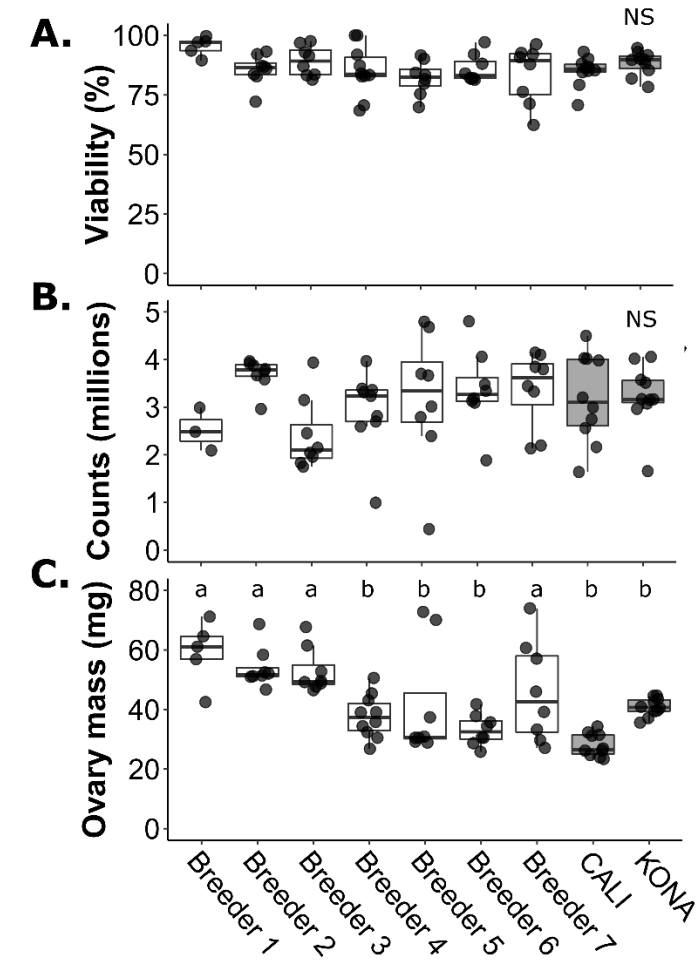


Supplementary figures: Trade-offs between sperm viability and immune protein expression in honey bee queens (*Apis mellifera*)

