

Author-supplied pdf for *American Naturalist* article:

**Queen Longevity and Fecundity Affect Conflict with Workers over Resource Inheritance in a Social Insect**

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### 1. Supplementary results for experiment (a), i.e. experiment to determine effect of queen longevity on workers' reproductive success

**Table S1a.** Effect of queen longevity on queen sexual production (figure S1). Parameter estimates and hypothesis tests of all effects included in the 'best' LMM according to model selection using AICc (table S1b). Term, variable name; Estimate, parameter estimate; SE, standard error of estimate;  $t$ , test statistic;  $p$ , p-value.

Term	Estimate	SE	$t$	$p$
(Intercept)	1.57	101.62	0.02	0.99
treatment	85.12	145.44	0.59	0.56
longevity	8.65	1.66	5.20	< 0.0001
cohort	874.47	2952.42	0.30	0.77
w.rate	-1.91	13.64	-0.14	0.89
treatment:longevity	-4.65	3.55	-1.31	0.19
treatment:cohort	-445.24	3199.42	-0.14	0.89
longevity:cohort	-23.10	71.07	-0.32	0.75
treatment:w.rate	32.22	31.00	1.04	0.30
longevity:w.rate	-0.26	0.26	-1.00	0.32
cohort:w.rate	-333.58	1144.56	-0.29	0.77
treatment: longevity: cohort	11.58	74.52	0.16	0.88
treatment:longevity:w.rate	0.09	0.82	0.11	0.91
longevity:cohort:w.rate	6.94	27.10	0.26	0.80

Cohort: cohort 1 or 2 (see main text, 'Methods'); longevity: time before queen removal or death, dated from the switch point; w.rate: mean rate of worker production; treatment: queen removed or died naturally. Queen sexual production was measured as the total number of males and young queens produced by a queen during her tenure of the colony.

**Table S1b.** Model selection summary of 17 highest ranked models from candidate LMMs using Akaike's Information Criteria for models to estimate effect of queen longevity on queen sexual production; all possible combinations of variables and interactions were compared (167 candidate models) and candidate models within 10 AIC points of the best model are shown. Effects of variables (as defined in table S1a): parameter estimates are given for covariates; + denotes a factor is included in that model; NA, variable not included; d.f., model degrees of freedom; logLik, Log Likelihood; AICc, second-order Akaike's Information Criterion; delta, difference in AICc relative to the best model; weight, sum of Akaike weights. In this and all similar tables, interaction terms not included in any of the shown models are not included.

