Larval thermal windows in native and hybrid *Pseudoboletia* progeny (Echinoidea) as potential drivers of the hybridization zone

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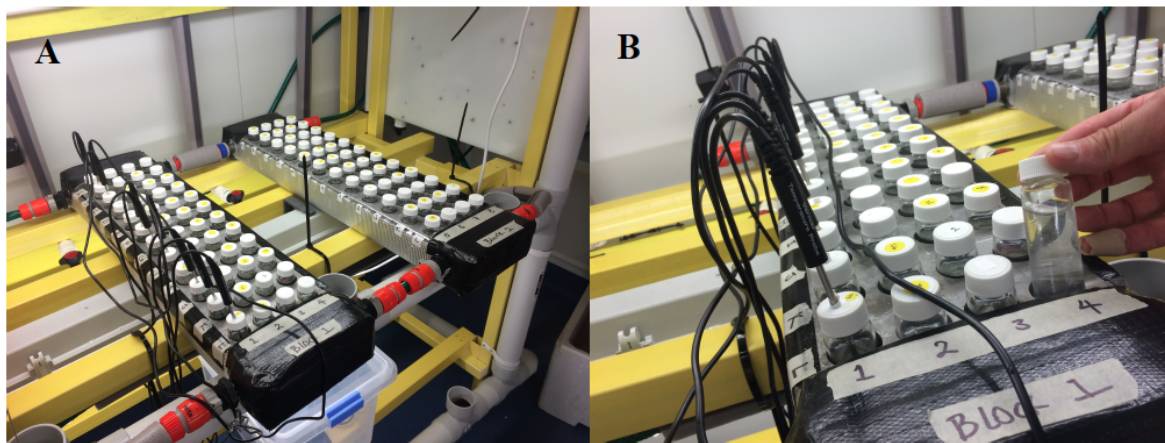
Supplement 1: supplementary figures

Figure S1. Thermal heat blocks (A), with culture vials in place (B) and temperatures being recorded in vials in the first column of vials (B).
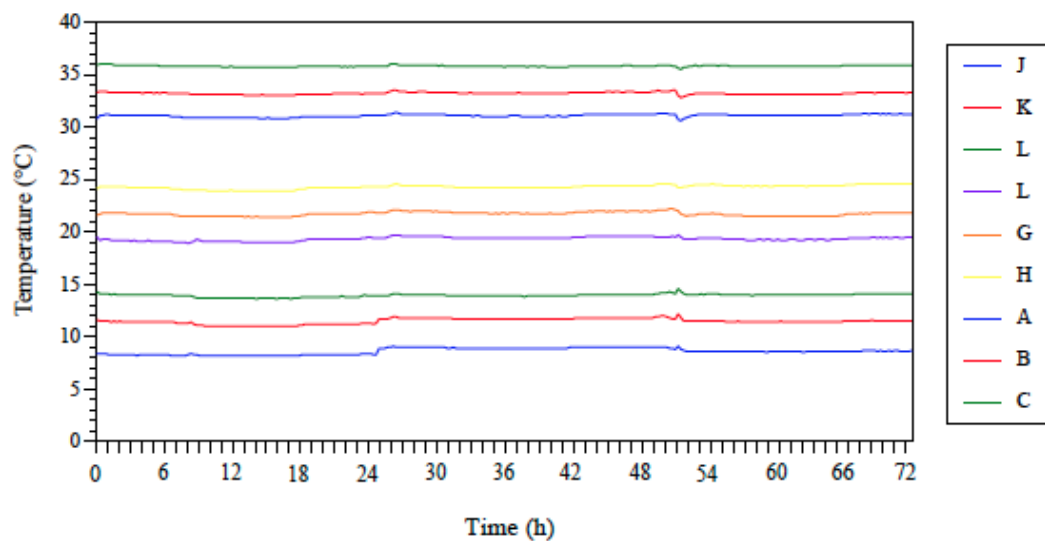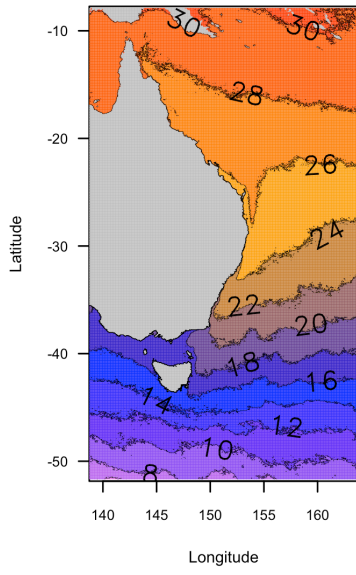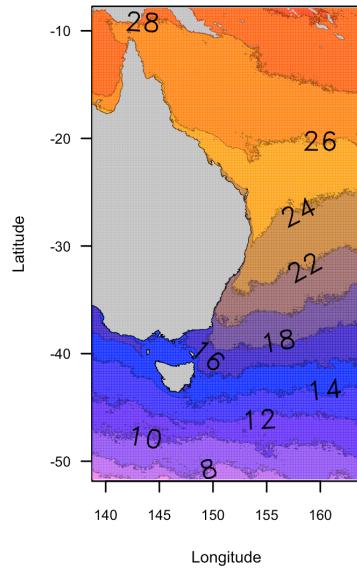

Figure S2. Logged temperatures in nine temperature treatments within the heat block thermal gradient over a 72 h period. Average temperatures logged are 8.6°C (J), 11.5°C (K), 13.9°C (L), 19.3°C (F), 21.7°C (G), 24.3°C (H), 31.1°C (A), 33.2°C (B) and 35.8°C (C). Summary details of the thermal regime in the heat block are given in Table 1.

A)

Monthly average 2003 - 2016 SST (°C) in April   **Monthly average 2003 - 2016 SST (°C) in May**   **Average 2003 - 2016 SST (°C) in June**
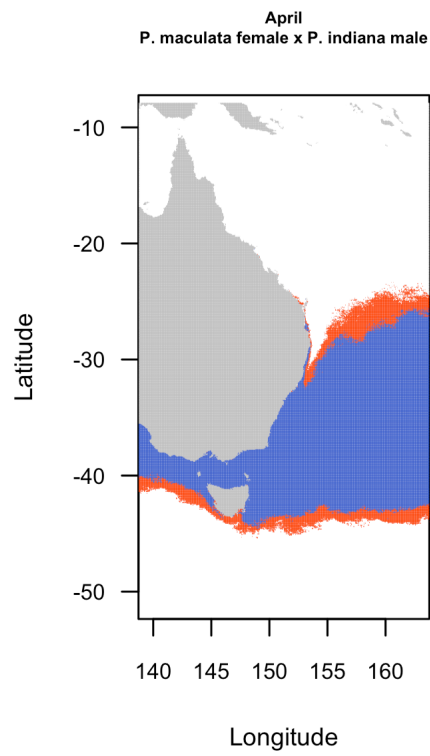
B)

April
P. indiana

April
P. maculata

April
P. indiana female x P. maculata male

April
P. maculata female x P. indiana male

C)