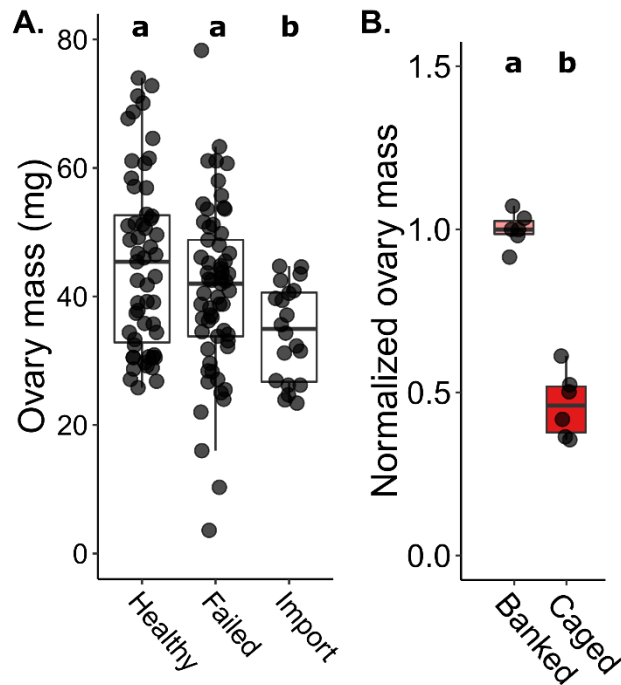
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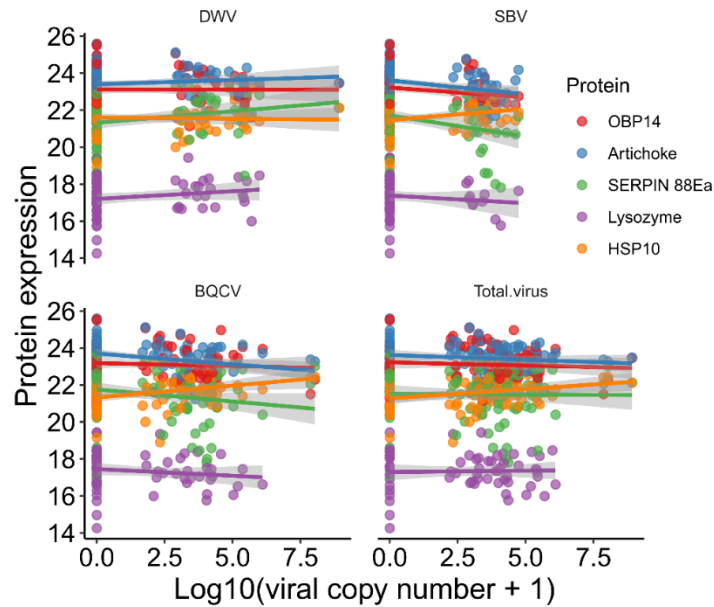
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Supplementary Figure 1. Data presented in **Figure 1**, separated by queen source. A) Sperm viability, B) sperm counts, and C) ovary mass metrics for healthy $n = 52$ healthy queens donated by 7 queen producers within British Columbia, $n = 10$ queens imported from California, and $n = 10$ queen imported from Hawaii. No differences in sperm viability nor counts were detected. Ovary masses varied significantly (one-way ANOVA), but this was likely an artifact of differences of caging time between producers. Lowercase letters indicate significant differences ($p < 0.05$). In all cases, boxes represent the bounds between the 2nd and 3rd interquartile range, midlines represent the median, and whiskers are extended by 1.5 times the interquartile range.



Supplementary Figure 2. Queen ovary masses. A) Ovary mass data from healthy (n = 52), failed (n = 53), and imported (n = 20) queens (data also presented in **Figure 1**). Ovary mass of imported queens was significantly lower than healthy and failed. Lowercase letters indicate significant differences ($p < 0.05$). See **Table 1** for summary statistics. B) Ovary masses from imported Californian queens were either measured upon arrival ('Caged,' n = 6) or banked in a queenless colony ('Banked,' n = 6) for two weeks prior to measuring ovary mass. Lowercase letters indicate significant differences (t test, $p < 0.05$). In all cases, boxes represent the bounds between the 2nd and 3rd interquartile range, midlines represent the median, and whiskers are extended by 1.5 times the interquartile range.



Supplementary Figure 3. No associations between expression of top five proteins and viral factors. N = 94 queens had complete sets of viability, proteomics, and viral data. We evaluated relationships for each protein separately using a least squares linear model, including sperm viability, protein expression, queen status (levels: healthy, failed, imported), and viral copy numbers for deformed wing virus (DWV), sacbrood virus (SBV), black queen cell virus (BQCV), and total load as fixed effects. No significant associations were identified ($p > 0.05$), except for protein expression with sperm viability, which we already determined previously ($p < 0.005$). Shaded grey bands represent the 95% confidence interval. Complete statistical summaries for each protein are below.