(Intercept)	cohort	longevity	treatment	w.rate	cohort: longevity	cohort: treatment	cohort: w.rate	longevity: treatment	longevity: w.rate	treatment: w.rate	cohort: longevity: treatment	cohort: longevity: w.rate	longevity: treatment: w.rate	d.f.	logLik	AICc	delta	weight
1.57	+	8.65	85.12	-1.91	+	+	+	-4.65	-0.26	32.22	+	+	0.09	18	-291.86	637.26	0.00	0.31
62.27	+	7.29	39.16	-13.90	+	+	+	-3.74	NA	38.22	+	NA	NA	14	-300.07	637.91	0.65	0.22
3.85	+	8.60	75.13	-2.36	+	+	+	-4.34	-0.25	35.37	+	+	NA	16	-296.83	638.93	1.68	0.13
67.10	+	7.18	3.30	-13.81	+	+	+	-2.56	NA	36.33	NA	NA	NA	13	-303.08	640.43	3.17	0.06
85.08	+	6.77	-70.24	-13.49	+	+	+	NA	NA	31.69	NA	NA	NA	12	-305.20	641.33	4.08	0.04
14.87	+	8.35	32.07	-3.26	+	+	+	-2.96	-0.23	33.46	NA	+	NA	15	-300.01	641.45	4.20	0.04
3.29	+	8.61	90.15	-2.25	+	+	+	-4.78	-0.25	35.61	+	NA	NA	15	-300.03	641.49	4.23	0.04
78.83	+	6.91	14.25	-13.60	NA	+	+	-3.09	NA	37.48	NA	NA	NA	12	-305.59	642.12	4.86	0.03
52.04	+	7.51	-58.54	-5.97	+	+	+	NA	-0.16	29.40	NA	+	NA	14	-302.41	642.59	5.33	0.02
99.74	+	6.43	-77.87	-13.22	NA	+	+	NA	NA	32.43	NA	NA	NA	11	-307.92	643.59	6.33	0.01
1.72	+	8.64	98.66	-1.94	+	+	+	-5.04	-0.26	33.70	+	NA	0.06	16	-299.33	643.92	6.67	0.01
13.76	+	8.37	38.50	-3.02	+	+	+	-3.16	-0.23	31.77	NA	+	0.05	16	-299.33	643.92	6.67	0.01
14.30	+	8.36	36.75	-3.26	+	+	+	-3.09	-0.23	33.47	NA	NA	NA	14	-303.13	644.03	6.78	0.01
51.84	+	7.51	-57.87	-6.00	+	+	+	NA	-0.16	29.17	NA	NA	NA	13	-305.50	645.26	8.01	0.01
63.24	+	7.29	39.42	-14.16	+	+	+	-3.84	NA	39.05	+	NA	NA	13	-305.62	645.51	8.26	0.00
39.21	+	7.80	44.64	-5.38	NA	+	+	-3.66	-0.18	35.49	NA	NA	NA	13	-305.84	645.94	8.69	0.00
12.84	+	8.39	44.81	-2.95	+	+	+	-3.34	-0.24	31.30	NA	NA	0.06	15	-302.46	646.34	9.08	0.00

**Table S2a.** Effect of queen longevity on worker reproductive success (measured as the proportion of total males produced by workers; figure 2). Parameter estimates and hypothesis tests of all effects included in the 'best' LMM according to model selection using AICc (table S2b). Variables and column headings are as in table S1a.

Term	Estimate	SE	<i>t</i>	<i>p</i>
(Intercept)	0.841	0.061	13.85	< 0.0001
longevity	-0.012912	0.001351	9.56	< 0.0001

**Table S2b.** Model selection summary of 5 highest ranked models from 167 candidate LMMs using Akaike's Information Criteria for models to estimate effect of queen longevity on worker reproductive success (measured as the proportion of total males produced by workers); all possible combinations of variables and interactions were compared (167 candidate models), and candidate models within 10 AIC points of the best model are shown. Effects of variables (as defined in table S1a). Column headings and other abbreviations are as in table S1b.

(Intercept)	cohort	longevity	treatment	w.rate	d.f.	logLik	AICc	delta	weight
0.84	NA	-0.01	NA	NA	4	7.91	-7.06	0.00	0.77
0.93	NA	-0.01	-0.10	NA	5	7.58	-4.01	3.05	0.17
0.84	+	-0.01	NA	NA	5	6.18	-1.20	5.85	0.04
0.93	+	-0.01	-0.10	NA	6	5.80	2.05	9.10	0.01
0.85	NA	-0.01	NA	0.00	5	4.20	2.75	9.81	0.01