May
P. indiana

May
P. maculata

May
P. indiana female x P. maculata male

May
P. maculata female x P. indiana male
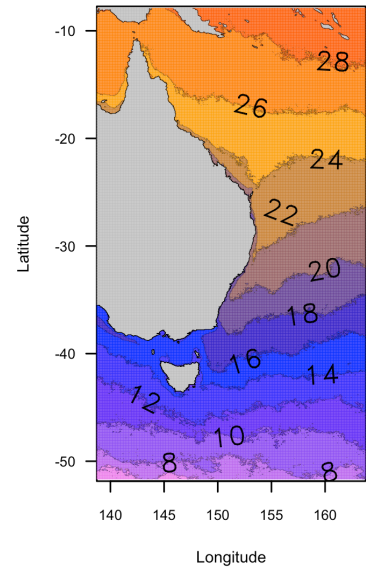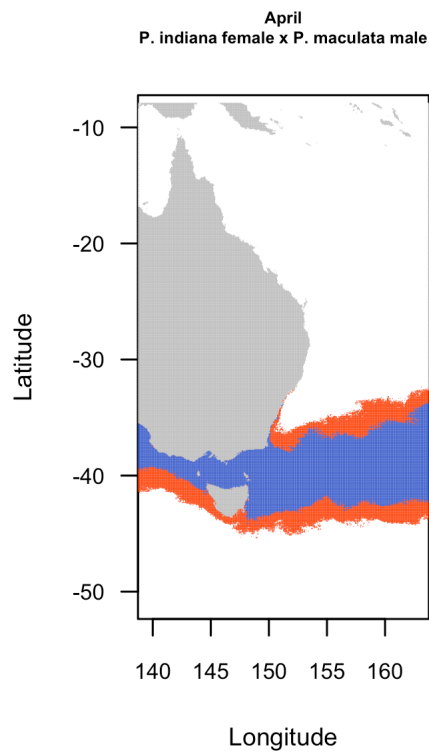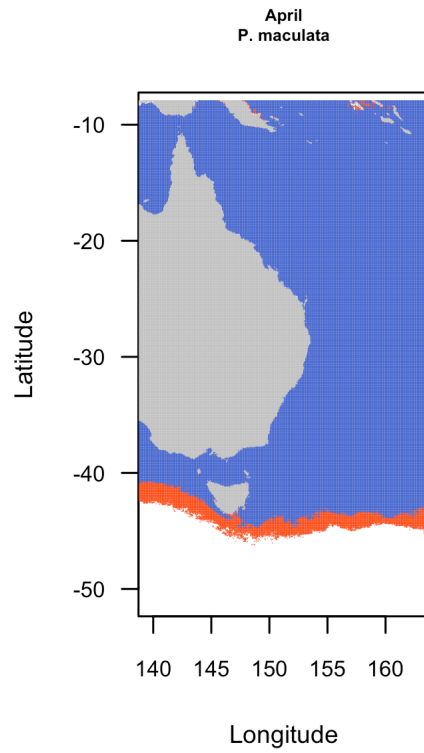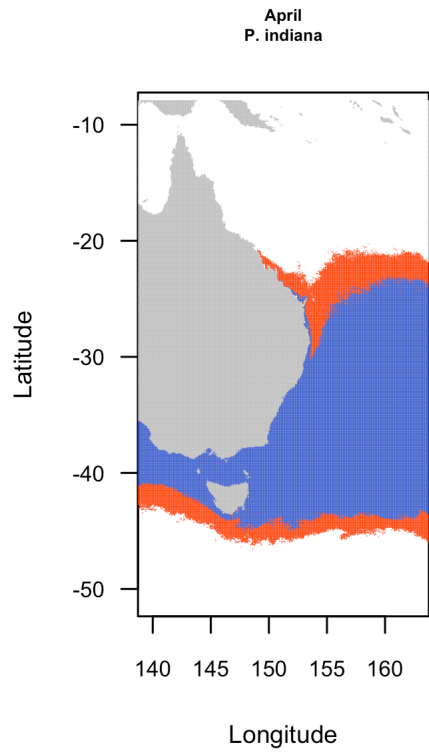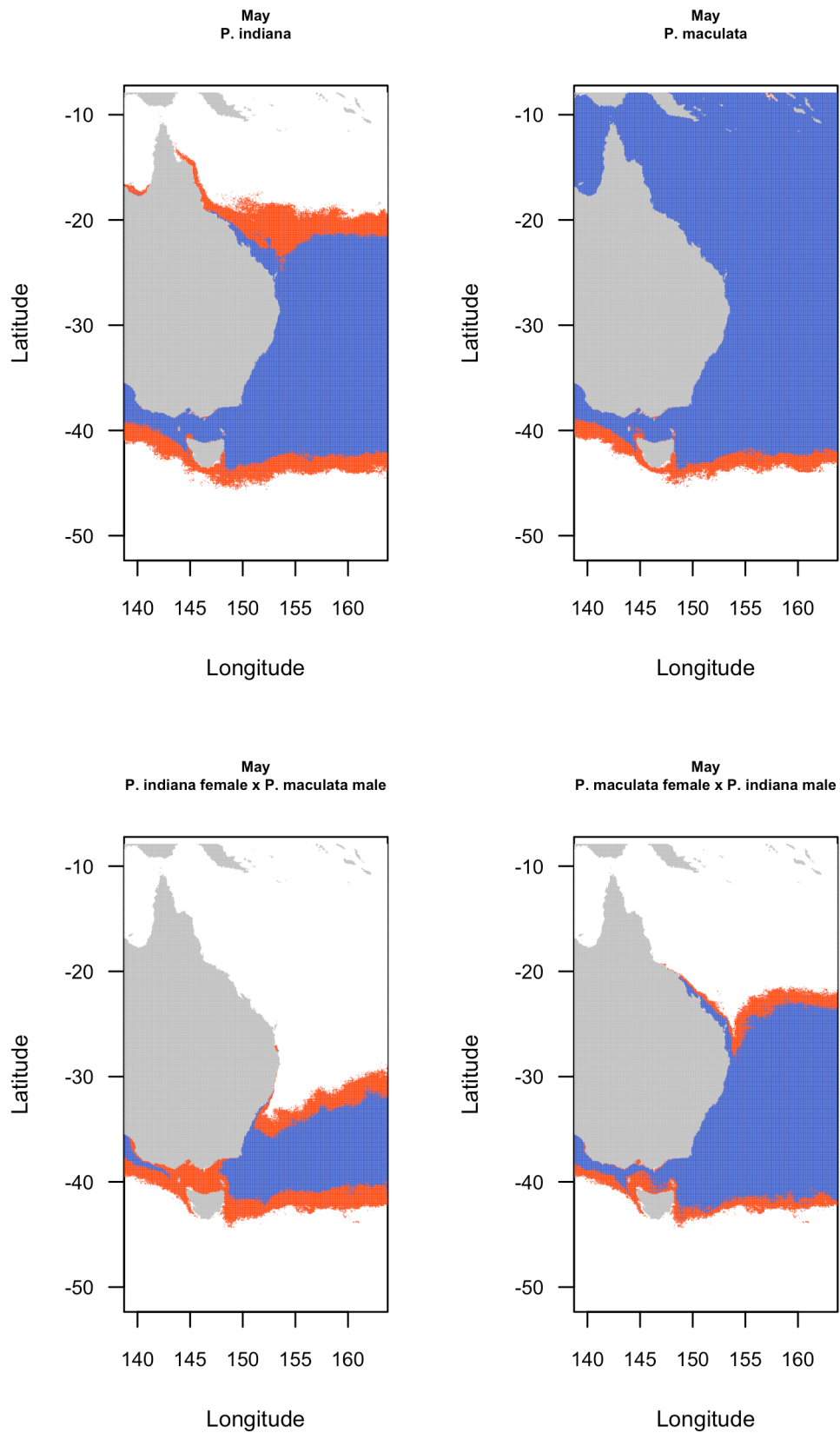
D)

Figure S3. Average sea surface temperatures for April, May and June between 2003 and 2016 along eastern Australia (Fig. S3A), and the distribution of sea temperatures in April (Fig. S3B), May (Fig. S3C) and June (Fig. S3D) that correspond to the thermal windows for 50% and 90% normal development in native and hybrid *Pseudoboletia* progeny.

