Supplemental Information for:

Concordant asymmetries are consistent with rapid reinforcement of reproductive isolation in a damselfly hybrid region

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Table S1. Sample size, gene flow and absolute reproductive isolation for each type of cross per rearing year experiment for five isolation barriers.

Fitness component	Cross	2000–2001 experiment			2019–2020 experiment		
		N	Success (Proportion/ Mean±SE)	Absolute RI	N	Success (proportion/ Mean±SE)	Absolute RI
Mechanical-I (tandem attempts)							
F0: Allopatric conspecific species	E♂ E♀	25	0.92	0.08			
F0: Allopatric conspecific species	G♂G♀	8	0.88	0.13			
F0: Sympatric conspecific crosses	E♂ E♀	NA	NA	NA	53	0.64	0.36
	G♂G♀	NA	NA	NA	10	1.00	0.00
F0: Sympatric heterospecific crosses	E♂G♀	23	1.00	0.00	92	0.59	0.41
	G♂E♀	44	0.11	0.89	22	0.05	0.95
F1: Sympatric F1-hybrids and backcrosses	H♂H♀	46	0.15	0.85	8	0.88	0.12
	H♂G♀	8	0.63	0.38	4	1.00	0.00
	G♂H♀	10	0.10	0.90	NA	NA	NA
	H♂E♀	14	0.14	0.86	1	1.00	0.00
	E♂H♀	9	1.00	0.00	11	0.45	0.55
F2: Sympatric F2-hybrids and backcrosses	H♂H♀	10	0.90	0.10	11	0.82	0.18
	H♂G♀	21	0.90	0.10	2	0.50	0.50
	G♂H♀	NA	NA	NA	3	0.33	0.67
	H♂E♀	NA	NA	NA	22	0.09	0.91
	E♂H♀	14	1.00	0.00	8	0.75	0.25
Mechanical-II (copula attempts)							
F0: Allopatric conspecific species	E♂ E♀	23	0.91	0.09			
F0: Allopatric conspecific species	G♂G♀	7	0.86	0.14			
F0: Sympatric conspecific crosses	E♂ E♀	NA	NA	NA	34	0.56	0.44
	G♂G♀	NA	NA	NA	10	1.00	0.00
F0: Sympatric heterospecific crosses	E♂G♀	23	0.87	0.13	54	0.67	0.33
	G♂E♀	5	0.60	0.40	1	0.00	**1.00**

F1: Sympatric F1-hybrids and backcrosses	H♂H♀	7	0.43	0.57	7	1.00	0.00
	H♂G♀	5	0.60	0.40	4	0.75	0.25
	G♂H♀	1	0.00	**1.00**	NA	NA	NA
	H♂E♀	2	1.00	0.00	1	0.00	**1.00**
	E♂H♀	9	1.00	0.00	5	1.00	0.00
F2: Sympatric F2-hybrids and backcrosses	H♂H♀	9	0.89	0.11	9	1.00	0.00
	H♂G♀	19	0.68	0.32	1	1.00	0.00
	G♂H♀	NA	NA	NA	1	1.00	0.00
	H♂E♀	NA	NA	NA	2	1.00	0.00
	E♂H♀	14	0.86	0.14	6	0.83	0.17
Gametic-I (oviposition)							
F0: Allopatric conspecific species	E♂ E♀	35	0.97	0.03			
F0: Allopatric conspecific species	G♂G♀	14	0.93	0.07			
F0: Sympatric conspecific crosses	E♂ E♀	10	0.80	0.20	21	0.86	0.14
	G♂G♀	4	1.00	0.00	12	1.00	0.00
F0: Sympatric heterospecific crosses	E♂G♀	18	0.67	0.33	25	0.96	0.04
	G♂E♀	3	1.00	0.00	Not possible (completed RI)		
F1: Sympatric F1-hybrids and backcrosses	H♂H♀	3	0.33	0.67	12	0.92	0.08
	H♂G♀	2	1.00	0.00	3	1.00	0.00
	G♂H♀	Not possible (completed RI)			NA	NA	NA
	H♂E♀	2	0.00	**1.00**	Not possible (completed RI)		
	E♂H♀	2	1.00	0.00	6	1.00	0.00
F2: Sympatric F2-hybrids and backcrosses	H♂H♀	9	1.00	0.00	8	0.75	0.25
	H♂G♀	1	1.00	0.00	1	1.00	0.00
	G♂H♀	NA	NA	NA	1	1.00	0.00
	H♂E♀	8	1.00	0.00	2	1.00	0.00
	E♂H♀	12	0.92	0.08	5	0.60	0.40
Gametic-II (fecundity)							
F0: Allopatric conspecific species	E♂ E♀	34	160.67±16.53	0.00			
F0: Allopatric conspecific species	G♂G♀	13	224.23±18.59	0.00			
F0: Sympatric conspecific crosses	E♂ E♀	8	200.60±42.03	-0.25	18	99.26±12.67	0.38