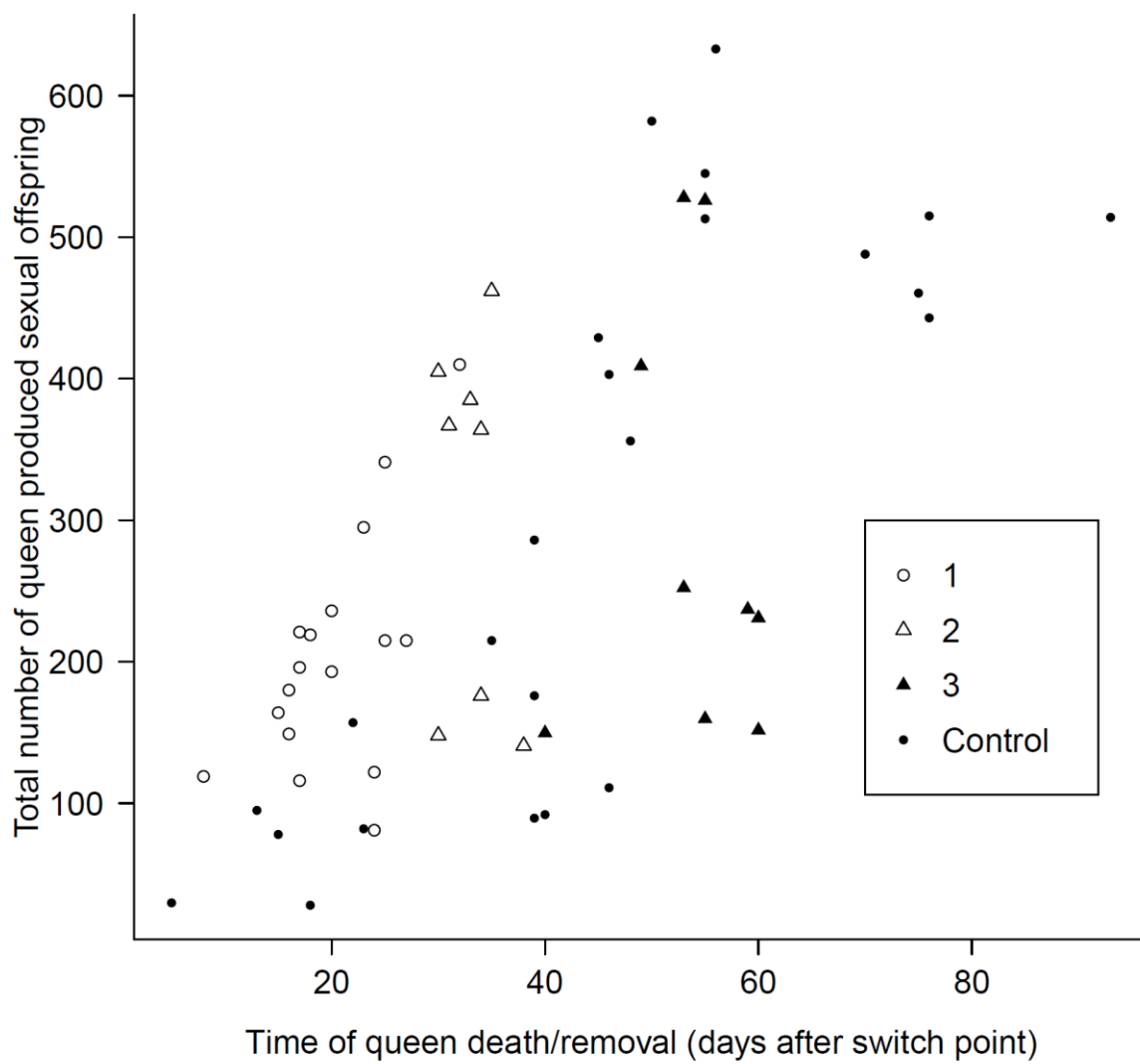
**Table S2c.** Effect of queen longevity on worker reproductive success (measured as the per-worker proportion of total males produced by workers). Parameter estimates and hypothesis tests of all effects included in the 'best' LMM according to model selection using AICc (table S2d). Variables and column headings are as in table S1a.

Term	Estimate	SE	<i>t</i>	<i>p</i>
(Intercept)	5.15	0.56	9.13	< 0.0001
cohort	-2.74	0.72	-3.80	0.0001
treatment	-1.19	0.43	-2.80	0.0051
longevity	-0.06	0.01	-6.23	< 0.0001
cohort:treatment	2.45	0.99	2.48	0.0132

**Table S2d.** Model selection summary of 9 highest ranked models from 167 candidate LMMs using Akaike's Information Criteria for models to estimate effect of queen longevity on worker reproductive success (measured as the per-worker proportion of total males produced by workers). Effects of variables (as defined in table S1a). Column headings and other abbreviations are as in table S1b.

