We were unable to obtain adequate fits of this model to the data, and qq-plots displayed significant inflation of p-values. Despite this caveat, the trend of genes showing significant expression differences was essentially a subset of those found by edgeR (results not shown).

Effect of *Wolbachia* on post-mating gene expression changes

Of the five Global Diversity Lines used in this study, four lines carried the bacterial endosymbiont *Wolbachia pipientis* (only the Netherlands line is uninfected). This raised the concern that observed female x male genotypic effects on post-mating transcriptional changes might have been caused by the presence or absence of *Wolbachia*, rather than true genotype interaction effects.

To control for *Wolbachia* effects, we divided the 24 female x male combinations into four groups, based on the presence or absence of *Wolbachia* in female and male (table 1). We used edgeR to find genes that were differentially expressed after mating in each of the four groups. We set up four contrasts to compare mated females of each group with their respective virgin females (fig. 1).

No genes were found to be differentially expressed after mating in females that were not infected with *Wolbachia* (Netherlands females).

We found 170 genes that were differentially expressed after mating if both female and male carried *Wolbachia* ($q< 0.05$; this includes all crosses except crosses with Netherlands flies). Of these 170 genes, one gene was among the genes that were found to be involved in female x male mating interactions:

- *Ect3* was down-regulated in IxB ($\log_2$ fold change= -1.2). It was also down-regulated in *Wolbachia* infected females mated to *Wolbachia* infected males, but this down-regulation was not as strong as in IxB (average $\log_2$ fold change= -0.4). This suggests that the strong down-regulation of *Ect3* in IxB is due to the I and B genotypes, and not solely due to the presence of *Wolbachia*.

In addition to this, we found 200 genes that were differentially expressed after mating a *Wolbachia* infected female with an uninfected male ($q< 0.05$). Of these 200 genes, only one gene was also found among the genes involved in female x male genotype interactions (IxN). *Obp49a* was up-regulated in *Wolbachia* infected females mated to uninfected males ($\log_2$ fold change= 1, this includes BxN, IxN, TxN and ZxN). However, *Obp49a* was up-regulated more strongly in IxN

(log_2 fold change = 1.9). This suggests that the stronger up-regulation observed in IxN is mediated by genotype interactions, rather than the presence of *Wolbachia*.

In short, the variation in presence/absence of *Wolbachia* across the five *Drosophila* lines used in this study did not appear to generate false positive calls for genes whose expression levels are robustly impacted by mating.

Table 2: The 24 female x male mating combinations can be subdivided into four groups, based on the presence or absence of *Wolbachia*. In parentheses are the numbers of genes that are DE after mating in each of the four groups.

Female \ Male	*Wolbachia* present	*Wolbachia* absent
Wolbachia present	BB, BI, BT, BZ, IB, II, IT, TB, TI, TT, TZ, ZB, ZI, ZT, ZZ (170 DE genes)	BN, IN, TN, ZN (200 DE genes)
Wolbachia absent	NB, NI, NT, NZ (0 DE genes)	NN (0 DE genes)

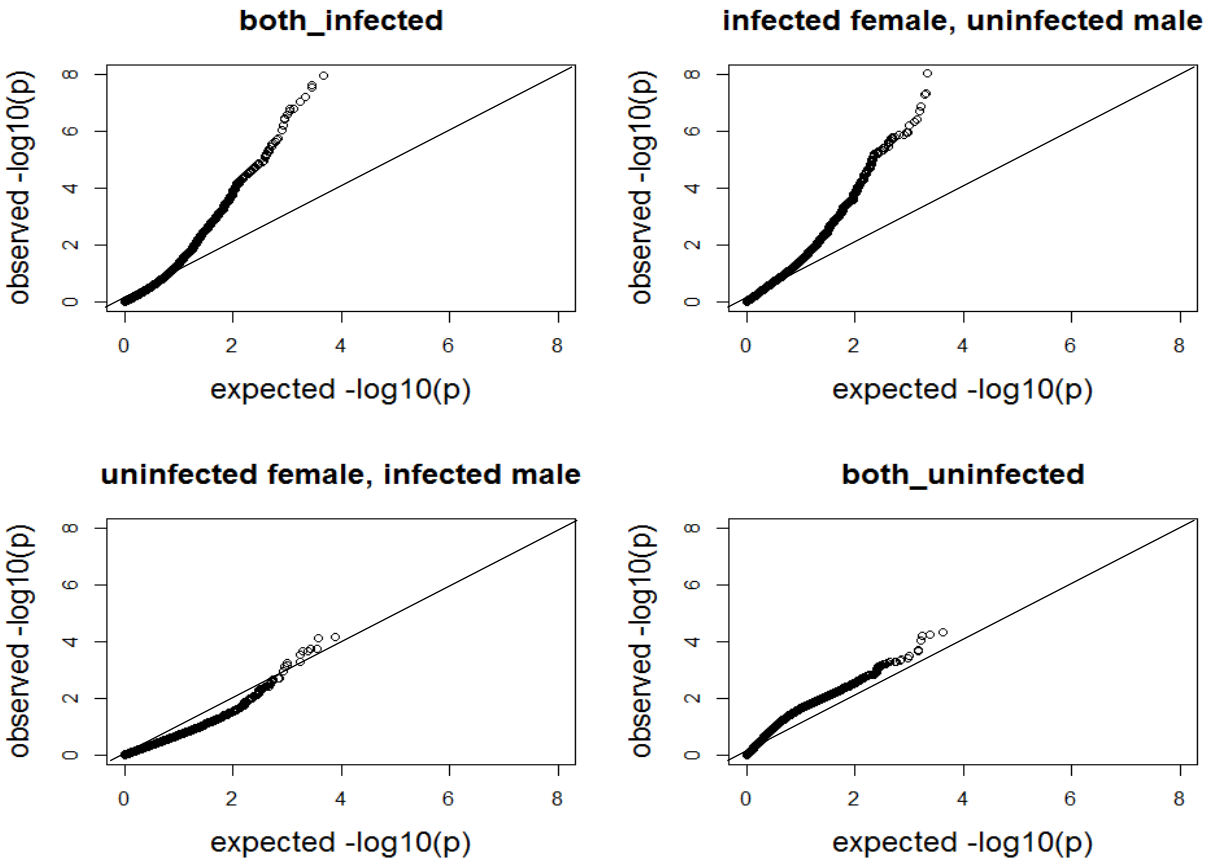

Fig. 1: QQ-plots of p-values for differential expression tests conducted using edgeR. The solid diagonal represents the distribution of p-values under the null hypothesis (no DE genes). Circles represent observed p-values for each gene in our dataset. Circles that lie above the diagonal have smaller p-values compared to what is expected under the null hypothesis. Shown here are qq-plots for all four mating combinations of *Wolbachia* infected and uninfected females and males. Contrasts were set up in edgeR to find genes that were differentially regulated post-mating, for each of the four groups.

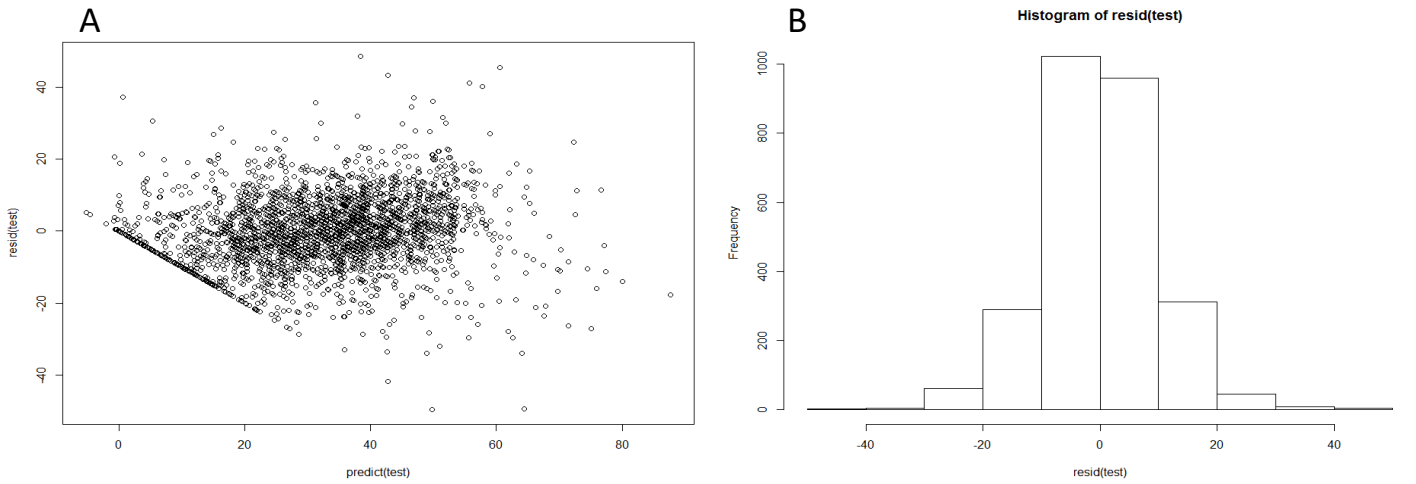

Fig. S13: Diagnostic plots to check assumptions of the lmer model used to analyze fecundity data. A: Residuals plot for homogeneity of variance. B: Residuals follow a normal distribution.