Supplement 2: supplementary tables

Table S1. Average egg diameter for *P. maculata* ♀ x *P. maculata* ♂; *P. indiana* ♀ x *P. indiana* ♂; *P. maculata* ♀ x *P. indiana* ♂, and; *P. indiana* ♀ x *P. maculata* ♂ experiments. One-way ANOVA of egg diameter indicated a significant difference among progeny (ln(x) transformed data, $F_{(3, 36)} = 176.52$, $p < 0.001$). Tukey's post-hoc comparis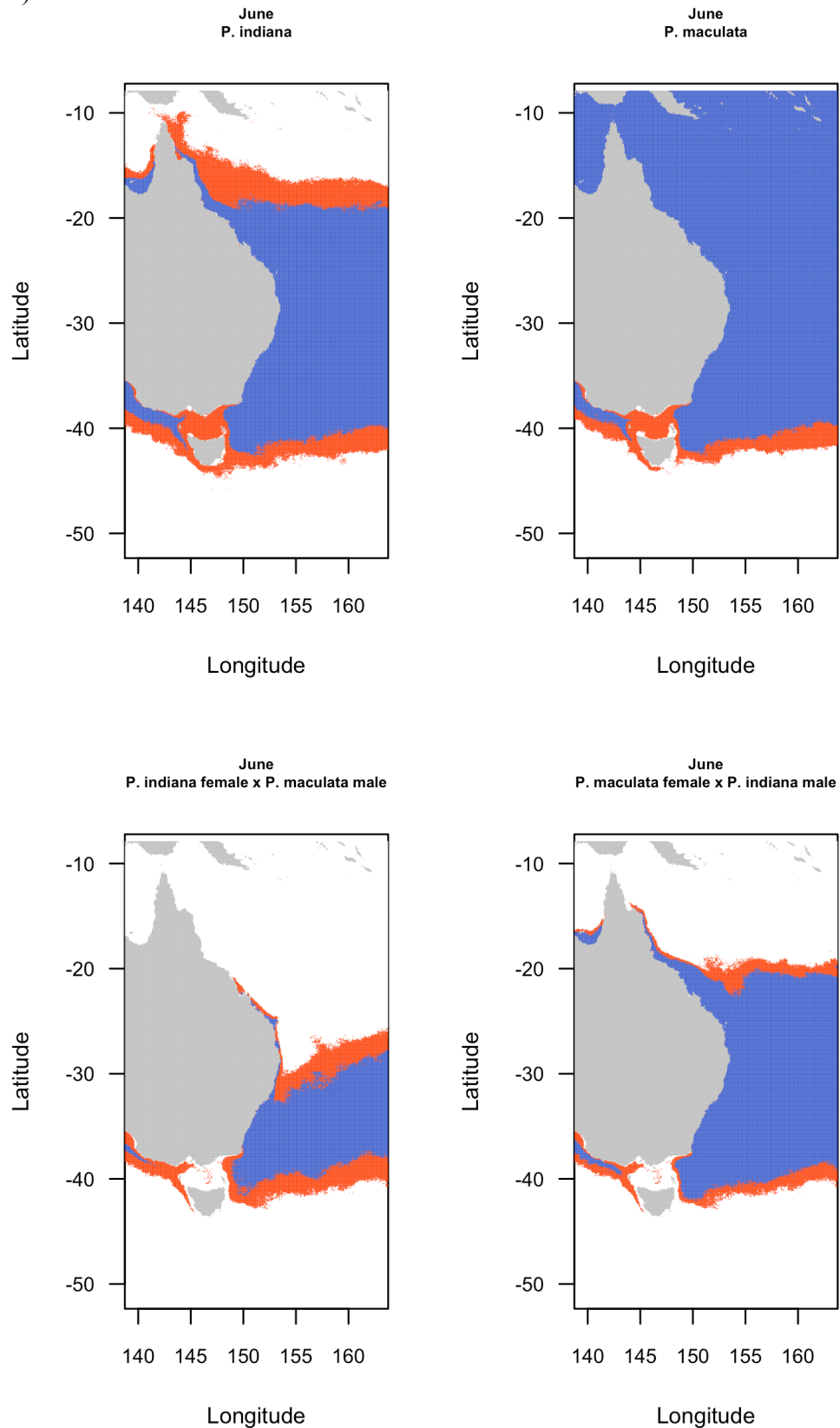ons amongst progeny indicate those groups not significantly different (sharing the same lower case lettering). N = 10 for each group.

Progeny egg	*P. maculata* ♀	*P. indiana* ♀	*P. maculata* ♀ x *P. indiana* ♂	*P. indiana* ♀ x *P. maculata* ♂
Average (μm)	84.41	103.96	85.16	104.42
SE	1.13	1.09	0.38	0.45
Tukey's *Post-hoc* comparisons	a	b	a	b

Table S2. Temperature measurements in 30 mL culture vessels placed in 9 positions along the thermal block temperature gradients. Summary statistics are for measurements were made every 15 min for 72 hours.

Temperature	Row J	K	L	F	G	H	A	B	C
Average (°C)	8.6	11.5	13.9	19.3	21.7	24.3	31.1	33.2	35.8
Maximum (°C)	8.2	11.0	13.6	18.9	21.4	23.9	30.6	32.8	35.5
Minimum(°C)	9.1	12.2	14.6	19.7	22.2	24.6	31.4	33.5	36.0
Medium(°C)	8.6	11.5	14.0	19.4	21.7	24.3	31.1	33.2	35.8
Range(°C)	0.9	1.2	1.0	0.8	0.7	0.7	0.7	0.8	0.5

Table S3. Measured temperature (A) and oxygen concentration (B) in experimental vials incubated in heat block gradients across 12 temperatures regimes. Measurements are for four columns (2 per heat block), with the average temperature (and range reported). Oxygen concentrations are reported for the four columns, and the predicted O_2 saturation state is calculated based on the average temperature for each temperature treatment. The measured % O_2 saturation is calculated as: (Average $[O_2]$/Predicted saturation $[O_2]$) x100%.