(Intercept)	cohort	longevity	treatment	w.rate	cohort: longevity	cohort: treatment	cohort: w.rate	longevity: treatment	d.f.	logLik	AICc	delta	weight
5.15	+	-0.06	-1.19	NA	NA	+	NA	NA	7	-98.13	212.50	0.00	0.67
5.43	+	-0.07	-1.30	NA	+	+	NA	NA	8	-98.93	216.80	4.30	0.08
4.47	+	-0.05	-0.64	NA	NA	NA	NA	NA	6	-101.99	217.63	5.13	0.05
3.96	+	-0.05	NA	NA	NA	NA	NA	NA	5	-103.27	217.70	5.20	0.05
5.21	+	-0.07	-1.08	NA	+	NA	NA	NA	7	-100.88	218.01	5.51	0.04
5.31	+	-0.06	-1.22	-0.05	NA	+	NA	NA	8	-99.65	218.23	5.73	0.04
4.39	+	-0.06	NA	NA	+	NA	NA	NA	6	-103.31	220.27	7.77	0.01
5.37	+	-0.07	-1.68	NA	NA	+	NA	0.01	8	-100.82	220.57	8.07	0.01
5.30	+	-0.06	-1.22	-0.05	NA	+	+	NA	9	-99.44	220.62	8.12	0.01

**Figure S1.** Relationship of queen reproductive success (total number of queens' sexual offspring) in *Bombus terrestris* colonies as a function of queen longevity/tenure (first experiment). Queens were removed at a mean 20, 33 and 54 days after the switch point in Groups 1 ( $n = 17$ , white circles), 2 ( $n = 8$ , white triangles) and 3 ( $n = 9$ , black triangles), respectively. Control group ( $n = 24$ , black circles): queen allowed to die naturally. For statistical analysis, see tables S1a, S1b.



## 2. Supplementary results for experiment (b), i.e. experiment to determine queen fecundity as a function of time

**Table S3a.** Effect of time since the switch point on queen fecundity (measured as the frequency of queen egg-laying events; figure 3a). Parameter estimates and hypothesis tests of all effects included in the 'best' GLMM according to model selection using AICc (table S3b). Term, variable name; Estimate, parameter estimate; SE, standard error of estimate; Z value, test statistic;  $p$ , p-value.

Term	Estimate	SE	Z value	$p$
(Intercept)	0.85064	0.55488	1.533	0.125
time	-0.02744	0.01409	1.948	0.051

**Table S3b.** Model selection summary of 5 candidate binomial GLMMs using Akaike's Information Criteria for models to estimate effect of time since the switch point on queen fecundity (measured as the frequency of queen egg-laying events). Effects of variables (defined below). Column headings and other abbreviations are as in table S1b.

(Intercept)	time	male.eclosed	time:male.eclosed	d.f.	logLik	AICc	delta	weight
0.85	-0.03	NA	NA	3	-42.14	90.67	0.00	0.45
0.88	-0.04	0.49	NA	4	-41.95	92.59	1.92	0.17
-0.13	NA	NA	NA	2	-44.24	92.67	2.00	0.16
2.23	-0.12	-1.29	0.09	5	-41.20	93.43	2.76	0.11
0.26	NA	-0.61	NA	3	-43.56	93.53	2.86	0.11

Time: time since switch point in days; male.eclosed: binary factor coding whether males had begun to eclose or not.



**Table S4a.** Effect of time since the switch point on queen fecundity (measured as the number of eggs laid per egg-laying event; figure 3b). Parameter estimates and hypothesis tests of all effects included in the 'best' GLMM according to model selection using AICc (table S4b). Variables are as below and column headings are as in table S3a. The Poisson GLMM used a log link function and was not subject to significant overdispersion (ratio of residual deviance to residual degrees of freedom = 0.78,  $\chi^2 = 7.01$ , d.f. = 9,  $p = 0.64$ ).

Term	Estimate	SE	Z value	<i>p</i>
(Intercept)	2.60	0.19	13.54	< 0.0001
time	-0.03	0.00653	-3.85	0.0001
cells	0.67	0.12	5.65	< 0.0001

Cells: number of egg cells built; time: time since switch point in days.