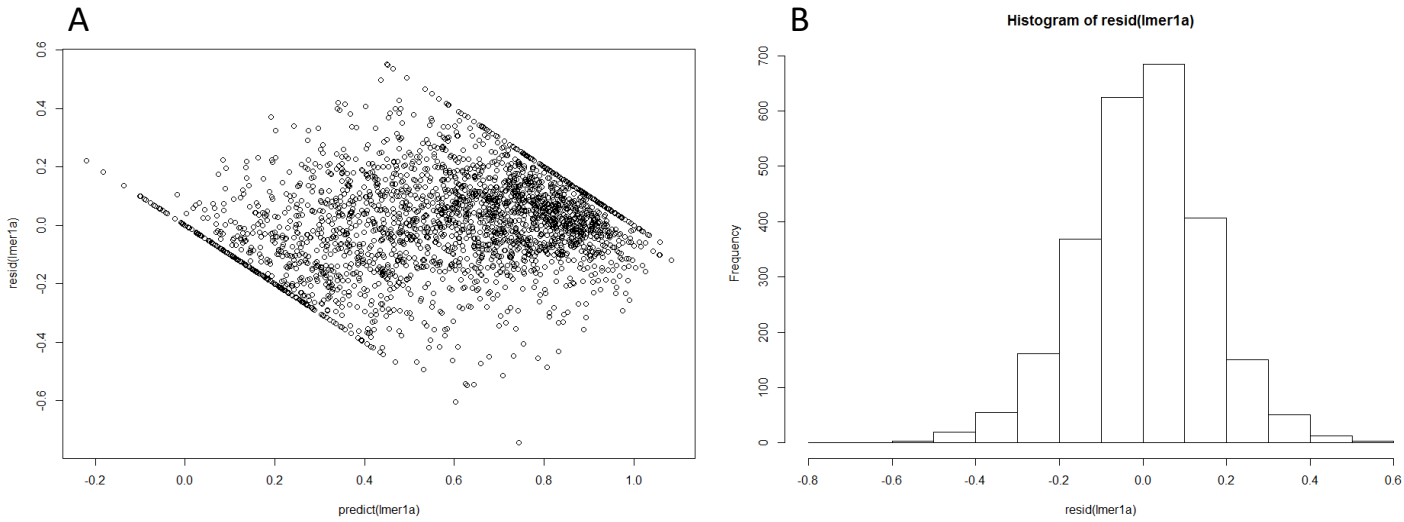

Fig. S14: Diagnostic plots to check assumptions of the lmer model used to analyze hatchability data. A: Residuals plot for homogeneity of variance. B: Residuals follow a normal distribution. When using a GLM with binomial error distribution, instead of lmer with normal distribution, our data was over-dispersed. Adding an observation level random effect (Harrison 2014) did not sufficiently reduce the over-dispersion. An alternative was to use a quasi-binomial model, which accounts for over-dispersion, but this model did not allow us to add random effects (which meant we would have to analyze each block separately). Because of this, and because the diagnostic plots for the normal lmer model looked good, we decided to use lmer instead.

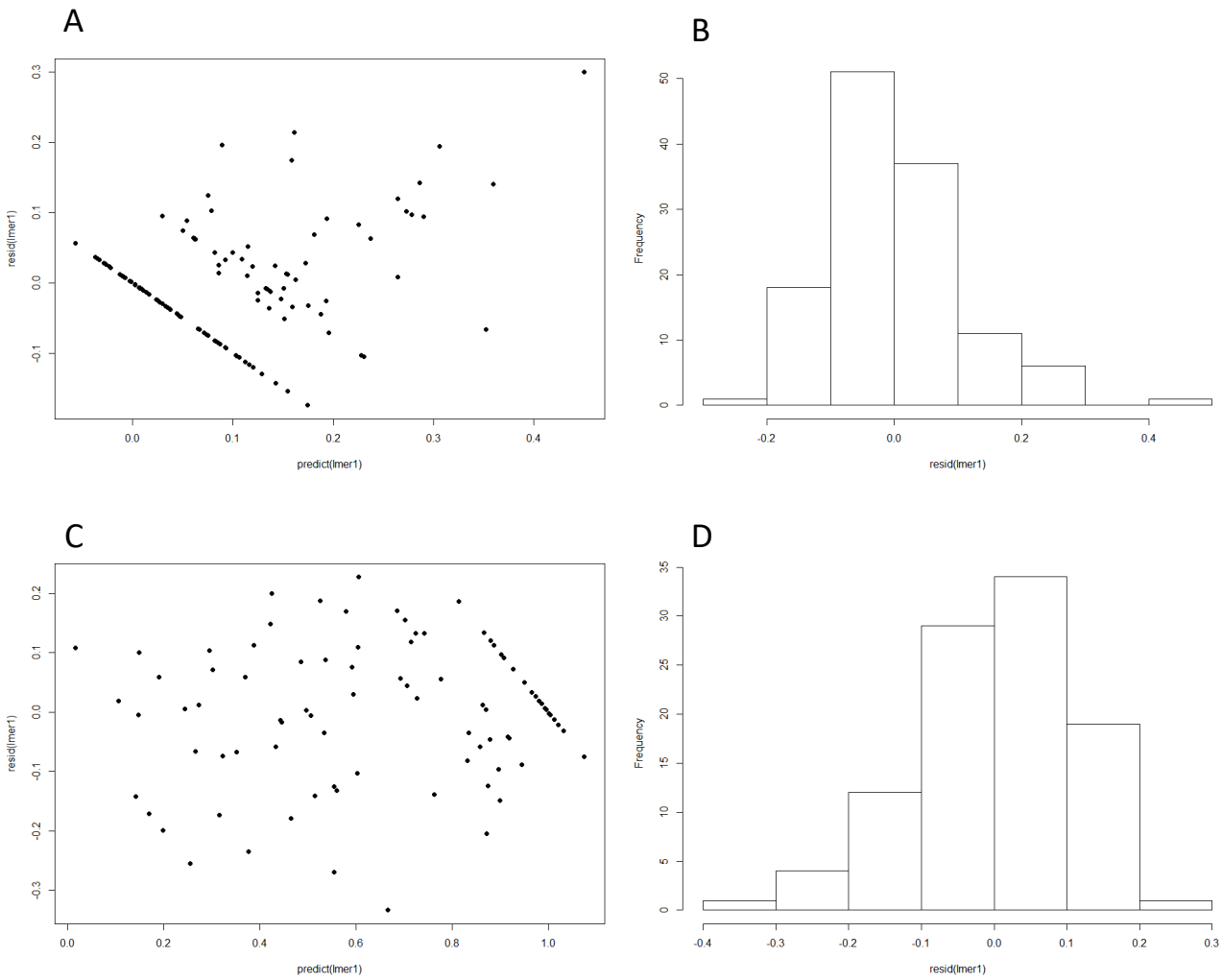

Fig. S15: Diagnostic plots to check assumptions of the lmer model used to analyze receptivity data.

A: Residuals plot for homogeneity of variance, for female refractoriness at day 1. B: Residuals follow a normal distribution for female refractoriness at day 1. C: Residuals plot for homogeneity of variance, for female refractoriness at day 4. D: Residuals follow a normal distribution for female refractoriness at day 4. To analyze female refractoriness to re-mating, we added three random effects to the lmer models: (1) block, (2) the interaction between block and female genotype, and (3) the interaction between block and male genotype. Adding these random interaction terms to the model did not affect the results for refractoriness to re-mating on day 4. However, he results changed for refractoriness to re-mating on day 1. A model with “block” as the only random effect, found significant effects of female and male genotype on re-mating rate on day 1. Adding additional random interaction terms removed these significant effects. These random interaction terms consider how the overall effect of female genotype, or the overall effect of male genotype, changes across blocks. We did no longer find significant effects of female or male genotype due to large variability between the blocks for 1 day refractoriness to re-mating.

Results:

Table S3: Permutation tests were performed to determine the likelihood to find the observed number of differentially expressed (DE) genes by chance. This table contains the observed number of differentially expressed genes in the original RNAseq dataset for each of the 24 combinations. The proportion of how often this number, or a larger number, of differentially expressed genes was observed in any of the 500 randomized datasets is shown in parentheses. This proportion can be considered as a permutation p-value. All permutation p-values were < 0.05. Intra-population crosses are highlighted in yellow.

MALE **FEMALE**	**B**	**I**	**N**	**N**	**Z**
B	2 (0.014)	22 (0.004)	1 (0)	0 (0.01)	0 (0.01)
I	1 (0.002)	0 (0.014)	10 (0.004)	0 (0.008)	/
N	0 (0.002)	0 (0.004)	0 (0)	2 (0.014)	6 (0.012)
T	18 (0)	14 (0.002)	2 (0.008)	8 (0)	5 (0.002)
Z	0 (0.002)	0 (0.004)	0 (0)	0 (0.004)	0 (0.002)

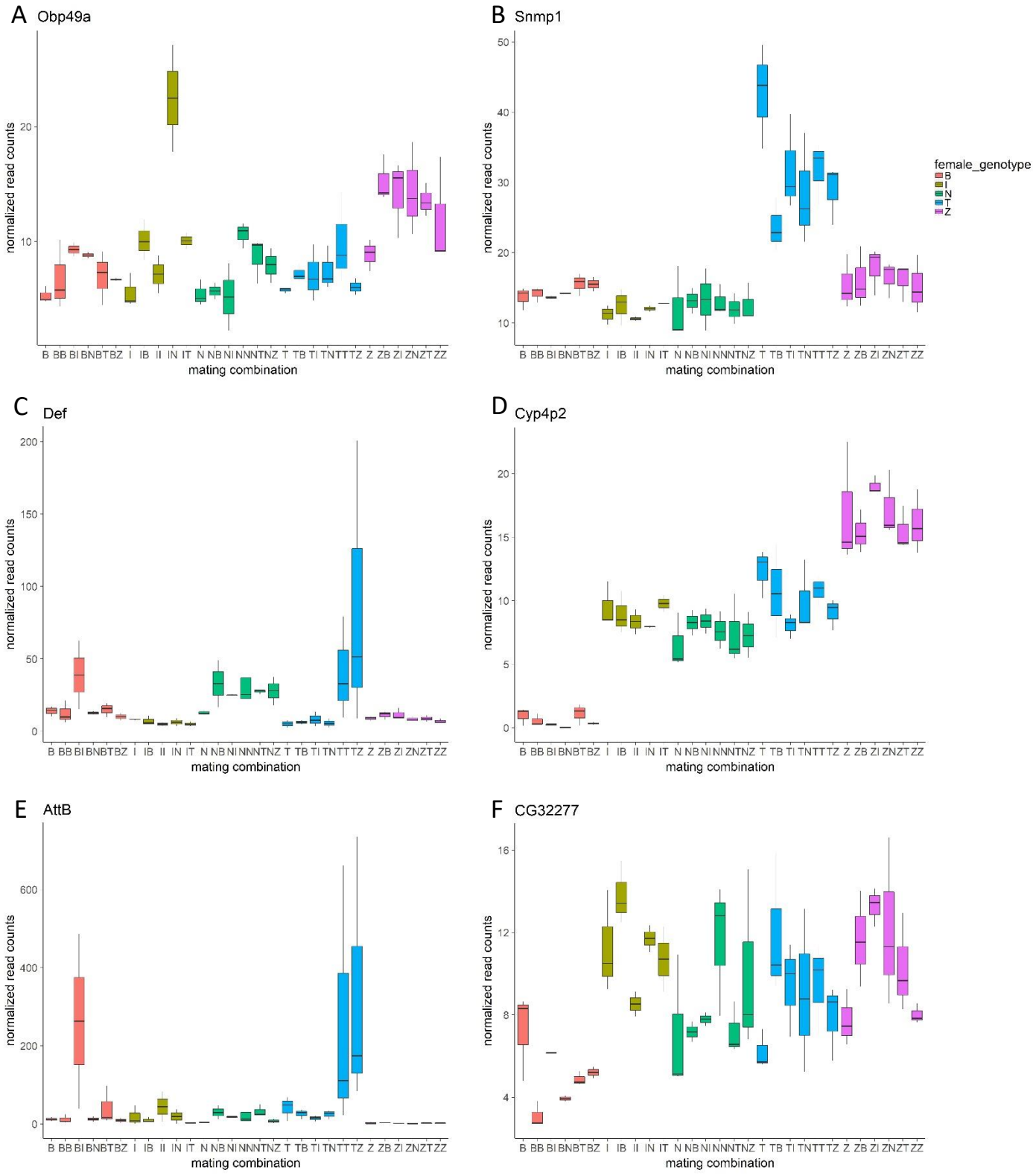
A Obp49a
B Snmp1
C Def
D Cyp4p2
E AttB
F CG32277
normalized read counts
mating combination
B BB BI BN BT BZ I IB II IN IT N NB NI NN NT NZ T TB TI TN TT TZ Z ZB ZI ZN ZT ZZ
female_genotype
B
I
N
T
Z

Fig. S16: Six genes for which the post-mating fold changes were impacted by female x male genotype interactions. Boxplots represent normalized read counts (cpm) across all replicates, for virgin and mated females of all genotypes. A: *Obp49a* transcript levels were up-regulated higher than average in I x N (q= 0.015). B: *Snmp1* transcript levels were down-regulated more than average in T x B (q= 0.048). C: *Def* transcript levels were up-regulated more than average in T x T (q= 0.19) and T x Z (q= 6 x 10^{-6}). D: *Cyp4p2* mRNA levels were down-regulated more in B x N (q= 1.5 x 10^{-5}). E: *AttB* transcripts were up-regulated more than average in B x I (q= 0.041). F: *CG32277* mRNA was down-regulated more than average in B x B (q= 0.006).