	G♂G♀	4	126.25±52.83	0.44	12	91.88±19.86	0.59
F0: Sympatric heterospecific crosses	E♂G♀	12	112.22±23.38	0.42	24	122.22±17.62	0.36
	G♂E♀	3	134.00±1.50	0.30	Not possible (completed RI)		
F1: Sympatric F1-hybrids and backcrosses	H♂H♀	1	127.00	0.34	11	78.88±29.13	0.59
	H♂G♀	2	190.50±113.50	0.01	3	119.44±14.81	0.38
	G♂H♀	Not possible (completed RI)			NA	NA	NA
	H♂E♀	Not possible (completed RI)			Not possible (completed RI)		
	E♂H♀	4	160.67±53.55	0.17	6	175.06±51.16	0.09
F2: Sympatric F2-hybrids and backcrosses	H♂H♀	9	163.59±19.74	0.15	6	51.83±10.70	0.73
	H♂G♀	1	23.67	0.88	1	83.33	0.57
	G♂H♀	NA	NA	NA	1	38.67	0.80
	H♂E♀	8	120.77±41.90	0.37	2	71.17±69.83	0.63
	E♂H♀	11	163.27±26.15	0.15	3	22.89±14.77	0.88
Gametic-III (fertility)							
F0: Allopatric conspecific species	E♂E♀	34	0.59±0.06	0.41			
F0: Allopatric conspecific species	G♂G♀	13	0.98±0.00	0.02			
F0: Sympatric conspecific crosses	E♂E♀	8	0.83±0.04	0.17	10	0.92±0.06	0.08
	G♂G♀	4	0.93±0.02	0.07	4	0.49±0.28	0.51
F0: Sympatric heterospecific crosses	E♂G♀	12	0.69±0.09	0.31	23	0.65±0.08	0.35
	G♂E♀	3	0.75±0.06	0.25	Not possible (completed RI)		
F1: Sympatric F1-hybrids and backcrosses	H♂H♀	1	0.85	0.15	NA	NA	NA
	H♂G♀	2	0.66±0.09	0.34	NA	NA	NA
	G♂H♀	Not possible (completed RI)			NA	NA	NA
	H♂E♀	Not possible (completed RI)			NA	NA	NA
	E♂H♀	4	0.52±0.09	0.49	NA	NA	NA
F2: Sympatric F2-hybrids and backcrosses	H♂H♀	9	0.67±0.04	0.33	6	0.20±0.08	0.80
	H♂G♀	1	0.27	0.73	1	0.07	0.93
	G♂H♀	NA	NA	NA	1	0.14	0.86
	H♂E♀	8	0.37±0.16	0.63	2	0.49±0.49	0.51
	E♂H♀	11	0.76±0.04	0.24	3	0.65±0.21	0.35

Table S2. *P* values for statistical comparisons between breeding experiments across all five reproductive isolation barriers measured.

Group	Cross	Comparison	Tandem	Copula	Oviposition	Fecundity	Fertility
F0: heterospecific crosses	E♂G♀	2000–2001 *vs* 2019–2020	**<0.001***	0.094	**0.015***	0.749	0.702
	G♂E♀	2000–2001 *vs* 2019–2020	0.655	1	NA	NA	NA
F1: hybrids and backcrosses	H♂H♀	2000–2001 *vs* 2019–2020	**<0.001***	0.070	0.081	0.385	NA
	H♂G♀	2000–2001 *vs* 2019–2020	0.490	1	1	1	NA
	G♂H♀	2000–2001 *vs* 2019–2020	NA	NA	NA	NA	NA
	H♂E♀	2000–2001 *vs* 2019–2020	0.200	0.333	NA	NA	NA
	E♂H♀	2000–2001 *vs* 2019–2020	**0.014***	1	1	0.915	NA
F2: hybrids and backcrosses	H♂H♀	2000–2001 *vs* 2019–2020	1	1	0.206	**<0.010***	**<0.010***
	H♂G♀	2000–2001 *vs* 2019–2020	0.249	1	1	1	1
	G♂H♀	2000–2001 *vs* 2019–2020	NA	NA	NA	NA	NA
	H♂E♀	2000–2001 *vs* 2019–2020	NA	NA	1	0.361	0.676
	E♂H♀	2000–2001 *vs* 2019–2020	0.121	1	0.191	0.043	1
*Statistically significant ($p<0.05$).							