**Table S4b.** Model selection summary of 5 candidate Poisson GLMMs using Akaike's Information Criteria for models to estimate effect of time since the switch point on queen fecundity (measured as the number of eggs laid per egg-laying event). Effects of variables (table S4a). Column headings and other abbreviations are as in table S1b.

(Intercept)	cells	time	cells:time	d.f.	logLik	AICc	delta	weight
2.60	0.68	-0.03	NA	4	-35.82	84.63	0.00	0.86
2.99	0.41	-0.05	0.02	5	-34.92	88.40	3.77	0.13
2.40	0.53	NA	NA	3	-42.84	94.34	9.71	0.01
3.16	NA	NA	NA	2	-52.21	109.62	24.99	0.00
3.35	NA	-0.01	NA	3	-50.75	110.17	25.54	0.00

### **3. Supplementary results for experiment (c), i.e. experiment to determine effect of queen fecundity on worker-to-queen aggression**

#### *Effect of queen activity rate*

During the egg-manipulation period, there was no significant difference between treatment and control groups either in distance travelled by the queen per minute (d.f. = 1,  $p = 0.56$ ) or in encounter rate of queens with workers (d.f. = 1,  $p = 0.18$ ). These findings showed that greater rates of worker aggression (wing buzzing) towards queens in treatment colonies during egg manipulation (see main text, 'Results') did not stem from differences in queen activity.

**Table S5a.** Effect of treatment on rate of worker aggression to queen (measured as non-contact aggression; figure 4). Parameter estimates and hypothesis tests of all effects included in the 'best' LMM according to model selection using AICc (table S5b). Variables are as below and column headings are as in table S3a.

Term	Estimate	SE	t value	<i>p</i>
(Intercept)	1.82	0.46	3.93	< 0.001
treatment	0.25	0.65	0.39	0.70
time period – pre-CP	-0.82	0.48	-1.69	0.09
time period – start	-0.56	0.67	-0.85	0.40
treatment:time period – pre-CP	2.05	0.68	2.99	0.003
treatment:time period – start	0.71	1.01	0.70	0.48

Time period: start, at start of egg-manipulation period (period of egg removals); pre-CP (near end of egg-manipulation period, i.e. shortly before the competition point); or post-CP (following the egg-manipulation period, i.e. after competition point); for full details of time period, see main text, 'Statistical analysis'; treatment: queen (a) eggs removed before competition point or (b) removed and returned;  $n = 369$  wing buzzes from 24 colonies. There was no effect of number of workers on rate of worker aggression to queen (number of workers was not retained in the best model; table S5b).

**Table S5b.** Model selection summary of 10 candidate LMMs using Akaike's Information Criteria for models to estimate effect of treatment on rate of worker aggression to queen (measured as non-contact aggression). Effects of variables (as defined in table S5a and below). Further column headings and other abbreviations are as in table S1b.

(Intercept)	treatment	time period	workers	treatment: time period	treatment: workers	time period: workers	treatment:time period:workers	d.f.	logLik	AICc	delta	weight
1.82	+	+	NA	+	NA	NA	NA	9	-544.30	1107.40	0.00	0.81
1.44	+	NA	NA	NA	NA	NA	NA	5	-550.56	1111.38	3.98	0.11
1.98	NA	NA	NA	NA	NA	NA	NA	4	-552.67	1113.51	6.11	0.04
1.39	+	+	NA	NA	NA	NA	NA	7	-550.15	1114.80	7.40	0.02
2.66	+	+	-0.009	+	NA	NA	NA	10	-547.61	1116.21	8.82	0.01
1.93	NA	+	NA	NA	NA	NA	NA	6	-552.22	1116.80	9.41	0.01
1.69	+	NA	-0.003	NA	NA	NA	NA	6	-554.67	1121.71	14.31	0.00
2.27	NA	NA	-0.004	NA	NA	NA	NA	5	-556.68	1123.63	16.23	0.00
2.13	+	+	-0.008	NA	NA	NA	NA	8	-553.59	1123.82	16.42	0.00
2.35	+	+	-0.006	+	+	NA	NA	11	-550.65	1124.50	17.10	0.00

Workers: number of workers at time of measurements.