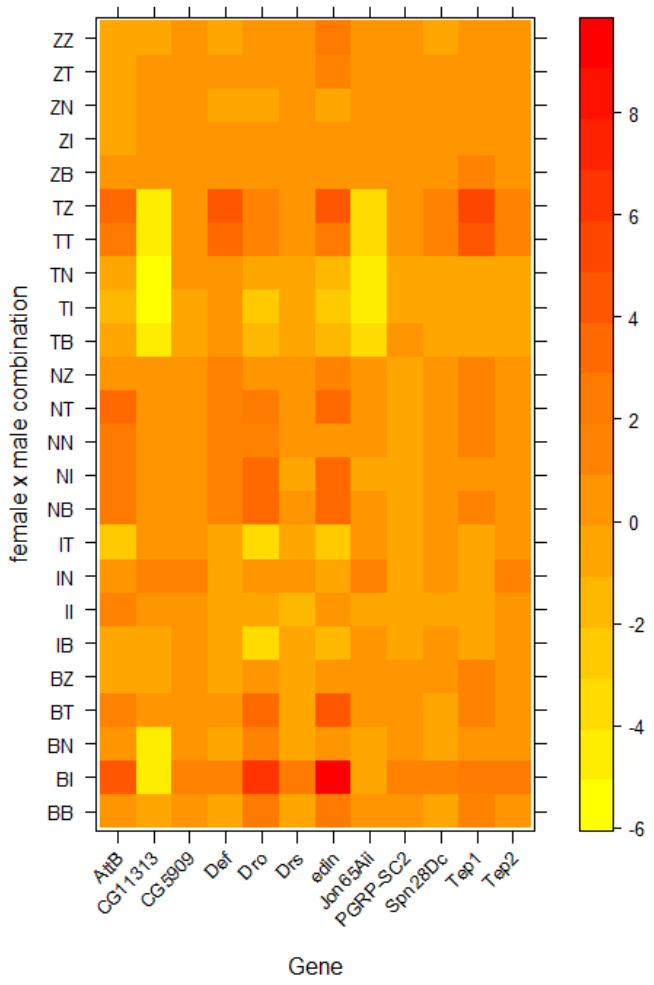

Fig. S17: Post-mating $\log_2$ fold changes for immune transcripts in all 24 female x male mating combinations. A significant up-regulation of immune gene transcripts was seen in B x I (*AttB*, *Dro*, *Drs*, *PGRP-SC2*, *edin*, *Tep2*), T x Z (*Tep1*, *Def*) and T x T (*Def*, *Spn28Dc*). A significant down-regulation of *CG11313* was observed in T x I, while *Jon65Aii* was significantly down-regulated in T x B, T x I, T x N and T x T.

Table S4: Permutation tests were performed to determine the likelihood to find the observed number of differentially expressed (DE) genes by chance. This table contains the observed number of differentially expressed genes in the original RNAseq dataset for each of the five female genotypes. The proportion of how often this number, or a larger number, of differentially expressed genes was observed in any of the 500 randomized datasets is shown in parentheses. Of the 35 differentially expressed genes indicated below, eleven genes were also differentially expressed for the female x male interaction effects, leaving 24 genes differentially expressed depending on female genotype only.

Female	**B**	**I**	**N**	**T**	**Z**
DE genes (permutation p-value)	1 (0.23)	2 (0.14)	1 (0.19)	30 (0.03)	1 (0)

Table S5: Permutation tests were performed to determine the likelihood to find the observed number of differentially expressed (DE) genes by chance. This table contains the observed number of differentially expressed genes in the original RNAseq dataset for each of the five male genotypes. The proportion of how often this number, or a larger number, of differentially expressed genes was observed in any of the 500 randomized datasets is shown in parentheses. Of the seven differentially expressed genes indicated below, five genes were also differentially expressed for the female x male interaction effects, leaving two genes differentially expressed depending on male genotype only: *CG16743* in females mated to Zimbabwe males, and *Diedel* in females mated to Tasmania males.

CG16743 transcripts were on average up-regulated after mating, confirming findings from earlier studies (McGraw et al. 2004; McGraw, Clark, and Wolfner 2008; Zhou, Mackay, and Anholt 2014; Hollis, Houle, and Kawecki 2016). However, in females that mated to a male from the Zimbabwe line, the mRNA levels of *CG16743* did not change. The post-mating up-regulation of *Diedel* transcripts was found to be significantly higher than average if a female mated to a male from the Tasmania line. Yet, permutation tests showed that the number of differentially regulated genes depending on male genotype, was not significantly different from the number of differentially regulated genes found by chance.

Male	**B**	**I**	**N**	**T**	**Z**
DE genes (permutation p-value)	0 (1)	0 (1)	0 (1)	1 (0.43)	6 (0.074)

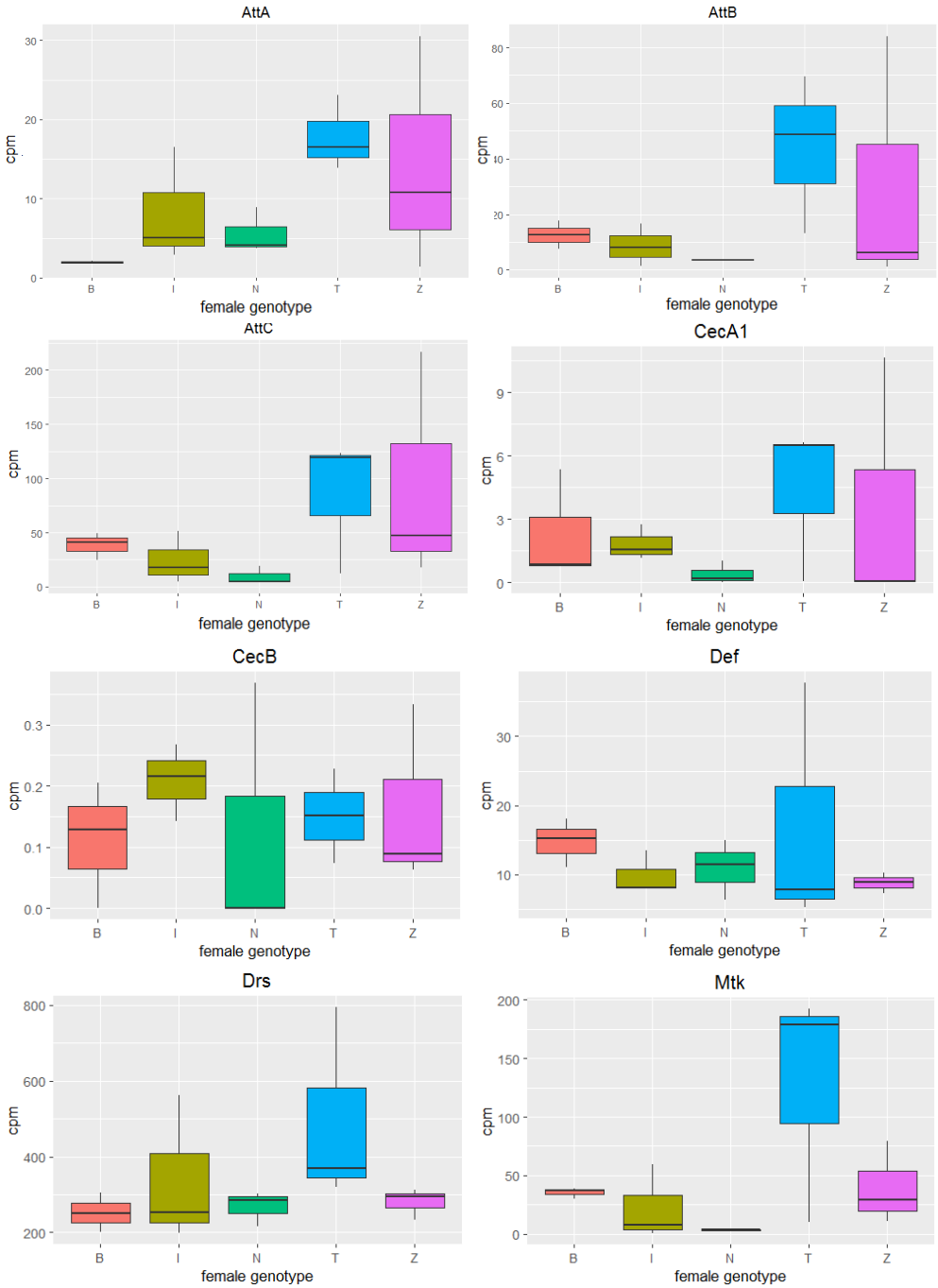

Fig. S18: Normalized read counts (cpm, counts per million) for virgin females of each genotype (each boxplot represents data from three biological replicates). Virgin females from the Tasmania and Zimbabwe lines had higher cpm values for a range of antimicrobial peptides.