A. Measured temperature

Row	Column 1 Temperature (°C)	Column 2 Temperature (°C)	Column 3 Temperature (°C)	Column 4 Temperature (°C)	Average Temperature (°C)	Temperature range (± °C)
A	36.1	36.1	36.4	36.8	36.3	0.7
B	33.3	33.5	33.8	34	33.6	0.7
C	31.4	31.2	31.5	31.4	31.3	0.3
D	29	29.1	29.0	28.9	29.0	0.2
E	26.4	26.4	26.8	26.7	26.5	0.4
F	24.4	24.2	24.2	24.0	24.2	0.4
G	21.9	21.8	21.8	21.7	21.8	0.2
H	19.3	19.3	19.4	19.5	19.3	0.2
I	16.8	16.9	16.7	16.7	16.7	0.2
J	14.2	14.6	14.2	14.4	14.3	0.4
K	11.8	11.9	11.8	11.9	11.8	0.1
L	8.4	8.5	8.9	8.6	8.6	0.5

B. Dissolved Oxygen

Row	Column 1 $[O_2]$ mg O_2 :L^{-1}	Column 2 $[O_2]$ mg O_2 :L^{-1}	Column 3 $[O_2]$ mg O_2 :L^{-1}	Column 4 $[O_2]$ mg O_2 :L^{-1}	Predicted Saturation $[O_2]$ mg O_2 :L^{-1}	Measured % saturation
A	5.36	5.6	5.31	5.6	5.67	96.43
B	5.63	6.02	5.35	5.88	5.92	96.62
C	5.78	6.18	6.03	5.8	6.1	97.50
D	6.3	5.98	6.41	5.96	6.34	97.20
E	6.03	6.01	6.97	6.56	6.61	96.71
F	6.67	6.8	7.35	6.89	6.84	101.28
G	7.42	7.06	7.65	7.1	7.15	102.20
H	7.61	7.89	8.02	8.17	7.49	105.77
I	8.55	8.46	8.24	8.59	7.85	107.77
J	9.13	8.78	8.71	8.74	8.27	106.89
K	9.46	8.36	8.61	8.29	8.68	100.00

Supplement 3: generalized linear mixed model parameters and validations for the proportion of larvae at stage (embryo, gastrula, pluteus) for four *Pseudobolitia* progeny across a thermal gradient (12 temperatures between 8.6° and 36.4°C) at three times (10, 24 and 48 h).

Embryo
Family: binomial distribution.
Link: logit.
Formula: (embryo | other stages) ~ poly(temperature, 3)*poly(time, 2) + poly(time, 2):species + poly(temperature, 3):species + time:species + (1|vial ID)

Control: glmerControl(optimizer = "bobyqa")

AIC	BIC	logLik	deviance	df.resid
1788.2	1875.2	-875.1	1750.2	701

Scaled residuals:

Min	1Q	Median	3Q	Max
-13.74	-0.01	0.00	0.00	152.84

Random effects:

Groups Name	Variance	Std.Dev.
vial ID (Intercept)	1.329	1.153

Number of obs: 720, groups: replicates, 84

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-36.6581	4.3393	-8.448	< 2e-16 ***
poly(Temp, 3)1	-709.4828	62.0209	-11.439	< 2e-16 ***
poly(Temp, 3)2	-404.3460	41.7493	-9.685	< 2e-16 ***
poly(Temp, 3)3	-136.4236	16.4746	-8.281	< 2e-16 ***
poly(time, 2)1	-294.9856	66.4648	-4.438	9.07e-06 ***
poly(time, 2)2	14.8570	1.0866	13.672	< 2e-16 ***
poly(Temp, 3)1: *Sp P. maculata*	446.7944	23.5571	18.966	< 2e-16 ***
poly(Temp, 3)2: *Sp P. maculata*	566.8256	36.1397	15.684	< 2e-16 ***
poly(Temp, 3)3: *Sp P. maculata*	274.2362	19.2532	14.244	< 2e-16 ***
poly(Temp, 3)1: *Sp P. indiana (f) x P. maculata (m)*	442.2307	34.4035	12.854	< 2e-16 ***
poly(Temp, 3)2: *Sp P. indiana (f) x P. maculata (m)*	563.2814	53.5077	10.527	< 2e-16 ***
poly(Temp, 3)3: *Sp P. indiana (f) x P. maculata (m)*	248.5232	28.0297	8.866	< 2e-16 ***
poly(Temp, 3)1: *Sp P. maculata (f) x P. Indiana (m)*	428.0428	53.0159	8.074	6.81e-16 ***
poly(Temp, 3)2: *Sp P. maculata (f) x P. Indiana (m)*	657.7193	64.5448	10.190	< 2e-16 ***
poly(Temp, 3)3: *Sp P. maculata (f) x P. Indiana (m)*	387.9024	39.7491	9.759	< 2e-16 ***
Sp P. indiana:time	0.5071	0.1578	3.214	0.001309 **
Sp P. maculata:time	0.5812	0.1582	3.673	0.000239 ***
Sp P. indiana (f) x P. maculata (m):time	0.5705	0.1582	3.605	0.000312 ***

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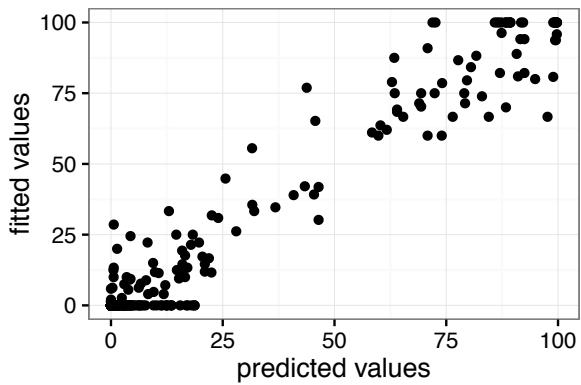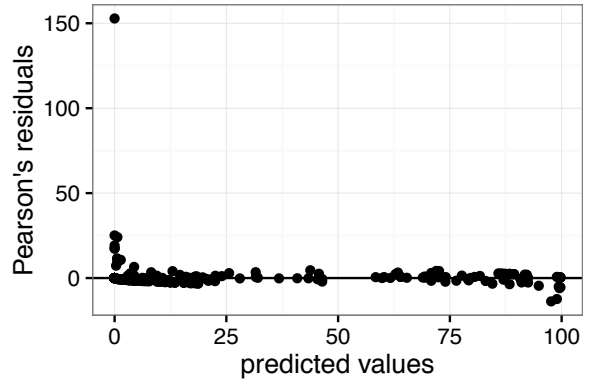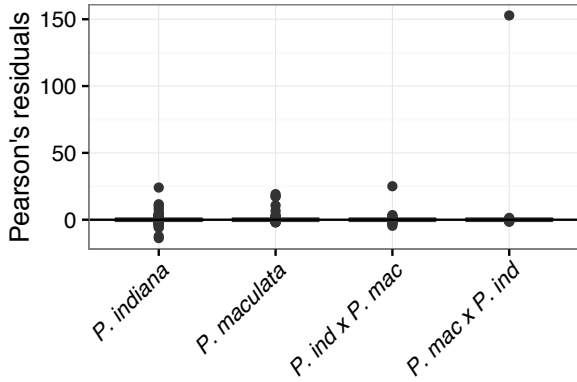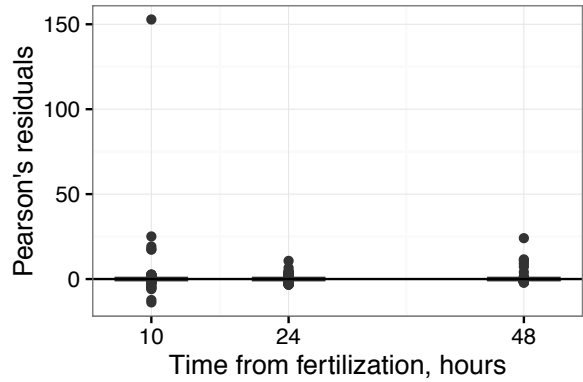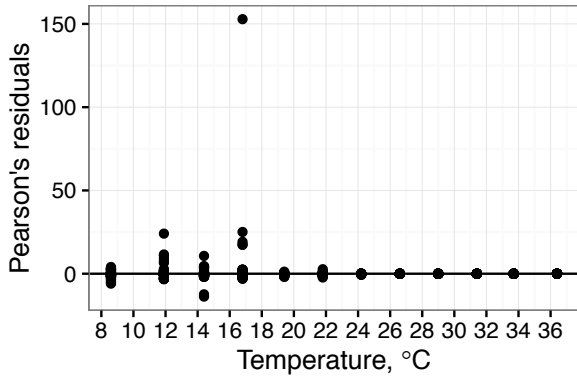
Gastrula