**Table S5c.** Effect of treatment on rate of worker aggression to queen (measured as contact aggression). Parameter estimates and hypothesis tests of all effects included in the 'best' LMM that included 'treatment' (delta = 3.92) according to model selection using AICc (table S5d). Variables are as in table S5a and column headings are as in table S3a.

Term	Estimate	SE	t value	<i>p</i>
(Intercept)	0.34	0.11	3.006	0.0059
treatment	0.06	0.15	0.429	0.67

**Table S5d.** Model selection summary of 10 candidate LMMs using Akaike's Information Criteria for models to estimate effect of treatment on rate of worker aggression to queen (measured as contact aggression). Effects of variables (as defined in tables S5a and S5b). Further column headings and other abbreviations are as in table S1b.

(Intercept)	treatment	time period	workers	treatment: time period	treatment: workers	d.f.	logLik	AICc	delta	weight
0.376	NA	NA	NA	NA	NA	4	-326.5	661.09	0.00	0.84
0.345	+	NA	NA	NA	NA	5	-327.4	665.01	3.92	0.12
0.407	NA	+	NA	NA	NA	6	-327.6	667.63	6.54	0.03
0.376	+	+	NA	NA	NA	7	-328.6	671.60	10.51	0.00
0.361	NA	NA	0.00	+	NA	5	-331.6	673.51	12.42	0.00
0.369	+	+	NA	NA	NA	9	-328.9	676.69	15.60	0.00
0.317	+	NA	0.00	NA	NA	6	-332.5	677.40	16.31	0.00
0.472	NA	+	-0.00	NA	NA	7	-332.5	679.60	18.50	0.00
0.428	+	+	-0.00	NA	NA	8	-333.5	683.56	22.47	0.00
0.363	+	NA	-0.00	NA	+	7	-337.0	688.52	27.43	0.00

**Tables S6a-d.** Summary data on the association between *Bombus terrestris* workers' aggressive behavior to queens (contact and non-contact pooled) and worker reproductive status in the third experiment. (a) Workers' aggressive behavior occurring during any experimental period (i.e. egg-manipulation period or post-manipulation period), reproductive status measured as ovary activation; (b) Workers' aggressive behavior occurring during the egg-manipulation period only, reproductive status measured as ovary activation; (c) Workers' aggressive behavior occurring during any experimental period, reproductive status measured as observed egg-laying in the post-manipulation period; (d) Workers' aggressive behavior occurring during the egg-manipulation period only, reproductive status measured as observed egg-laying in the post-manipulation period. Treatment and control colonies are pooled (total  $n = 24$  colonies) in all cases; all cell entries are numbers of workers. Ovary activation was defined as a worker having at least one oocyte of length  $> 1.92$  mm. Statistical analyses were performed on the per-colony numbers of workers of the different classes.

(a) Any period	Ovary-activated	Not ovary-activated	Totals
Aggressive worker	238	57	295
Non-aggressive worker	189	158	347
Totals	427	215	642

(b) Egg-manipulation period	Ovary-activated	Not ovary-activated	Totals
Aggressive worker	236	49	285
Non-aggressive worker	186	113	299
Totals	422	162	584

(c) Any period	Observed egg-laying	Not observed egg-laying	Totals
Aggressive worker	89	206	295
Non-aggressive worker	57	290	347
Totals	146	496	642

(d) Egg-manipulation period	Observed egg-laying	Not observed egg-laying	Totals
Aggressive worker	89	196	285
Non-aggressive worker	56	243	299
Totals	145	439	584

**Table S7a.** Association between workers' aggression to queens (measured over any experimental period) and worker reproductive status (measured as ovary activation). Parameter estimates and hypothesis tests of all effects included in the 'best' GLMM according to model selection using AICc (table S7b). Variables are as defined below and column headings are as in table S3a.