Table S6: Summary statistics for qRT-PCR results. A t-test was performed to determine if post-mating fold changes (gene expression in mated females relative to gene expression in virgin females) differed significantly between female x male combinations. After removing outlier replicates, significant differences ($p < 0.05$) were found for all genes, except for *Dro*. For *Dro*, the trends did confirm the pattern observed in the RNAseq dataset. (SE=standard error)

Gene	**Female x male combination**	**Mean log_2 fold change**	**SE**	**p-value**
CG3088	B x N	-0.68	0.29	0.01
	B x T	0.14	0.1	
Cyp4p2	B x N	-0.76	0.25	0.02
	I x T	0.1	0.08	
***AttB* (all replicates)**	B x I	2.98	3.81	0.5
	T x I	1.36	1.64	
***AttB* (outlier replicates removed)**	B x I	4.88	0.16	0.02
	T x I	1.96	1.34	
***Dro* (all replicates)**	B x I	1.76	3.79	0.3
	T x I	-0.96	1.82	
***Dro* (outlier replicates removed)**	B x I	3.48	1.95	0.09
	T x I	-0.05	0.18	
Def	T x N	0.92	1.43	0.003
	T x T	4.76	0.67	
Obp49a	I x N	0.64	0.81	0.58
	T x N	0.64	0.61	

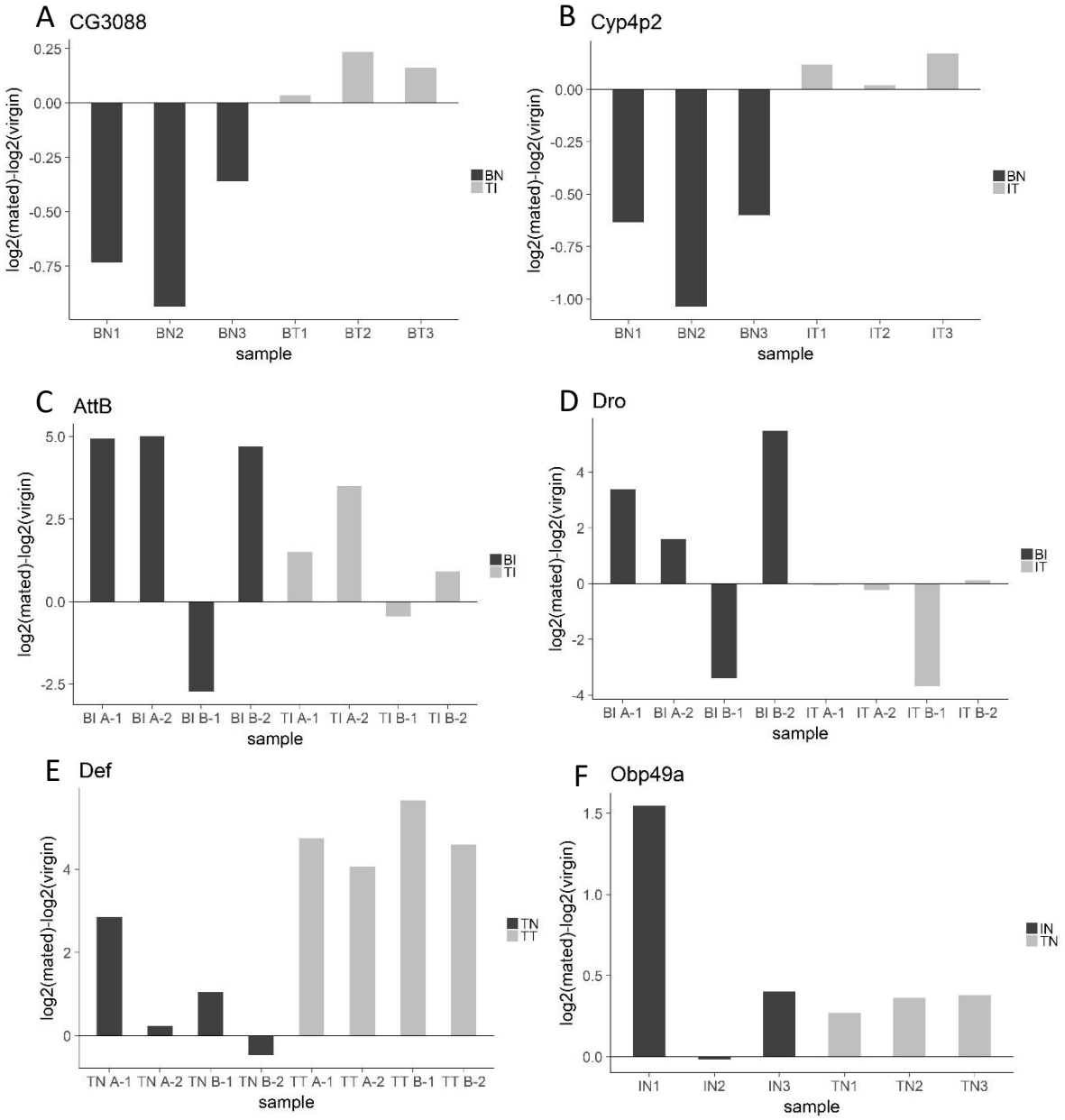
A CG3088
log2(mated)-log2(virgin)
0.25
0.00
-0.25
-0.50
-0.75
BN1 BN2 BN3 BT1 BT2 BT3
sample
BN
TI
B Cyp4p2
log2(mated)-log2(virgin)
0.00
-0.25
-0.50
-0.75
-1.00
BN1 BN2 BN3 IT1 IT2 IT3
sample
BN
IT
C AttB
log2(mated)-log2(virgin)
5.0
2.5
0.0
-2.5
BI A-1 BI A-2 BI B-1 BI B-2 TI A-1 TI A-2 TI B-1 TI B-2
sample
BI
TI
D Dro
log2(mated)-log2(virgin)
4
2
0
-2
-4
BI A-1 BI A-2 BI B-1 BI B-2 IT A-1 IT A-2 IT B-1 IT B-2
sample
BI
IT
E Def
log2(mated)-log2(virgin)
4
2
0
TN A-1 TN A-2 TN B-1 TN B-2 TT A-1 TT A-2 TT B-1 TT B-2
sample
TN
TT
F Obp49a
log2(mated)-log2(virgin)
1.5
1.0
0.5
0.0
IN1 IN2 IN3 TN1 TN2 TN3
sample
IN
TN

Fig. S19: QRT-PCR results for post-mating gene expression changes in six genes.

Variation in post-mating gene expression changes was validated for six genes using qRT-PCR. For each of the six genes, two female x male combinations were compared, that showed the largest difference in post-mating response based on the RNAseq data. Each bar represents one biological replicate (10 females pooled per replicate).

A-B: Based on the RNAseq data, *CG3088* and *Cyp4p2* were significantly down-regulated in B x N relative to the average response to mating in all female x male combinations. We ran qRT-PCR to compare post-mating transcript levels in B x N and B x T (*CG3088*) and B x N and I x T (*Cyp4p2*). QRT-PCR results confirmed the down-regulation of *CG3088* and *Cyp4p2* in the B x N combination. C: QRT-PCR confirmed an up-regulation of *Def* in T x T. D-E: qPCR results confirmed an up-regulation of antimicrobial peptides (AMPs) *AttB* and *Dro* in B x I for 3 out of 4 biological replicates. When investigating Ct values for the aberrant biological replicate, it became clear that the low fold change in this replicate was not due to the absence of AMP induction in mated females, but that it was due to higher AMP expression in virgin females (fig. S20). F: *Obp49a* was up-regulated in I x N in the RNAseq data. Only one out of three biological replicates confirmed this up-regulation using qRT-PCR.

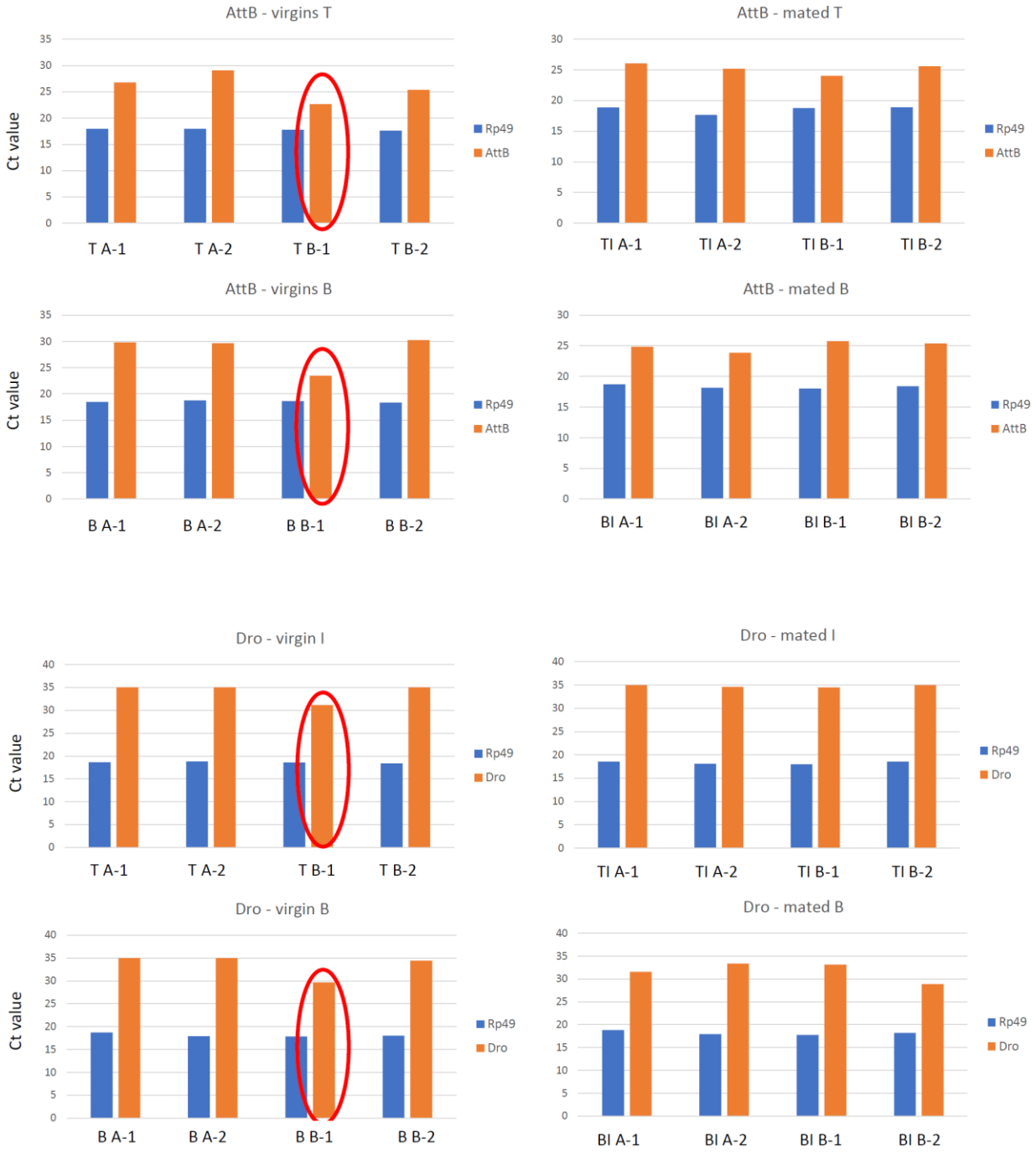

fig. S20: Ct values for mated and virgin females, for two antimicrobial peptides. Virgin females from replicates B-1 (bottle B, replicate 1) had lower Ct values for *Dro* and *AttB*, indicating an up-regulation of antimicrobial peptides in these virgin females.