Family: binomial distribution.
Link: logit.
Formula: (gastrula | other stages) ~ poly(temperature, 3)*poly(time, 2) + poly(time, 2):species + poly(temperature, 3):species + time:species + (1|vial ID)

Control: glmerControl(optimizer = "bobyqa")

AIC	BIC	logLik	deviance	df.resid
2296.6	2438.6	-1117.3	2234.6	689

Scaled residuals:

Min	1Q	Median	3Q	Max
-20.1896	-0.1815	-0.0003	0.0000	28.4224

Random effects:

Groups Name	Variance	Std.Dev.	
vial ID (Intercept)	0.8921		0.9445

Number of obs: 720, groups: replicates, 84

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-10.4507	0.5264	-19.85	< 2e-16 ***
poly(Temp, 3)1	-75.9766	24.9805	-3.04	0.002355 **
poly(Temp, 3)2	-311.5484	15.6436	-19.92	< 2e-16 ***
poly(Temp, 3)3	35.2422	15.5732	2.26	0.023636 *
poly(time, 2)1	22.7216	12.1729	1.87	0.061962 .
poly(time, 2)2	14.5890	11.2040	1.30	0.192874
Sp P. maculata	-9.8314	0.8460	-11.62	< 2e-16 ***
Sp P. indiana (f) x P. maculata (m)	-4.9072	0.9289	-5.28	1.27e-07 ***
Sp P. maculata (f) x P. Indiana (m)	-2.4295	0.4573	-5.31	1.08e-07 ***
poly(Temp, 3)1:poly(time, 2)1	-998.4481	439.1490	-2.27	0.022990 *
poly(Temp, 3)2:poly(time, 2)1	2021.3947	274.7104	7.36	1.86e-13 ***
poly(Temp, 3)3:poly(time, 2)1	3109.2633	176.6388	17.60	< 2e-16 ***
poly(Temp, 3)1:poly(time, 2)2	4439.3611	513.5358	8.64	< 2e-16 ***
poly(Temp, 3)2:poly(time, 2)2	2228.2921	318.9647	6.99	2.83e-12 ***
poly(Temp, 3)3:poly(time, 2)2	1863.9001	209.5847	8.89	< 2e-16 ***
poly(time, 2)1: *Sp P. maculata*	-159.9966	4.2837	-37.35	< 2e-16 ***
poly(time, 2)2: *Sp P. maculata*	71.0438	3.7654	18.87	< 2e-16 ***
poly(time, 2)1: *Sp P. indiana (f) x P. maculata (m)*	-188.7367	4.7053	-40.11	< 2e-16 ***
poly(time, 2)2: *Sp P. indiana (f) x P. maculata (m)*	79.5699	3.8240	20.81	< 2e-16 ***
poly(time, 2)1: *Sp P. maculata (f) x P. Indiana (m)*	-193.2530	4.9114	-39.35	< 2e-16 ***
poly(time, 2)2: *Sp P. maculata (f) x P. Indiana (m)*	96.1205	4.0332	23.83	< 2e-16 ***
poly(Temp, 3)1: *Sp P. maculata*	-481.2700	43.2039	-11.14	< 2e-16 ***
poly(Temp, 3)2: *Sp P. maculata*	-97.4392	23.7413	-4.10	4.06e-05 ***
poly(Temp, 3)3: *Sp P. maculata*	-43.3746	21.4583	-2.02	0.043245 *
poly(Temp, 3)1: *Sp P. indiana (f) x P. maculata (m)*	-167.0501	47.4933	-3.52	0.000436 ***
poly(Temp, 3)2: *Sp P. indiana (f) x P. maculata (m)*	136.5016	26.1534	5.22	1.80e-07 ***
poly(Temp, 3)3: *Sp P. indiana (f) x P. maculata (m)*	112.6014	24.6275	4.57	4.83e-06 ***
poly(Temp, 3)1: *Sp P. maculata (f) x P. Indiana (m)*-	135.2588	27.8408	-4.86	1.18e-06 ***
poly(Temp, 3)2: *Sp P. maculata (f) x P. Indiana (m)*	177.2036	15.0624	11.76	< 2e-16 ***
poly(Temp, 3)3: *Sp P. maculata (f) x P. Indiana (m)*	93.9240	17.4121	5.39	6.88e-08 ***