Term	Estimate	SE	Z value	<i>p</i>
(Intercept)	0.58	0.20	2.89	0.004
treatment	-0.75	0.26	-2.82	0.005
agg	1.35	0.19	6.96	< 0.0001

$n = 642$  workers from 24 colonies (table S6a); agg, worker aggression to queens; treatment: queen eggs (a) removed before competition point or (b) removed and returned; Workers from treatment colonies were significantly less likely to exhibit ovary activation ( $p = 0.005$ ), but the relationship between worker aggression to queens and ovary activation did not differ between treatment and control colonies (agg:treatment interaction was not retained in the best model; table S7b).

**Table S7b.** Model selection summary of all candidate binomial GLMMs using Akaike's Information Criteria for models to estimate association between workers' aggression to queens (measured over any experimental period) and worker reproductive status (measured as ovary activation). Effects of variables (as defined in table S7a). Further column headings and other abbreviations are as in table S1b.

(Intercept)	agg	treatment	agg:treat- ment	d.f.	logLik	AICc	delta	weight
0.58	1.35	+	NA	4	-374.89	757.85	0.00	0.68
0.54	1.47	+	+	5	-374.76	759.61	1.76	0.28
0.21	1.31	NA	NA	3	-378.87	763.78	5.94	0.04
1.04	NA	+	NA	3	-401.42	808.88	51.03	0.00
0.74	NA	NA	NA	2	-404.00	812.02	54.17	0.00



**Table S7c.** Association between workers' aggression to queens (measured over the egg-manipulation period only) and worker reproductive status (measured as ovary activation). Parameter estimates and hypothesis tests of all effects included in the 'best' GLMM according to model selection using AICc (table S7d). Variables are as in table S7a and column headings are as in table S3a.

Term	Estimate	SE	Z value	<i>p</i>
(Intercept)	0.99	0.23	4.38	0.0001
treatment	-0.88	0.29	-3.01	0.003
agg	1.20	0.21	5.70	< 0.0001

$n = 584$  workers from 24 colonies (table S6b). Workers from treatment colonies were significantly less likely to exhibit ovary activation ( $p = 0.003$ ), but the relationship between worker aggression to queens and ovary activation did not differ between treatment and control colonies (agg:treatment interaction was not retained in the best model; table S7d).

**Table S7d.** Model selection summary of all candidate binomial GLMMs using Akaike's Information Criteria for models to estimate association between workers' aggression to queens (measured over the egg-manipulation period only) and worker reproductive status (measured as ovary activation). Effects of variables (as defined in table S7a). Further column headings and other abbreviations are as in table S1b.

(Intercept)	agg	treatment	agg:treatment	d.f.	logLik	AICc	delta	weight
0.99	1.20	+	NA	4	-318.82	645.72	0.00	0.71
0.97	1.26	+	+	5	-318.79	647.69	1.97	0.27
0.57	1.16	NA	NA	3	-323.29	652.62	6.90	0.02
1.42	NA	+	NA	3	-336.29	678.61	32.90	0.00
1.05	NA	NA	NA	2	-339.56	683.13	37.42	0.00

**Table S7e.** Association between workers' aggression to queens (measured over any experimental period) and worker reproductive status (measured as observed egg-laying). Parameter estimates and hypothesis tests of all effects included in the 'best' GLMM according to model selection using AICc (table S7f). Variables are as in table S7a and column headings are as in table S3a.

Term	Estimate	SE	Z value	<i>p</i>
(Intercept)	-1.70	0.18	-9.30	< 0.0001
agg	0.22457	0.08014	2.802	0.0006

$n = 642$  workers from 24 colonies (table S6c). The proportion of workers that were egg-layers did not differ between treatment and control colonies (the effect of treatment was not retained in the best model; table S7f).

**Table S7f.** Model selection summary of all candidate binomial GLMMs using Akaike's Information Criteria for models to estimate association between workers' aggression to queens (measured over any experimental period) and worker reproductive status (measured as observed egg-laying). Effects of variables (as defined in table S7a). Further column headings and other abbreviations are as in table S1b.