Table S7: ANOVA output from the linear mixed effects model used to analyze fecundity data. Fecundity differed depending on interactions between female and male genotype, and depending on the day after mating ($p= 7.6 \times 10^{-6}$).

Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom

	Sum Sq	Mean Sq	NumDF	DenDF	F.value	Pr(>F)	
female	5043	1260.7	4	515.87	10.435	3.911e-08	***
male	12549	3137.2	4	515.87	25.966	< 2.2e-16	***
day	65041	16260.4	4	2070.92	134.582	< 2.2e-16	***
female:male	6384	399.0	16	515.88	3.302	1.601e-05	***
female:day	60575	3785.9	16	2070.91	31.335	< 2.2e-16	***
male:day	22163	1385.2	16	2070.92	11.465	< 2.2e-16	***
female:male:day	15357	240.0	64	2070.91	1.986	7.547e-06	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table S8: Pairwise comparisons were made between all 25 female x male combinations to evaluate differences in mean egg production per day. If egg production differed significantly between two female x male combinations, these combinations were assigned to groups with no numbers or letters in common. Comparisons across days were not made. (Lsmean= least square means calculated based on three replicates, with average n per female x male combination= 32; SE= standard error; df= degrees of f reedom; lower.CL and upper.CL= boundaries for 95% confidence interval)

day = 1:

female	male	lsmean	SE	df	lower.CL	upper.CL	.group
N	N	30.294057	3.849901	57.58	22.5864372	38.00168	1
N	Z	30.874599	3.612869	45.01	23.5979520	38.15125	1
N	I	31.786856	3.483175	39.02	24.7416035	38.83211	12
N	T	32.508880	3.545911	41.84	25.3521201	39.66564	12
T	I	33.493647	3.612869	45.01	26.2169996	40.77029	123
Z	I	35.111111	3.848352	57.53	27.4064710	42.81575	123
T	Z	35.205700	3.483173	39.02	28.1604512	42.25095	123
T	T	35.250000	3.424906	36.53	28.3074608	42.19254	123
N	B	36.701856	3.848867	57.55	28.9962254	44.40749	1234
T	N	37.083333	3.424906	36.53	30.1407941	44.02587	1234
Z	B	37.358562	3.684960	48.62	29.9518836	44.76524	1234
Z	N	38.020959	3.612859	45.01	30.7443310	45.29759	1234
T	B	38.359196	3.545536	41.83	31.2031453	45.51525	1234
Z	T	39.234593	3.612866	45.01	31.9579519	46.51123	12345
I	T	39.703097	3.483172	39.02	32.6578505	46.74834	12345
I	B	41.901259	3.612866	45.01	34.6246186	49.17790	123456
Z	Z	42.864548	3.685812	48.64	35.4562456	50.27285	123456
I	Z	46.000000	3.424906	36.53	39.0574608	52.94254	1234567
I	I	47.873812	3.483175	39.02	40.8285600	54.91906	234567
B	Z	50.190476	3.612466	45.00	42.9145938	57.46636	34567
I	N	52.708333	3.424906	36.53	45.7657941	59.65087	4567
B	N	54.708333	3.424906	36.53	47.7657941	61.65087	567
B	B	56.217136	3.612859	45.01	48.9405083	63.49376	67
B	I	56.857874	3.483173	39.02	49.8126252	63.90312	67
B	T	61.790053	3.483172	39.02	54.7448070	68.83530	7

day = 2:

female	male	lsmean	SE	df	lower.CL	upper.CL	.group
B	T	17.877010	3.483172	39.02	10.8317635	24.92226	1
Z	N	18.449530	3.612859	45.01	11.1729025	25.72616	1
B	Z	19.714286	3.612466	45.00	12.4384034	26.99017	12
B	N	20.833333	3.424906	36.53	13.8907941	27.77587	123
Z	Z	24.814548	3.685812	48.64	17.4062456	32.22285	1234
N	Z	25.303171	3.612869	45.01	18.0265234	32.57982	1234
Z	B	27.958562	3.684960	48.62	20.5518836	35.36524	1234
N	I	28.178160	3.483175	39.02	21.1329078	35.22341	1234
N	N	29.627391	3.849901	57.58	21.9197705	37.33501	1234
Z	I	31.666667	3.848352	57.53	23.9620266	39.37131	1234
T	N	31.916667	3.424906	36.53	24.9741274	38.85921	1234
I	N	32.416667	3.424906	36.53	25.4741274	39.35921	1234
N	T	32.645243	3.545911	41.84	25.4884837	39.80200	1234
B	I	32.857874	3.483173	39.02	25.8126252	39.90312	1234
Z	T	33.091736	3.612866	45.01	25.8150948	40.36838	1234
T	Z	33.683960	3.483173	39.02	26.6387121	40.72921	1234
T	I	33.731742	3.612869	45.01	26.4550948	41.00839	1234
I	Z	33.750000	3.424906	36.53	26.8074608	40.69254	1234
I	T	35.703097	3.483172	39.02	28.6578505	42.74834	234
T	B	36.586469	3.545536	41.83	29.4304180	43.74252	34
N	B	36.590745	3.848867	57.55	28.8851143	44.29638	234
B	B	37.788565	3.612859	45.01	30.5119369	45.06519	4
T	T	38.750000	3.424906	36.53	31.8074608	45.69254	4
I	I	40.830334	3.483175	39.02	33.7850818	47.87559	4
I	B	41.615545	3.612866	45.01	34.3389043	48.89219	4

day = 3:

female	male	lsmean	SE	df	lower.CL	upper.CL	.group
B	N	10.041667	3.424906	36.53	3.0991274	16.98421	1
B	Z	11.857143	3.612466	45.00	4.5812605	19.13303	12
Z	N	19.020959	3.612859	45.01	11.7443310	26.29759	123
Z	Z	20.514548	3.685812	48.64	13.1062456	27.92285	1234
N	Z	24.731742	3.612869	45.01	17.4550948	32.00839	12345
B	T	25.094401	3.483172	39.02	18.0491548	32.13965	12345
N	N	25.516280	3.849901	57.58	17.8086594	33.22390	123456
T	N	28.041667	3.424906	36.53	21.0991274	34.98421	23456
T	Z	29.162221	3.483173	39.02	22.1169730	36.20747	3456
B	I	31.597004	3.483173	39.02	24.5517556	38.64225	3456
N	T	32.190698	3.545911	41.84	25.0339382	39.34746	3456
I	Z	33.000000	3.424906	36.53	26.0574608	39.94254	3456
N	I	33.265116	3.483175	39.02	26.2198644	40.31037	3456
I	T	34.746575	3.483172	39.02	27.7013287	41.79182	3456
T	I	36.398409	3.612869	45.01	29.1217615	43.67506	456
T	B	37.995560	3.545536	41.83	30.8395089	45.15161	56
I	N	38.250000	3.424906	36.53	31.3074608	45.19254	56
I	I	38.612943	3.483175	39.02	31.5676905	45.65819	56
I	B	38.806021	3.612866	45.01	31.5293805	46.08266	56
T	T	39.000000	3.424906	36.53	32.0574608	45.94254	56
Z	B	39.158562	3.684960	48.62	31.7518836	46.56524	56
Z	I	40.555556	3.848352	57.53	32.8509155	48.26020	56
Z	T	42.615545	3.612866	45.01	35.3389043	49.89219	6
N	B	42.701856	3.848867	57.55	34.9962254	50.40749	6
B	B	42.836184	3.612859	45.01	35.5595559	50.11281	6

day = 4:

female	male	lsmean	SE	df	lower.CL	upper.CL	.group
B	N	6.208333	3.424906	36.53	-0.7342059	13.15087	1
B	Z	7.619048	3.612466	45.00	0.3431653	14.89493	12
N	Z	15.541266	3.612869	45.01	8.2646186	22.81791	123
Z	N	17.973340	3.612859	45.01	10.6967120	25.24997	123
Z	Z	20.914548	3.685812	48.64	13.5062456	28.32285	1234
N	N	21.349613	3.849901	57.58	13.6419927	29.05723	12345
B	T	23.224836	3.483172	39.02	16.1795896	30.27008	23456

B	I	25.205700	3.483173	39.02	18.1604512	32.25095	3456
N	I	27.047725	3.483175	39.02	20.0024731	34.09298	34567
T	N	28.041667	3.424906	36.53	21.0991274	34.98421	34567
T	Z	29.423091	3.483173	39.02	22.3778425	36.46834	34567
I	T	30.616140	3.483172	39.02	23.5708939	37.66139	34567
I	Z	30.833333	3.424906	36.53	23.8907941	37.77587	34567
N	T	31.099789	3.545911	41.84	23.9430292	38.25655	34567
T	I	35.588885	3.612869	45.01	28.3122377	42.86553	4567
I	N	36.333333	3.424906	36.53	29.3907941	43.27587	4567
T	B	37.041015	3.545536	41.83	29.8849635	44.19707	4567
I	I	37.656421	3.483175	39.02	30.6111687	44.70167	567
T	T	38.208333	3.424906	36.53	31.2657941	45.15087	567
I	B	38.567926	3.612866	45.01	31.2912853	45.84457	567
Z	B	38.708562	3.684960	48.62	31.3018836	46.11524	567
Z	T	38.948878	3.612866	45.01	31.6722376	46.22552	67
Z	I	39.944444	3.848352	57.53	32.2398044	47.64908	67
B	B	42.550470	3.612859	45.01	35.2738416	49.82710	7
N	B	43.090745	3.848867	57.55	35.3851143	50.79638	7

day = 5:

female	male	lsmean	SE	df	lower.CL	upper.CL	.group
B	N	5.583333	3.424906	36.53	-1.3592059	12.52587	1
B	Z	7.000000	3.612466	45.00	-0.2758823	14.27588	1
N	N	15.071835	3.849901	57.58	7.3642149	22.77946	12
N	Z	15.779361	3.612869	45.01	8.5027139	23.05601	12
Z	N	17.592388	3.612859	45.01	10.3157596	24.86902	123
B	I	18.205700	3.483173	39.02	11.1604512	25.25095	123
Z	Z	18.824692	3.736886	51.36	11.3238716	26.32551	1234
T	Z	23.640482	3.483173	39.02	16.5952339	30.68573	2345
B	T	23.790053	3.483172	39.02	16.7448070	30.83530	2345
N	I	25.265116	3.483175	39.02	18.2198644	32.31037	2345
T	N	26.000000	3.424906	36.53	19.0574608	32.94254	2345
N	T	26.099789	3.545911	41.84	18.9430292	33.25655	2345
I	Z	27.875000	3.424906	36.53	20.9324608	34.81754	2345
I	T	29.050923	3.483172	39.02	22.0056766	36.09617	2345
T	I	30.207933	3.612869	45.01	22.9312853	37.48458	2345
I	N	33.041667	3.424906	36.53	26.0991274	39.98421	345
T	B	34.177378	3.545536	41.83	27.0213271	41.33343	345
I	I	35.395551	3.483175	39.02	28.3502991	42.44080	45
T	T	36.625000	3.424906	36.53	29.6824608	43.56754	5
N	B	38.646301	3.848867	57.55	30.9406698	46.35193	5
Z	B	38.708562	3.684960	48.62	31.3018836	46.11524	5
I	B	39.329831	3.612866	45.01	32.0531900	46.60647	5
Z	T	39.425069	3.612866	45.01	32.1484281	46.70171	5
B	B	39.455231	3.612859	45.01	32.1786035	46.73186	5
Z	I	40.111111	3.848352	57.53	32.4064710	47.81575	5