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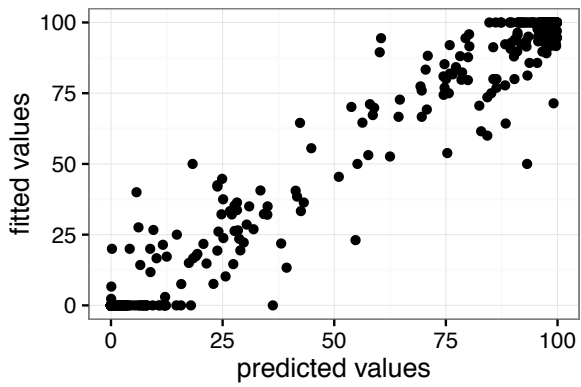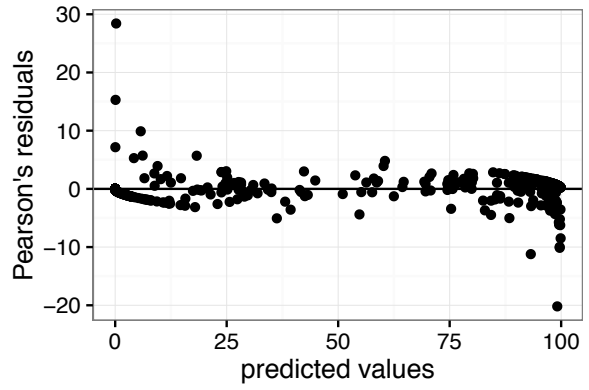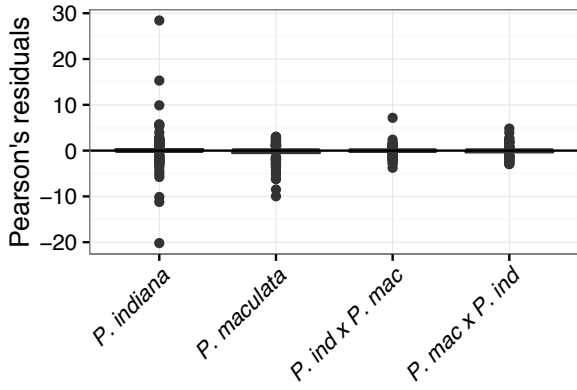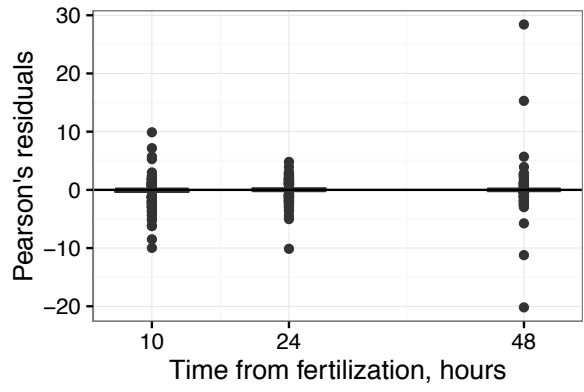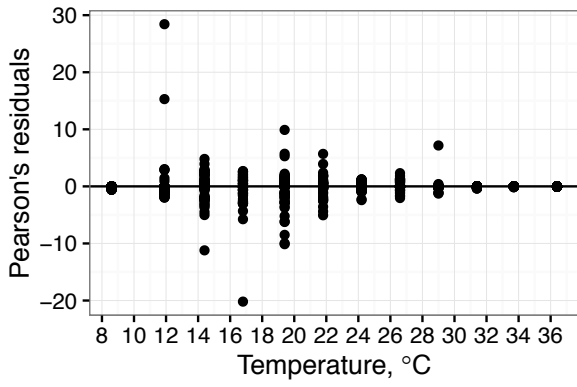
Pluteus
Family: binomial distribution.
Link: logit.
Formula: (plutei | other stages) ~ poly(temperature, 3)*poly(time, 2) + poly(time, 2):species + poly(temperature, 3):species + time:species + (1|vial ID)

Control: glmerControl(optimizer = "bobyqa")

AIC	BIC	logLik	deviance	df.resid
862.8	1004.8	-400.4	800.8	689

Scaled residuals:

Min	1Q	Median	3Q	Max
-14.4820	-0.0053	0.0000	0.0000	23.825

Random effects:

Groups	Name	Variance	Std.Dev.
vial ID	(Intercept)	0.3958	0.6291

Number of obs: 720, groups: replicates, 84

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-43.193	696.798	-0.004	0.996446
poly(Temp, 3)1	342.321	261.838	1.307	0.191085
poly(Temp, 3)2	-617.529	148.709	-4.153	3.29e-05 ***
poly(Temp, 3)3	-174.560	96.284	-1.813	0.069837
poly(time, 2)1	451.823	287417.404	0.002	0.998746
poly(time, 2)2	-442.085	229763.790	-0.002	0.998465
Sp P. maculata	-63.254	9696.802	-0.007	0.994795
Sp P. indiana (f) x P. maculata (m)	11.269	9696.796	0.001	0.999073
Sp P. maculata (f) x P. Indiana (m)	7.425	9696.796	0.001	0.999389
poly(Temp, 3)1:poly(time, 2)1	-22009.78	7518.185	-2.928	0.003417 **
poly(Temp, 3)2:poly(time, 2)1	-2092.295	4313.198	-0.485	0.627612
poly(Temp, 3)3:poly(time, 2)1	-5009.124	2621.491	-1.911	0.056031
poly(Temp, 3)1:poly(time, 2)2	1216.839	6030.311	0.202	0.840083
poly(Temp, 3)2:poly(time, 2)2	-3101.557	3359.839	-0.923	0.355941
poly(Temp, 3)3:poly(time, 2)2	-3260.387	2086.116	-1.563	0.118077
poly(time, 2)1:Sp *P. maculata*	206.816	287417.353	0.001	0.999426
poly(time, 2)2:Sp *P. maculata*	271.809	229763.749	0.001	0.999056
poly(time, 2)1: *Sp P. indiana (f) x P. maculata (m)* -	496.509	287417.352	-0.002	0.998622
poly(time, 2)2: *Sp P. indiana (f) x P. maculata (m)*	299.757	229763.749	0.001	0.998959
poly(time, 2)1: *Sp P. maculata (f) x P. Indiana (m)*	-407.251	287417.352	-0.001	0.998869
poly(time, 2)2: *Sp P. maculata (f) x P. Indiana (m)*	201.867	229763.749	0.001	0.999299
poly(Temp, 3)1:Sp *P. maculata*	3223.534	477.040	6.757	1.41e-11 ***
poly(Temp, 3)2:Sp *P. maculata*	-2364.591	327.105	-7.229	4.87e-13 ***
poly(Temp, 3)3:Sp *P. maculata*	929.763	168.027	5.533	3.14e-08 ***
poly(Temp, 3)1: *Sp P. indiana (f) x P. maculata (m)*	-360.128	119.513	-3.013	0.002584 **
poly(Temp, 3)2: *Sp P. indiana (f) x P. maculata (m)*	-126.544	51.947	-2.436	0.014850 *
poly(Temp, 3)3: *Sp P. indiana (f) x P. maculata (m)*	218.513	59.901	3.648	0.000264 ***
poly(Temp, 3)1: *Sp P. maculata (f) x P. Indiana (m)*	-566.798	99.774	-5.681	1.34e-08 ***
poly(Temp, 3)2: *Sp P. maculata (f) x P. Indiana (m)*	-323.213	61.660	-5.242	1.59e-07 ***
poly(Temp, 3)3: *Sp P. maculata (f) x P. Indiana (m)*	40.492	49.821	0.813	0.416359

Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ', 1
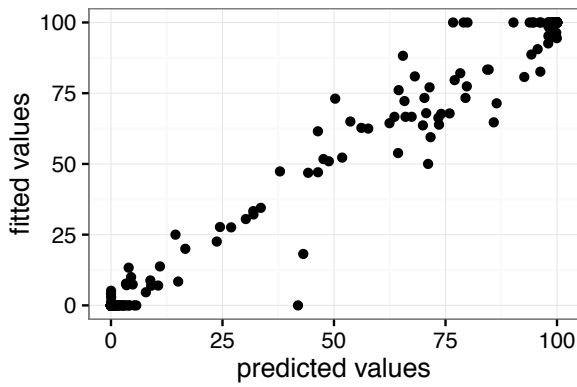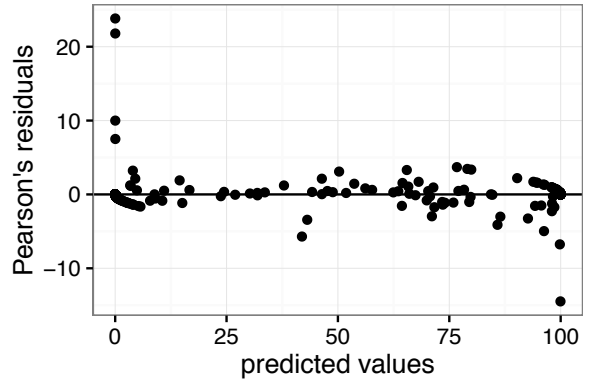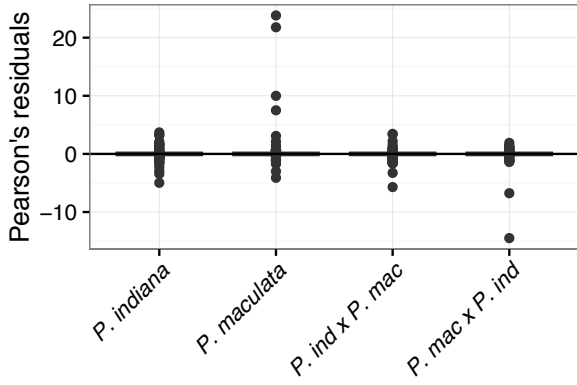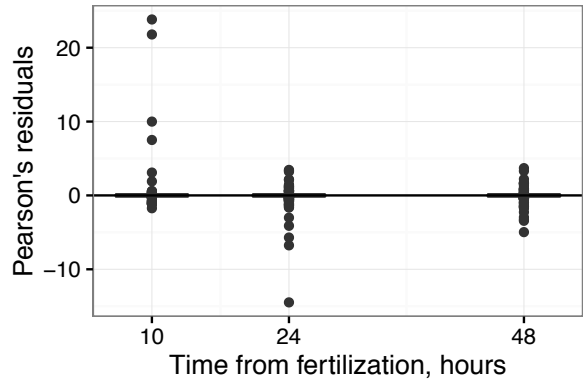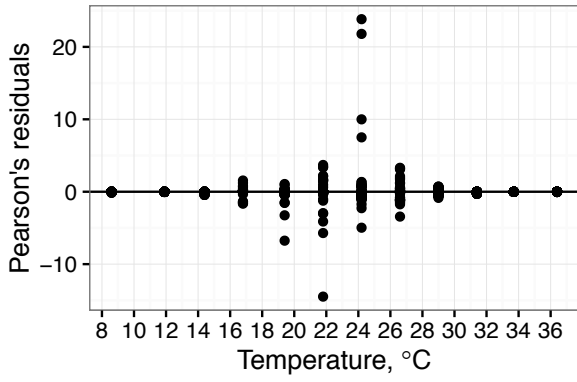
Supplement 4: generalized linear mixed model parameters and validations for the proportion of normally developed larvae for four *Pseudoboletia* progeny across a thermal gradient (12 temperatures between 8.6° and 36.4°C) at three times (10, 24 and 48 h).

Pseudoboletia indiana

Family: binomial distribution.
Link: logit.

Formula: (normal|abnormal) ~ poly(temperature, 3) * time + (1 | vial ID)

Control: glmerControl(optimizer = "bobyqa")

AIC	BIC	logLik	deviance	df.resid
1174.3	1205.3	-578.2	1156.3	222

Scaled residuals:

Min	1Q	Median	3Q	Max
-6.9938	-0.0799	0.0000	0.6093	9.4860

Random effects:

Groups Name	Variance	Std.Dev.
Vial ID (Intercept)	1.532	1.238

Number of obs: 231, groups: Reps, 77

Fixed effects:

	Estimate	*Std. Error*	*z value*	*Pr(>|z|)*
(Intercept)	-1.387e+00	1.041e-03	-1333	<2e-16 ***
poly(temperature, 3)1	-1.074e+02	1.040e-03	-103263	<2e-16 ***
poly(temperature, 3)2	-9.866e+01	1.040e-03	-94845	<2e-16 ***
poly(temperature, 3)3	-3.699e+01	1.040e-03	-35559	<2e-16 ***
time	-1.600e-01	1.016e-03	-157	<2e-16 ***
poly(temperature, 3)1:time	-2.497e+00	1.041e-03	-2399	<2e-16 ***
poly(temperature, 3)2:time	-1.766e+00	1.041e-03	1696	<2e-16 ***
poly(temperature, 3)3:time	1.867e-01	1.041e-03	179	<2e-16 ***

Signif. codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**
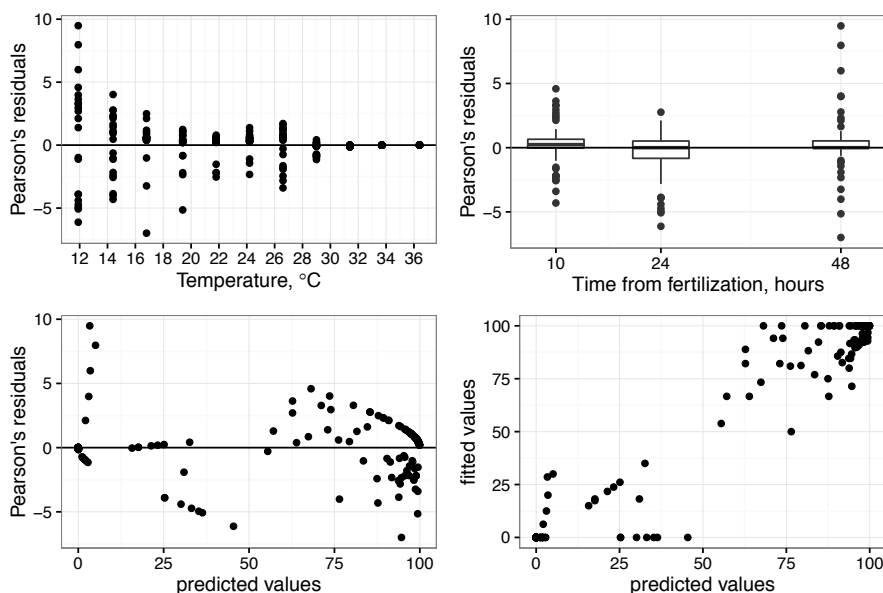
Pseudoboletia maculata

Family: binomial distribution.
Link: logit.

Formula: (normal|abnormal) ~ poly(temperature, 3) * time + (1 | vial ID)

Control: glmerControl(optimizer = "bobyqa")

AIC	BIC	logLik	deviance df.resid
674.2	705.2	-328.1	656.2 222

Scaled residuals:

Min	1Q	Median	3Q	Max
-7.6976	-0.0752	0.0004	0.2386	3.9767

Random effects:

Groups Name	Variance	Std.Dev.
vial ID (Intercept)	0.4263	0.653

Number of obs: 231, groups: Reps, 77

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-5.81174	0.75773	-7.670	1.72e-14 ***
poly(Temp, 3)1	-149.05913	19.52731	-7.633	2.29e-14 ***
poly(Temp, 3)2	-113.15699	18.85265	-6.002	1.95e-09 ***
poly(Temp, 3)3	-41.56252	8.67389	-4.792	1.65e-06 ***
time	0.08384	0.01873	4.476	7.62e-06 ***
poly(Temp, 3)1:time	-1.56474	0.60881	-2.570	0.0102 *
poly(Temp, 3)2:time	-4.13976	0.69728	-5.937	2.90e-09 ***
poly(Temp, 3)3:time	-1.51127	0.30364	-4.977	6.45e-07 ***

Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1
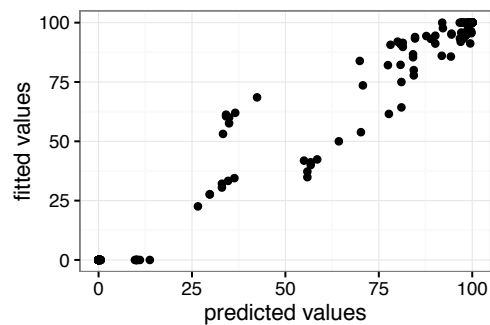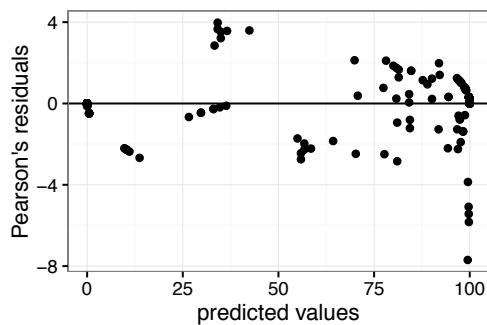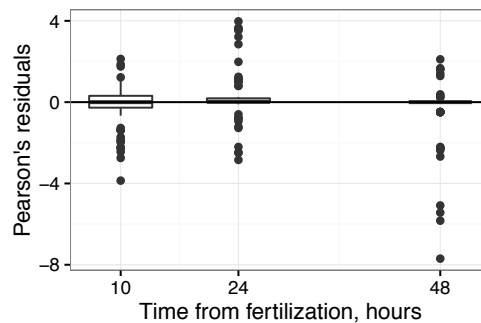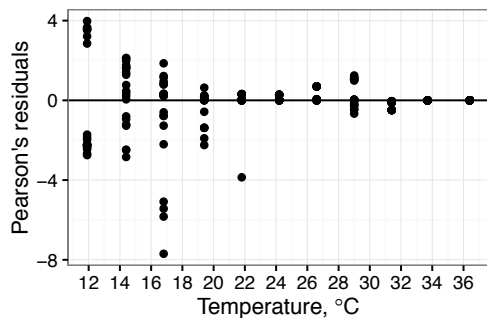
P. indiana (f) x P. maculata (m)

Family: binomial distribution.
Link: logit.

Formula: (normal|abnormal) ~ poly(temperature, 3) * time + (1 | vial ID)

Control: glmerControl(optimizer = "bobyqa")

	AIC	BIC	logLik	deviance	df.resid
	414.0	437.3	-198.0	396.0	90

Scaled residuals:

	Min	1Q	Median	3Q	Max
	-6.1887	-0.4059	0.0000	0.1724	2.6189

Random effects:

Groups Name	Variance	Std.Dev.
vial ID (Intercept)	0.5449	0.7382

Number of obs: 99, groups: Reps, 33

Fixed effects:

poly(Temp, 3)1	92.1313	20.8950	4.409	1.04e-05	***
poly(Temp, 3)2	46.3628	14.1517	3.276	0.00105	**
poly(Temp, 3)3	16.4769	6.0022	2.745	0.00605	**
time	-1.1946	0.1504	-7.942	1.98e-15	***
poly(Temp, 3)1:time	-13.9079	1.8906	-7.356	1.89e-13	***
poly(Temp, 3)2:time	-8.3985	1.1976	-7.013	2.34e-12	***
poly(Temp, 3)3:time	-1.9490	0.4126	-4.724	2.31e-06	***

Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1
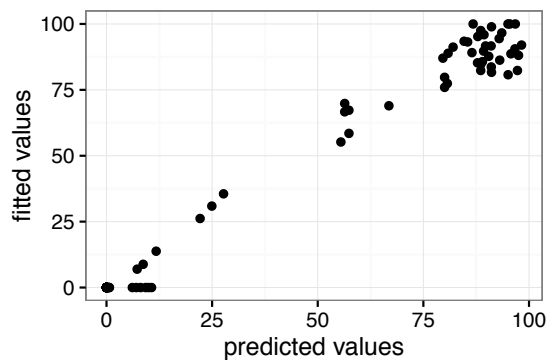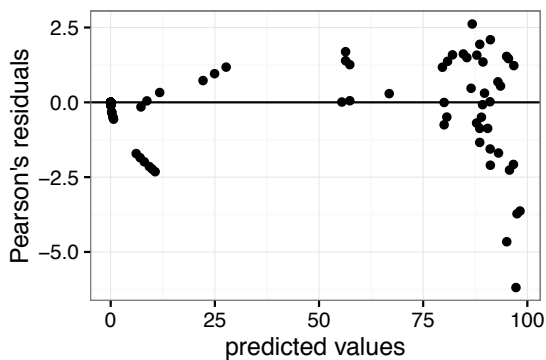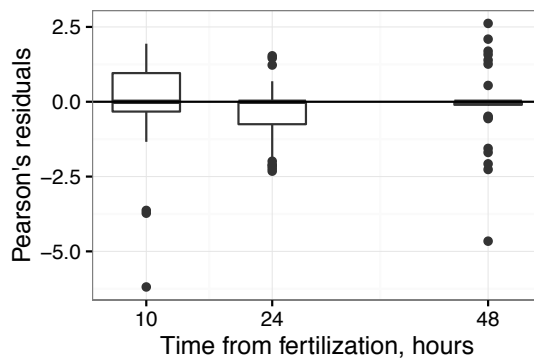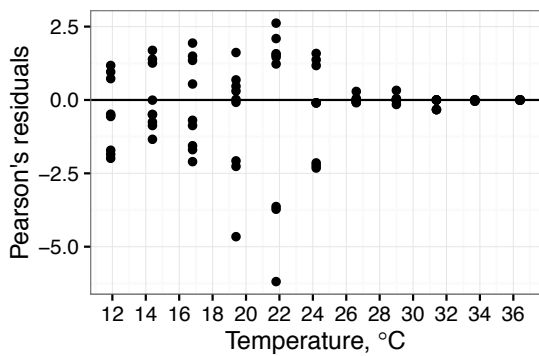
P. maculata (f) x P. indiana (m)

Family: binomial distribution.
Link: logit.

Formula: (normal|abnormal) ~ poly(temperature, 3) * time + (1 | vial ID)

Control: glmerControl(optimizer = "bobyqa")

AIC	BIC	logLik	deviance	df.resid
160.7	173.7	-75.4	150.7	94

Scaled residuals:

Min	1Q	Median	3Q	Max
-2.21820	-0.12798	0.00000	0.08357	2.89524

Random effects:

Groups Name	Variance	Std.Dev.
vial ID (Intercept)	7.164	2.677

Number of obs: 99, groups: Reps, 33

Fixed effects:

		Estimate	*Std. Error*	*z value*	*Pr(>|z|)*
(Intercept)		-28.494	6.965	-4.091	4.30e-05 ***
poly(Temp, 3)	1	-382.414	91.506	-4.179	2.93e-05 ***
poly(Temp, 3)	2	-288.802	66.997	-4.311	1.63e-05 ***
poly(Temp, 3)	3	-59.811	26.807	2.231	0.0257 *

Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1