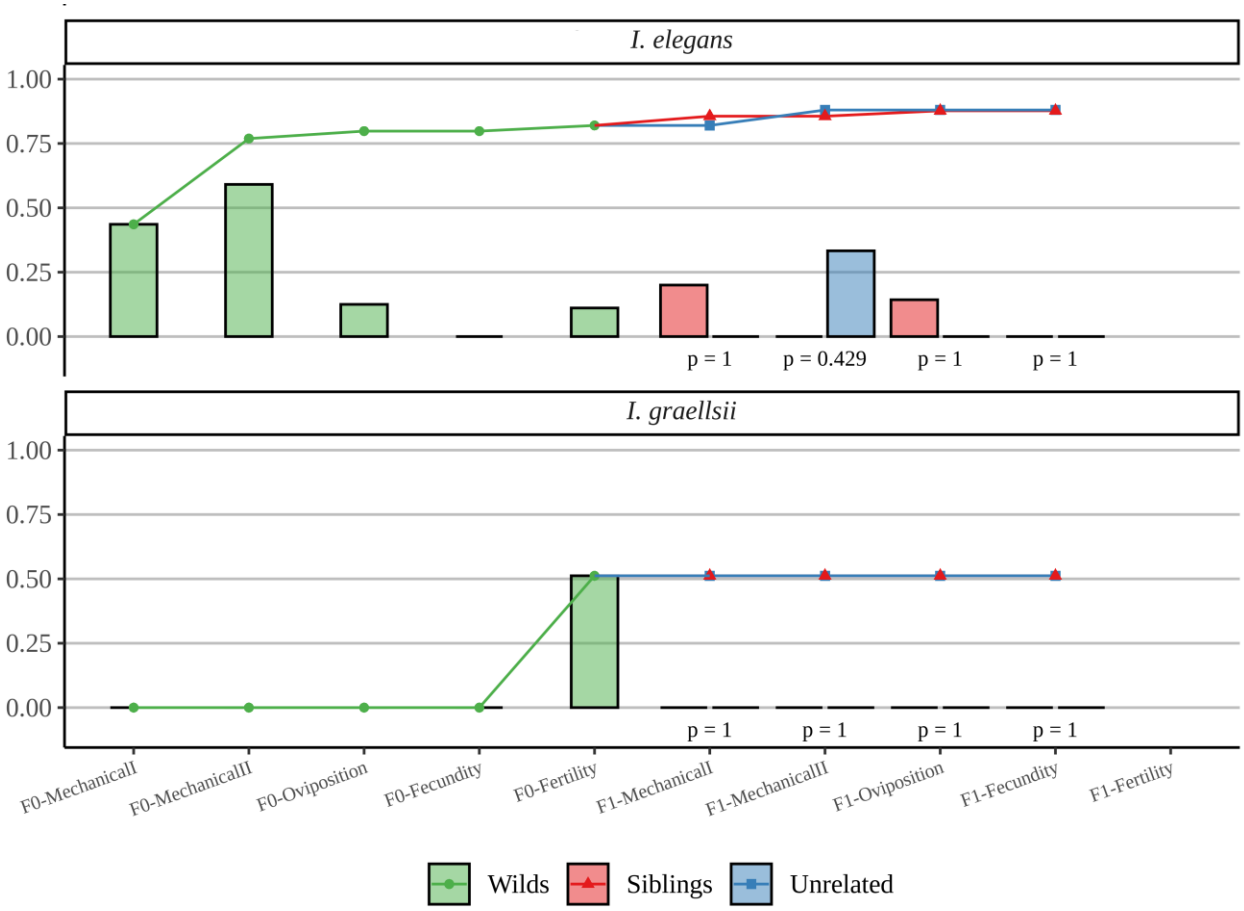

Figure S1. Reproductive isolation (bars) and cumulative absolute contribution (lines) per isolation barrier for 2019–2020 conspecific crosses. Reproductive isolation results are categorized in crosses between F0 wild samples (green), F1 siblings (red) and F1 unrelated individuals (blue). P values for statistical differences between sibling and unrelated families per isolation barrier are shown below the bars. No significant differences ($p > 0.05$) between siblings and unrelated crosses were found.