Degrees-of-freedom method: satterthwaite
Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 25 estimates
significance level used: alpha = 0.05

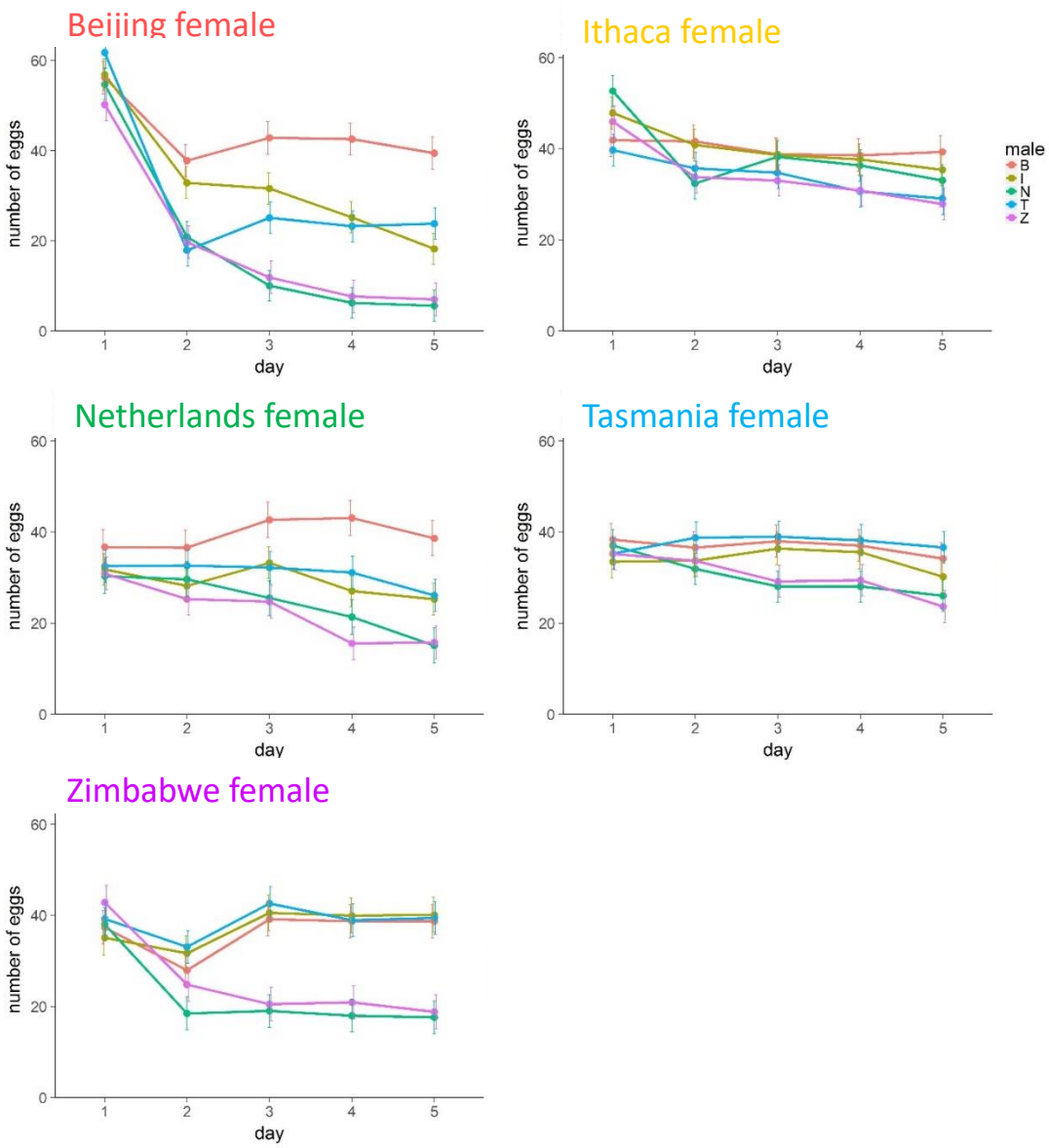

Fig. S21: Egg production over the course of five days differed depending on interactions between female and male genotype ($p= 7.6 \times 10^{-6}$). Shown here is mean egg production per day, for each of the 25 female x male combinations (mean based on three replicate experiments; average number of females (n) per combination across all replicates= 21.7). Error bars represent standard errors. Generally, egg production decreased over time. A high egg production persisted over the course of five days in Ithaca females. For other females, the decrease in egg production over time differed depending on the genotype of the female's mate. Specifically, males from the Netherlands and Zimbabwe lines were unable to stimulate a high egg production in females from Beijing, the Netherlands, Tasmania and Zimbabwe.

Table S9: ANOVA output from the linear mixed effects model used to analyze hatchability data. Hatchability differed depending on interactions between female and male genotype, and depending on the day after mating (p= 0.01).

Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom

	Sum Sq	Mean Sq	NumDF	DenDF	F.value	Pr(>F)	
female	4.5365	1.1341	4	511.41	36.861	< 2.2e-16	***
male	1.9811	0.4953	4	512.04	16.097	2.002e-12	***
day	15.9630	3.9908	4	1919.17	129.706	< 2.2e-16	***
female:male	1.0755	0.0672	16	511.29	2.185	0.004993	**
female:day	4.9412	0.3088	16	1915.87	10.037	< 2.2e-16	***
male:day	1.6658	0.1041	16	1918.16	3.384	6.060e-06	***
female:male:day	2.8701	0.0448	64	1914.02	1.458	0.011303	*

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table S10: Pairwise comparisons were made between all 25 female x male combinations, to evaluate differences in mean hatchability per day. If hatchability differed significantly between two mating combinations, these combinations were assigned to groups with no numbers or letters in common. Comparisons across days were not made. (Lsmean= least square means calculated based on three replicates, with average n per female x male combination= 32; SE= standard error; df= degrees of freedom; lower.CL and upper.CL= boundaries for 95% confidence interval)

day = 1:

female	male	lsmean	SE	df	lower.CL	upper.CL	.group
N	T	0.4194903	0.05769618	445.27	0.30609964	0.5328809	1
N	I	0.4249970	0.05645833	423.38	0.31402344	0.5359705	1
B	T	0.5632176	0.05645831	423.39	0.45224409	0.6741911	12
B	B	0.5852379	0.05901559	470.79	0.46927138	0.7012045	12
N	Z	0.5892693	0.05901567	470.73	0.47330256	0.7052361	12
I	T	0.6385622	0.05769133	446.86	0.52518221	0.7519422	12
Z	Z	0.6658774	0.06128383	520.06	0.54548306	0.7862716	12
T	N	0.6810033	0.05530253	401.82	0.57228482	0.7897217	12
T	I	0.6851937	0.05976305	492.17	0.56777150	0.8026159	12
N	B	0.6898153	0.06457491	585.13	0.56298845	0.8166421	12
B	I	0.6969494	0.05645832	423.39	0.58597588	0.8079229	12
B	Z	0.6973253	0.05901047	472.50	0.58136986	0.8132807	12
I	B	0.7004204	0.05901565	470.75	0.58445369	0.8163871	12
I	I	0.7172627	0.05645833	423.38	0.60628918	0.8282363	2
B	N	0.7187076	0.05719502	442.89	0.60630022	0.8311150	2
T	T	0.7376739	0.05530253	401.82	0.62895551	0.8463924	2
I	Z	0.7556239	0.05530253	401.82	0.64690549	0.8643424	2
N	N	0.7599003	0.06363883	550.51	0.63489569	0.8849050	2
I	N	0.7623784	0.05530253	401.82	0.65366001	0.8710969	2
T	B	0.7661748	0.05769133	446.86	0.65279478	0.8795548	2
T	Z	0.7965863	0.05645832	423.39	0.68561276	0.9075598	2
Z	T	0.8048408	0.05901565	470.75	0.68887414	0.9208075	2
Z	I	0.8090667	0.06566275	622.60	0.68011942	0.9380140	2
Z	N	0.8250476	0.05901559	470.79	0.70908101	0.9410141	2
Z	B	0.8499801	0.06043182	498.62	0.73124771	0.9687125	2

day = 2:

female	male	lsmean	SE	df	lower.CL	upper.CL	.group
N	I	0.2781944	0.05710650	441.42	0.16595997	0.3904288	1
B	T	0.3440058	0.05792380	464.05	0.23018039	0.4578313	12
B	B	0.3517544	0.05901559	470.79	0.23578781	0.4677209	12
B	I	0.3529996	0.05713743	442.15	0.24070493	0.4652943	12
N	T	0.3804452	0.05769618	445.27	0.26705459	0.4938359	123
I	T	0.4660945	0.05769133	446.86	0.35271444	0.5794745	1234
B	Z	0.4967956	0.06079152	522.74	0.37736995	0.6162213	12345
I	N	0.5626313	0.05530253	401.82	0.45391290	0.6713498	123456
N	B	0.5769009	0.06362567	555.47	0.45192458	0.7018772	12345678
N	Z	0.5789172	0.05901567	470.73	0.46295050	0.6948840	234567
I	Z	0.5947825	0.05530253	401.82	0.48606410	0.7035010	2345678
I	B	0.5985093	0.05901565	470.75	0.48254262	0.7144760	2345678
I	I	0.6226509	0.05645833	423.38	0.51167739	0.7336245	2345678
B	N	0.6275433	0.05719499	442.90	0.51513603	0.7399506	2345678
N	N	0.6427472	0.06363883	550.51	0.51774251	0.7677518	2345678
T	I	0.6710634	0.05976217	492.48	0.55364317	0.7884837	345678
T	T	0.6820586	0.05530253	401.82	0.57334013	0.7907770	45678
Z	Z	0.7671198	0.06128383	520.06	0.64672549	0.8875141	45678
T	B	0.7757467	0.05769133	446.86	0.66236668	0.8891267	5678
Z	I	0.7971796	0.06461182	588.79	0.67028195	0.9240773	5678
T	N	0.8078532	0.05530253	401.82	0.69913477	0.9165716	678
T	Z	0.8324095	0.05645832	423.39	0.72143605	0.9433830	678
Z	T	0.8353166	0.05998384	495.60	0.71746262	0.9531706	678
Z	N	0.8784012	0.05979790	492.95	0.76091099	0.9958914	78
Z	B	0.8852924	0.06043182	498.62	0.76656001	1.0040248	8