(Intercept)	agg	treatment	agg:treat- ment	d.f.	logLik	AICc	delta	weight
-1.70	0.80	NA	NA	3	-332.23	670.50	0.00	0.49
-1.54	0.81	+	NA	4	-331.53	671.12	0.62	0.36
-1.48	0.71	+	+	5	-331.37	672.82	2.32	0.15
-1.29	NA	NA	NA	2	-340.41	684.84	14.34	0.00
-1.16	NA	+	NA	3	-339.99	686.02	15.52	0.00

**Table S7g.** Association between workers' aggression to queens (measured over the egg-manipulation period only) and worker reproductive status (measured as observed egg-laying). Parameter estimates and hypothesis tests of all effects included in the 'best' GLMM according to model selection using AICc (table S7h). Variables are as in table S7a and column headings are as in table S3a.

Term	Estimate	SE	Z value	<i>p</i>
(Intercept)	-1.53	0.19	-8.13	< 0.0001
agg	0.69	0.20	3.40	0.0006

*n* = 584 workers from 24 colonies (table S6d). The proportion of workers that were egg-layers did not differ between treatment and control colonies (the effect of treatment was not retained in the best model; table S7h).

**Table S7h.** Model selection summary of all candidate binomial GLMMs using Akaike's Information Criteria for models to estimate association between workers' aggression to queens (measured over the egg-manipulation period only) and worker reproductive status (measured as observed egg laying). Effects of variables (as defined in table S7a). Further column headings and other abbreviations are as in table S1b.

(Intercept)	agg	treatment	agg:treatment	d.f.	logLik	AICc	delta	weight
-1.53	0.69	NA	NA	3	-317.63	641.29	0.00	0.49
-1.37	0.71	+	NA	4	-316.96	641.98	0.69	0.35
-1.31	0.58	+	+	5	-316.74	643.59	2.30	0.16
-1.17	NA	NA	NA	2	-323.48	650.98	9.68	0.00
-1.04	NA	+	NA	3	-323.07	652.18	10.89	0.00

**Table S8a.** Effect of treatment on queen egg-laying rate as a function of time (figure S2). Parameter estimates and hypothesis tests of all effects included in the 'best' GLMM according to model selection using AICc (table S8b). Variables are as below and column headings are as in table S3a.

Term	Estimate	SE	Z value	<i>p</i>
(Intercept)	1.70	0.14	12.19	< 0.0001
treatment	1.39	0.20	7.10	< 0.0001
time	-0.01	0.003	-5.57	< 0.0001
time:treatment	0.06	0.004	15.78	< 0.0001

Time: time relative to competition point; treatment: queen (a) eggs removed before competition point or (b) removed and returned.

**Table S8b.** Model selection summary of all candidate Poisson GLMMs using Akaike's Information Criteria for models to estimate effect of treatment on queen egg-laying rate as a function of time. Effects of variables (as defined in table S8a). Further column headings and other abbreviations are as in table S1b.

(Intercept)	time	treatment	time:treatment	d.f.	logLik	AICc	delta	weight
1.70	-0.01	+	+	5	-2707.06	5424.23	0.00	1.00
2.04	0.02	+	NA	4	-2832.91	5673.91	249.67	0.00
2.41	0.02	NA	NA	3	-2838.54	5683.13	258.89	0.00
1.86	NA	+	NA	3	-2872.93	5751.91	327.68	0.00
2.24	NA	NA	NA	2	-2878.06	5760.14	335.91	0.00

**Figure S2.** Egg-laying rate of *Bombus terrestris* queens (mean number of eggs laid per day) as a function of time during the egg-manipulation period (third experiment). Treatment: queens' eggs removed before competition point; Control: queens' eggs removed and returned. Data are shown from 22 days before the competition point (when  $n = 6$  control and  $n = 5$  treatment queens were laying) up to the competition point (when all  $n = 10$  queens for both treatment and control were laying). For illustrative purposes, separate fitted lines (from illustrative linear regressions) for each condition (solid line = treatment, dashed line = control) are plotted. For statistical analysis, see tables S8a, S8b.