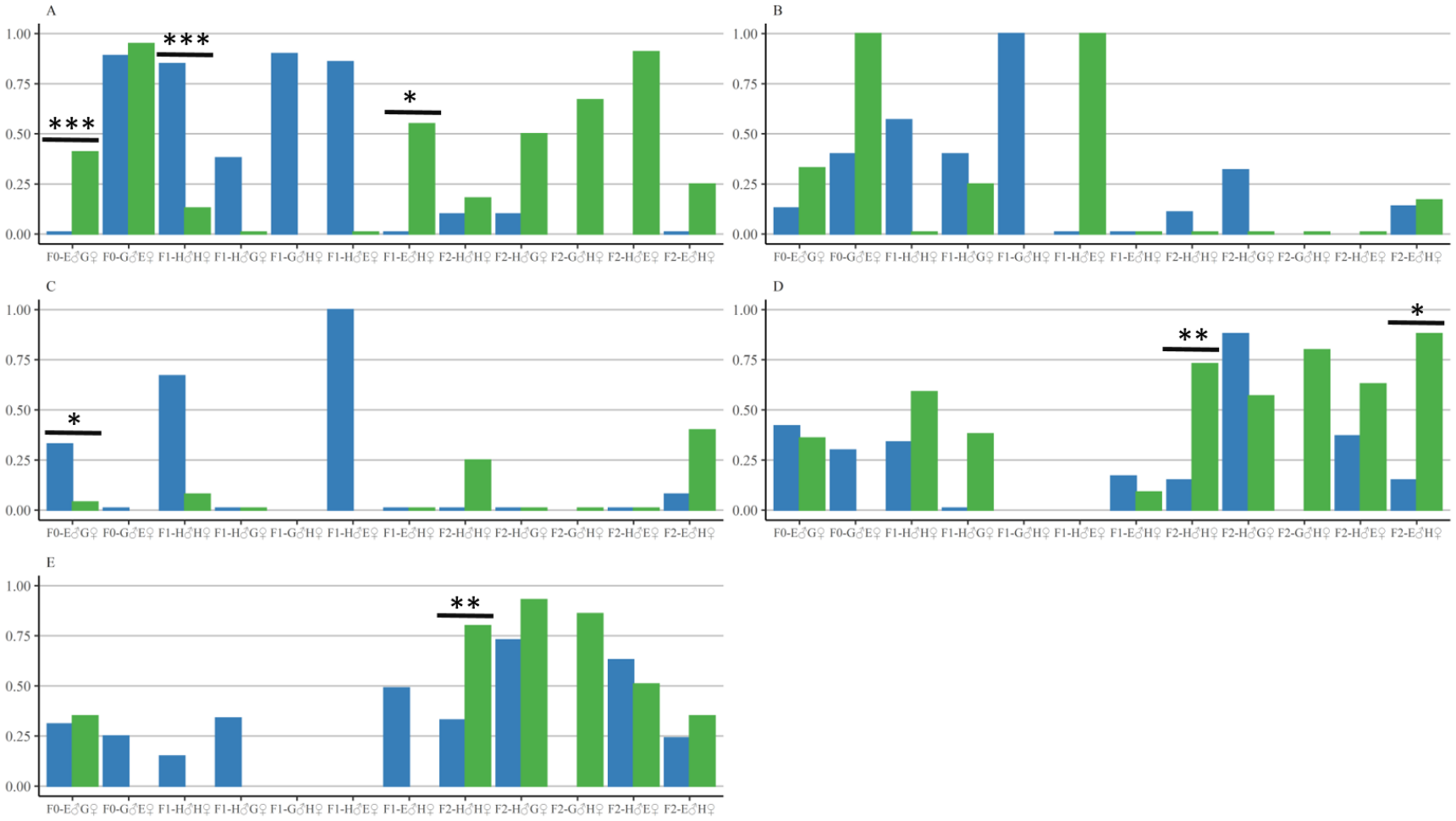

Figure S2. Tandem (A), copula (B), oviposition (C), fecundity (D) and fertility (E) reproductive isolation across three generations (F0, F1 and F2) of heterospecific crosses between 2000–2001 (blue) and 2019–2020 (green) experiments. G = *I. graellsii*; E = *I. elegans*; H = hybrid; *=p<0.05; **=p<0.01; ***=p<0.001.