day = 3:

female	male	lsmean	SE	df	lower.CL	upper.CL	.group
N	T	0.2902202	0.05932566	490.37	0.17365634	0.4067841	1
N	I	0.3418949	0.05788986	462.41	0.22813508	0.4556547	12
B	T	0.3680092	0.05967224	514.79	0.25077812	0.4852402	123
I	T	0.3866151	0.05769133	446.86	0.27323509	0.4999951	123
B	B	0.4464609	0.05901559	470.79	0.33049439	0.5624275	1234
B	Z	0.4564762	0.06659159	709.55	0.32573603	0.5872163	12345
B	I	0.4810186	0.05876287	487.03	0.36555858	0.5964787	123456
I	I	0.4815016	0.05645833	423.38	0.37052802	0.5924751	12345
N	Z	0.4896951	0.05985543	494.45	0.37209276	0.6072975	123456
I	B	0.5071951	0.05901565	470.75	0.39122841	0.6231618	123456
T	I	0.5127628	0.05901567	470.73	0.39679602	0.6287295	123456
I	N	0.5383742	0.05530253	401.82	0.42965576	0.6470926	1234567
N	B	0.5660507	0.06362567	555.47	0.44107435	0.6910270	12345678
T	T	0.5903215	0.05530253	401.82	0.48160303	0.6990399	2345678
I	Z	0.6247202	0.05530253	401.82	0.51600173	0.7334386	2345678
B	N	0.6310872	0.08026139	1213.30	0.47362066	0.7885537	12345678
T	B	0.6395818	0.05769133	446.86	0.52620183	0.7529619	345678
N	N	0.6748564	0.06363883	550.51	0.54985172	0.7998610	345678
Z	Z	0.6959610	0.06044285	494.79	0.57720474	0.8147173	45678
Z	I	0.7379216	0.06361911	557.96	0.61295940	0.8628839	45678
T	N	0.7543737	0.05593698	418.79	0.64442150	0.8643259	5678
Z	T	0.7647565	0.05998384	495.60	0.64690252	0.8826105	5678
T	Z	0.7732067	0.05645832	423.39	0.66223320	0.8841802	678
Z	N	0.8333320	0.06260556	578.45	0.71037011	0.9562940	78
Z	B	0.8565819	0.06043182	498.62	0.73784955	0.9753143	8

day = 4:

female	male	lsmean	SE	df	lower.CL	upper.CL	.group
N	T	0.2425241	0.05932566	490.37	0.12596019	0.3590879	1
I	T	0.2757884	0.05769133	446.86	0.16240839	0.3891684	12
N	I	0.3096278	0.05788986	462.41	0.19586800	0.4233876	12
I	I	0.3151156	0.05645833	423.38	0.20414202	0.4260891	12
B	T	0.3216267	0.06061911	543.62	0.20255031	0.4407031	123
T	T	0.4012689	0.05530253	401.82	0.29255051	0.5099874	1234

I	B	0.4102463	0.05901565	470.75	0.29427956	0.5262130	12345
B	B	0.4223037	0.05979629	493.31	0.30481683	0.5397905	123456
B	I	0.4435920	0.06154882	572.73	0.32270306	0.5644809	123456
N	B	0.4508833	0.06362567	555.47	0.32590697	0.5758596	123456
B	Z	0.4527480	0.07202632	903.19	0.31138957	0.5941064	1234567
T	I	0.4687728	0.05901567	470.73	0.35280600	0.5847395	123456
N	Z	0.5282993	0.06261435	579.58	0.40532066	0.6512780	123456789
I	N	0.5291339	0.05530253	401.82	0.42041548	0.6378523	12345678
T	B	0.5558958	0.05769133	446.86	0.44251576	0.6692758	23456789
B	N	0.5968285	0.07704753	1098.48	0.44565150	0.7480054	23456789
I	Z	0.6029322	0.05530253	401.82	0.49421381	0.7116507	3456789
T	N	0.6394727	0.05590916	418.18	0.52957473	0.7493708	456789
N	N	0.6821544	0.06363883	550.51	0.55714976	0.8071591	456789
Z	Z	0.6887689	0.06044285	494.79	0.57001260	0.8075252	456789
Z	I	0.7154202	0.06361911	557.96	0.59045802	0.8403825	56789
Z	T	0.7180919	0.05998384	495.60	0.60023795	0.8359459	6789
T	Z	0.7828354	0.05645832	423.39	0.67186194	0.8938089	789
Z	B	0.7999257	0.06127373	523.73	0.67955319	0.9202981	89
Z	N	0.8322533	0.06070677	519.60	0.71299238	0.9515141	9

day = 5:

female	male	lsmean	SE	df	lower.CL	upper.CL	.group
I	T	0.1488861	0.05769133	446.86	0.03550605	0.2622661	1
N	I	0.1707032	0.05788986	462.41	0.05694343	0.2844630	12
I	I	0.1815859	0.05645833	423.38	0.07061236	0.2925594	12
N	T	0.1974591	0.06014723	514.33	0.07929459	0.3156235	123
B	T	0.3068773	0.06055527	541.84	0.18792541	0.4258291	1234
N	B	0.3123832	0.06362567	555.47	0.18740686	0.4373595	1234
I	B	0.3166736	0.05901565	470.75	0.20070686	0.4326403	1234
B	Z	0.3332434	0.07201057	901.98	0.19191561	0.4745711	12345
T	T	0.3344615	0.05530253	401.82	0.22574307	0.4431799	1234
T	I	0.3692235	0.05901567	470.73	0.25325679	0.4851903	12345
B	B	0.3977074	0.06150220	544.73	0.27689693	0.5185180	123456
B	I	0.4225168	0.06155022	572.14	0.30162484	0.5434088	1234567
I	N	0.4424146	0.05530253	401.82	0.33369618	0.5511330	234567
I	Z	0.4577074	0.05530253	401.82	0.34898896	0.5664258	234567
N	Z	0.4999725	0.06370127	613.38	0.37487343	0.6250715	345678
B	N	0.5090285	0.07700021	1099.90	0.35794458	0.6601124	23456789
T	B	0.5176236	0.05769133	446.86	0.40424360	0.6310036	45678
T	N	0.5516529	0.05593698	418.79	0.44170072	0.6616052	456789
Z	T	0.5871719	0.05998384	495.60	0.46931792	0.7050259	456789
N	N	0.5930215	0.06363883	550.51	0.46801686	0.7180262	456789
Z	I	0.6408348	0.06361911	557.96	0.51587255	0.7657970	56789
Z	Z	0.6829654	0.06124819	518.79	0.56264044	0.8032904	6789
Z	B	0.7365184	0.06127373	523.73	0.61614596	0.8568909	789
T	Z	0.7534480	0.05645832	423.39	0.64247446	0.8644215	89
Z	N	0.8305521	0.05985560	494.51	0.71294944	0.9481548	9

Degrees-of-freedom method: satterthwaite
Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 25 estimates
significance level used: alpha = 0.05

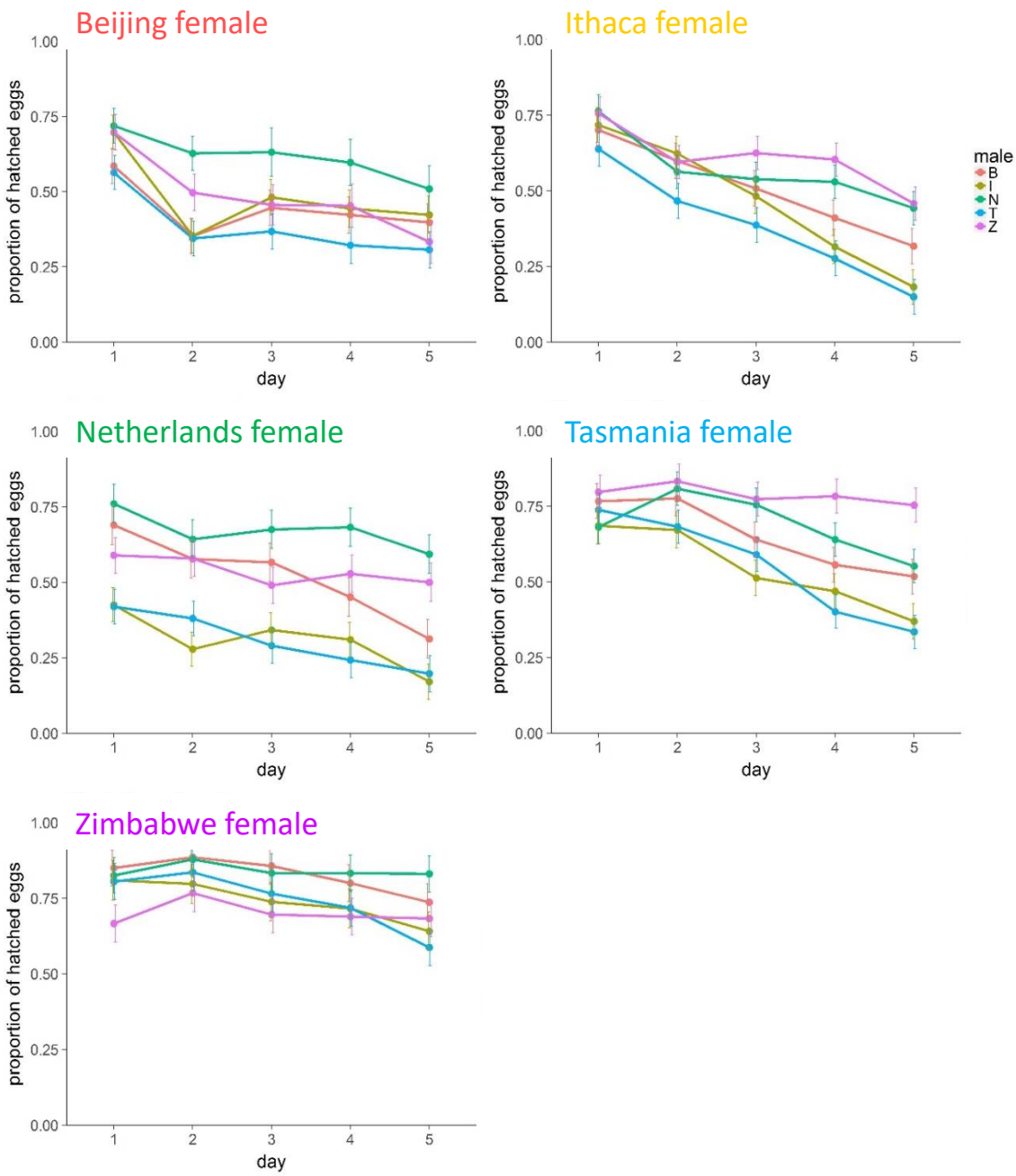
Beijing female
Ithaca female
Netherlands female
Tasmania female
Zimbabwe female
proportion of hatched eggs
day
1.00
0.75
0.50
0.25
0.00
1
2
3
4
5
male
B
I
N
T
Z