OBP14:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	25.681812	0.686255	37.423	< 2e-16 ***
Viability	-0.030919	0.009021	-3.428	0.000936 ***
DWV	-0.001888	0.086668	-0.022	0.982666
SBV	-0.090281	0.080970	-1.115	0.267958
BQCV	-0.011570	0.093597	-0.124	0.901907
Total	-0.009420	0.110389	-0.085	0.932191
GroupHealthy	0.256250	0.305502	0.839	0.403916
GroupImport	-0.165068	0.391746	-0.421	0.674539

Lysozyme:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	20.11135	0.77888	25.821	< 2e-16 ***
Viability	-0.04008	0.01031	-3.886	0.000285 ***
DWV	-0.03115	0.10554	-0.295	0.769041
SBV	0.01943	0.10169	0.191	0.849205
BQCV	-0.14229	0.11599	-1.227	0.225321
Total	0.12993	0.13632	0.953	0.344849
GroupHealthy	0.49760	0.32472	1.532	0.131366
GroupImport	0.88335	0.46990	1.880	0.065628 .

HSP10:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	19.403701	0.610623	31.777	< 2e-16 ***
Viability	0.024937	0.008026	3.107	0.00256 **
DWV	-0.039723	0.077116	-0.515	0.60780
SBV	0.005754	0.072046	0.080	0.93653
BQCV	0.059086	0.083282	0.709	0.47995

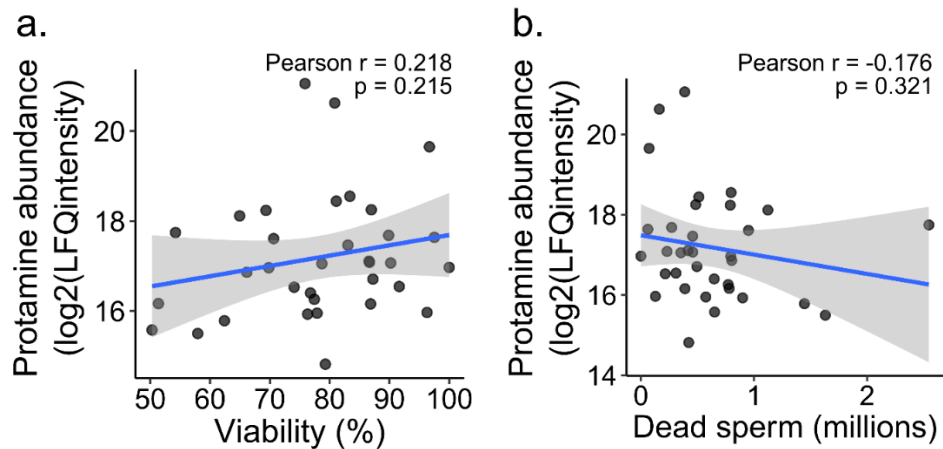
Total	0.078047	0.098223	0.795	0.42904
GroupHealthy	-0.253591	0.271832	-0.933	0.35349
GroupImport	-0.337728	0.348572	-0.969	0.33532

Serpin 88Ea:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	24.92732	0.88853	28.054	< 2e-16 ***
Viability	-0.04134	0.01168	-3.540	0.000649 ***
DWV	0.04284	0.11221	0.382	0.703604
SBV	-0.17599	0.10484	-1.679	0.096847 .
BQCV	-0.18008	0.12119	-1.486	0.140947
Total	0.11954	0.14293	0.836	0.405252
GroupHealthy	-0.13615	0.39555	-0.344	0.731543
GroupImport	0.56905	0.50722	1.122	0.265026

Artichoke:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	25.554697	0.554395	46.095	< 2e-16 ***
Viability	-0.024315	0.007287	-3.337	0.00125 **
DWV	-0.006204	0.070015	-0.089	0.92960
SBV	-0.086659	0.065412	-1.325	0.18874
BQCV	-0.144337	0.075613	-1.909	0.05961 .
Total	0.062854	0.089178	0.705	0.48283
GroupHealthy	0.184737	0.246801	0.749	0.45619
GroupImport	-0.042140	0.316474	-0.133	0.89438



Supplementary Figure 4. Pearson correlations of Protamine-like protein (XP_026294833.1; identified in N = 33 samples) with sperm viability (a) and number of dead sperm (b). Shaded grey bands represent the 95% confidence interval.