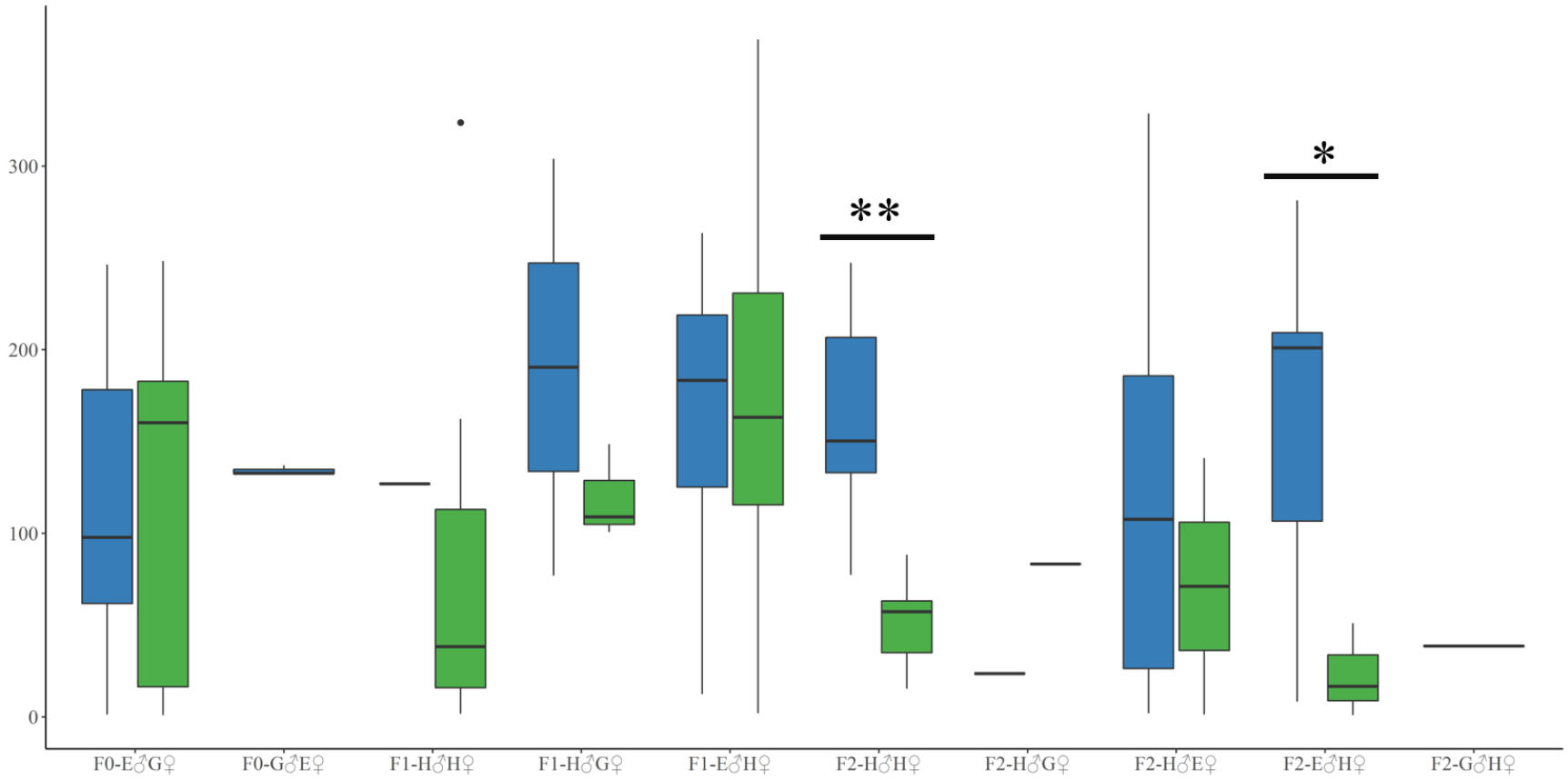

Figure S3. Fecundity boxplots showing mean number of eggs per batch. Data is grouped between 2000–2001 (blue) and 2019–2020 (green) experiments. Lower and upper hinges correspond to the first and third quartiles, while whiskers extend from the hinge to the largest or smallest value no further than 1.5 * inter-quartile range from the hinge. G = *I. graellsii*; E = *I. elegans*; H = hybrid; *=p<0.05; **=p<0.01; ***=p<0.001.