Fig. S22: Hatchability (number of pupae/ number of eggs) over the course of five days differed depending on interactions between female and male genotype (p= 0.01). Shown here is mean hatchability per day, for each of the 25 female x male combinations (based on three replicate experiments; average number of females (n) per combination across all replicates= 21.7). Error bars represent standard errors. Hatchability in Zimbabwe females was consistently high. In the four remaining female genotypes, hatchability decreased strongly over time. This decrease was more pronounced females mated to Ithaca or Tasmania males. Egg production was low in females mated to males from the Netherlands or Zimbabwe lines, but hatchability was high in these mating combinations. Low hatchability in intra-population crosses could be due to inbreeding effects. The Netherlands line is the only line not infected with *Wolbachia*. Low hatchability was observed in Netherlands females mated to *Wolbachia*-infected males, likely due to cytoplasmic incompatibility.

Table S11: ANOVA output from the linear mixed effects model used to analyze female refractoriness to re-mating, one day after the first mating. Refractoriness on day 1 after mating did not differ depending on female or male genotype.

Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom

	Sum Sq	Mean Sq	NumDF	DenDF	F.value	Pr(>F)	
female	0.081360	0.020340	4	16	2.0533	0.13501	
male	0.093787	0.023447	4	16	2.3669	0.09645	.
female:male	0.177722	0.011108	16	64	1.1213	0.35567	

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table S12: ANOVA output from the linear mixed effects model used to analyze female refractoriness to re-mating, four days after the first mating. Re-mating rate at day 4 after the first mating differed significantly depending on interactions between female and male genotype.

Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom

	Sum Sq	Mean Sq	NumDF	DenDF	F.value	Pr(>F)	
female	0.83747	0.209368	4	60	10.9928	9.407e-07	***
male	1.12139	0.280347	4	12	14.7196	0.0001409	***
female:male	1.26232	0.078895	16	60	4.1424	2.835e-05	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table S13: Pairwise comparisons were made between all 25 female x male combinations, to evaluate differences in female refractoriness to re-mating, four days after the first mating. If two mating combinations differed significantly from each other, these combinations were assigned to groups with no numbers or letters in common. (Lsmean= least square means calculated based on four replicates, with average n per female x male combination= 32; SE= standard error; df= degrees of freedom; lower.CL and upper.CL= boundaries for 95% confidence interval)

female	male	lsmean	SE	df	lower.CL	upper.CL	.group
Z	B	0.1651786	0.09636012	31.42	-0.03124312	0.3616003	1
Z	T	0.3169643	0.09636012	31.42	0.12054260	0.5133860	12345
Z	I	0.3169643	0.09636012	31.42	0.12054260	0.5133860	12367
T	B	0.3187500	0.09636012	31.42	0.12232831	0.5151717	12468
B	B	0.3392857	0.09636012	31.42	0.14286403	0.5357074	123456789
I	B	0.3928571	0.09636012	31.42	0.19643545	0.5892788	1234567890
I	T	0.4657738	0.09636012	31.42	0.26935212	0.6621955	1234567890A
T	T	0.4770833	0.09636012	31.42	0.28066164	0.6735050	1234567890AB
N	T	0.5982143	0.09636012	31.42	0.40179260	0.7946360	1234567890ABC
T	I	0.6339286	0.09636012	31.42	0.43750688	0.8303503	1234567890ABC
B	I	0.6553571	0.09636012	31.42	0.45893545	0.8517788	1234567890ABC
T	Z	0.6934524	0.09636012	31.42	0.49703069	0.8898741	234567890ABC
N	B	0.7089286	0.09636012	31.42	0.51250688	0.9053503	35790ABC
I	I	0.7232143	0.09636012	31.42	0.52679260	0.9196360	45890ABC
B	T	0.7455357	0.09636012	31.42	0.54911403	0.9419574	67890ABC
B	N	0.8770833	0.09636012	31.42	0.68066164	1.0735050	0ABC
T	N	0.8854167	0.09636012	31.42	0.68899498	1.0818384	0ABC
N	I	0.8958333	0.09636012	31.42	0.69941164	1.0922550	ABC
N	Z	0.9062500	0.09636012	31.42	0.70982831	1.1026717	ABC
N	N	0.9642857	0.09636012	31.42	0.76786403	1.1607074	BC
B	Z	1.0000000	0.09636012	31.42	0.80357831	1.1964217	C
I	Z	1.0000000	0.09636012	31.42	0.80357831	1.1964217	C
Z	Z	1.0000000	0.09636012	31.42	0.80357831	1.1964217	C
I	N	1.0000000	0.09636012	31.42	0.80357831	1.1964217	C
Z	N	1.0000000	0.09636012	31.42	0.80357831	1.1964217	C

Degrees-of-freedom method: satterthwaite

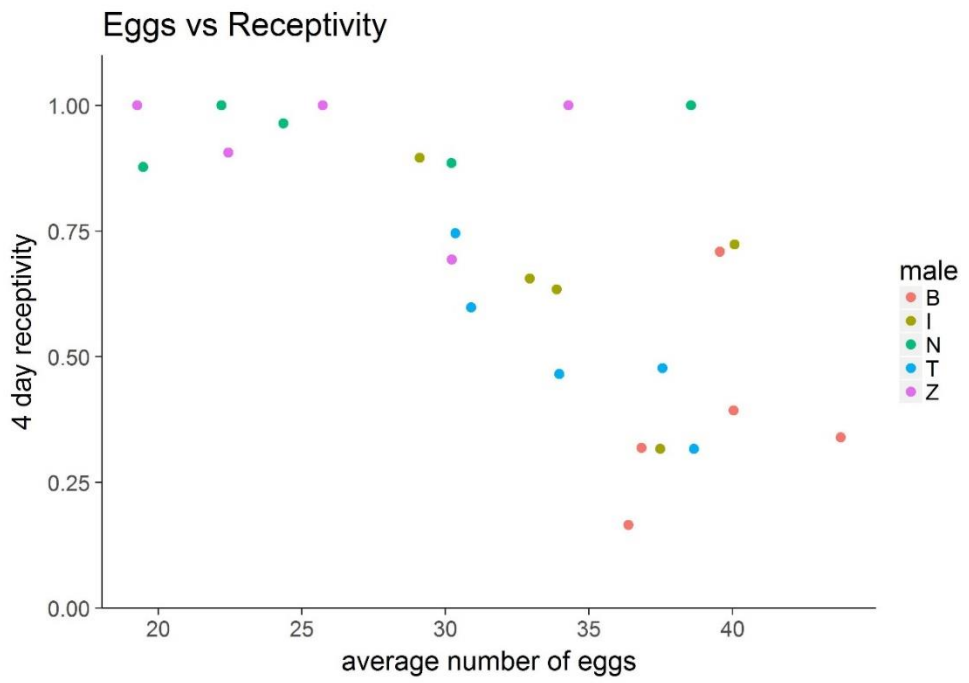

Fig S23: Using a Spearman correlation test, a negative correlation was found between (1) the average number of eggs produced per day, and (2) the proportion of females that re-mated with a standard male, four days after the first mating with a male from the Global Diversity Lines (p= 0.001). Generally, egg production was low, and receptivity to re-mating was high in females mated to males from the Netherlands or Zimbabwe lines. Colors indicate the genotype of the first male a female mated with (B= Beijing, I= Ithaca, N= Netherlands, T=Tasmania, Z= Zimbabwe).

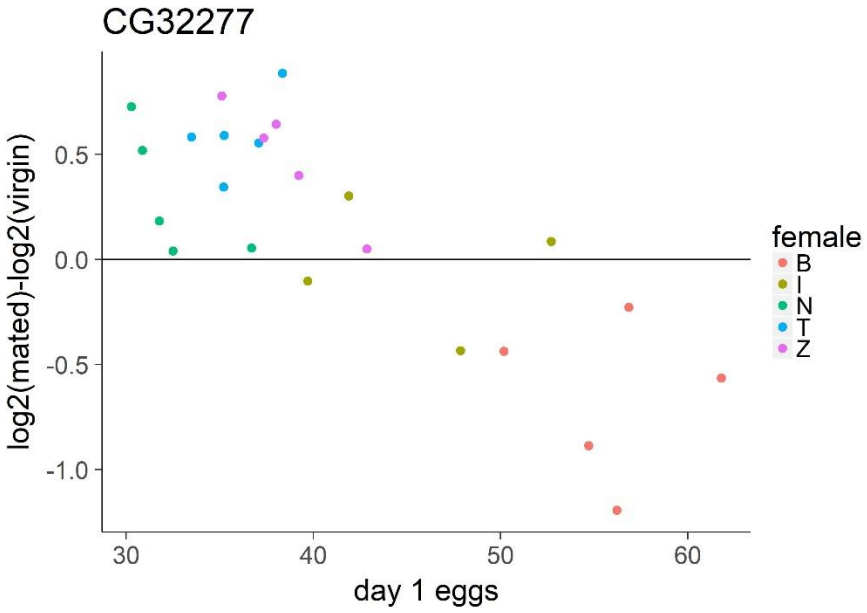

Fig. S24: Using a Spearman correlation test and Benjamini-Hochberg correction for multiple testing, a significant correlation was found between the post-mating fold change of *CG32277* and the average number of eggs produced on day 1 after mating (q= 0.03; B= Beijing, I= Ithaca, N= Netherlands, T=Tasmania, Z=Zimbabwe). Beijing females, which produced a high number of eggs on day 1 after mating, underwent a strong post-mating down-regulation of *CG32277* transcripts.

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