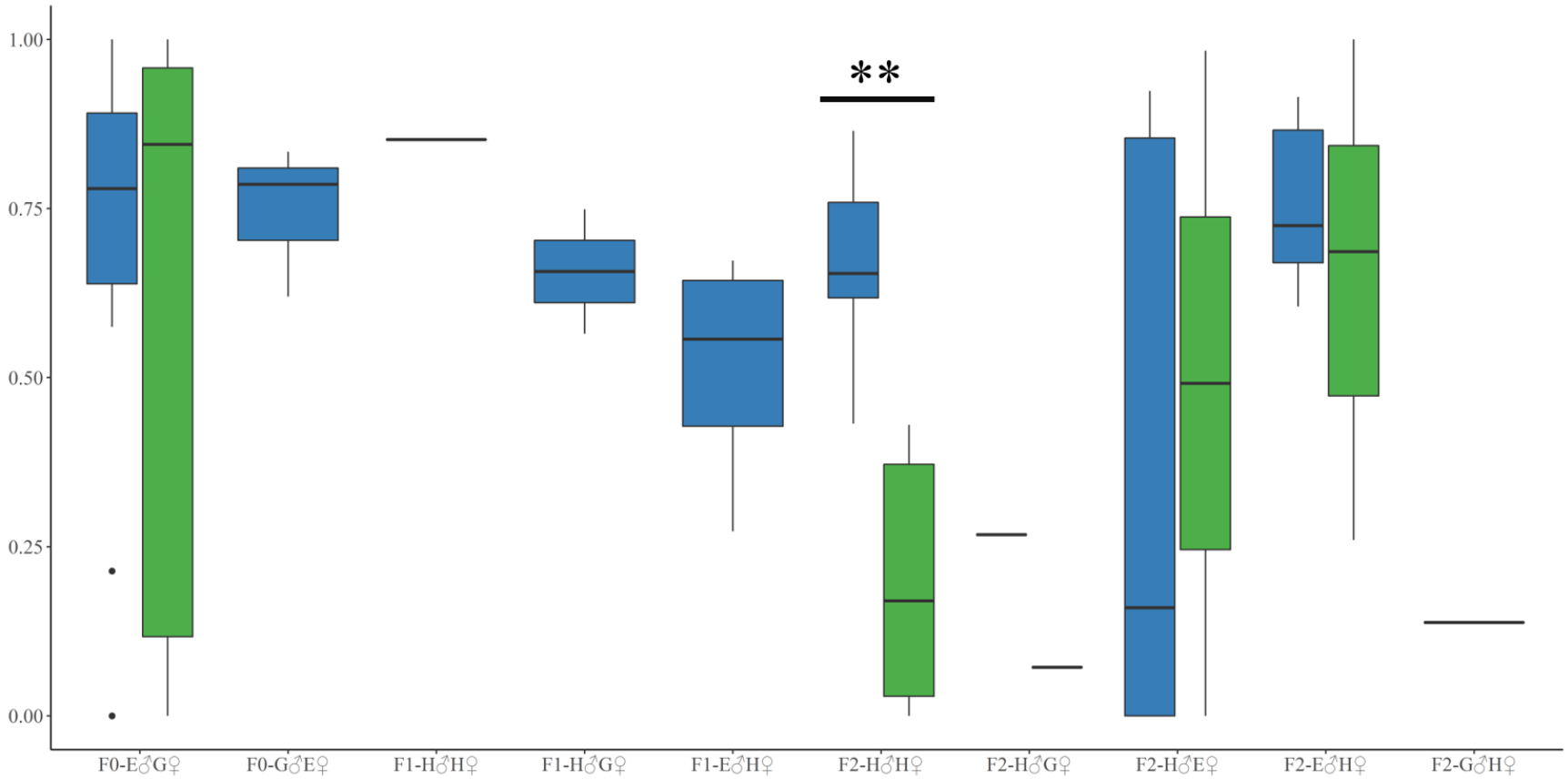

Figure S4. Fertility boxplots showing mean number of eggs per batch. Data is grouped between 2000–2001 (blue) and 2019–2020 (green) experiments. Lower and upper hinges correspond to the first and third quartiles, while whiskers extend from the hinge to the largest or smallest value no further than 1.5 * inter-quartile range from the hinge. G = *I. graellsii*; E = *I. elegans*; H = hybrid; *=p<0.05; **=p<0.01; ***=p<0.001.