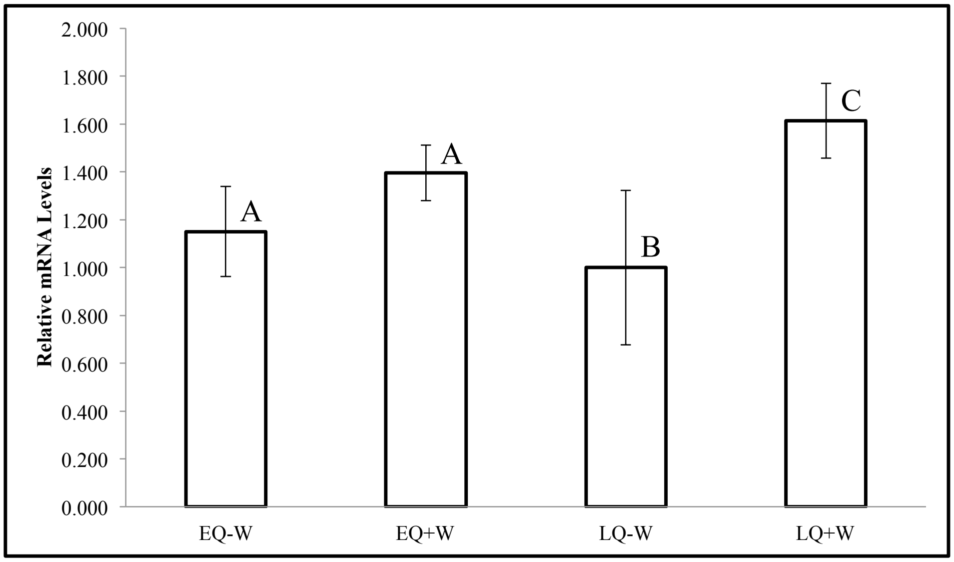
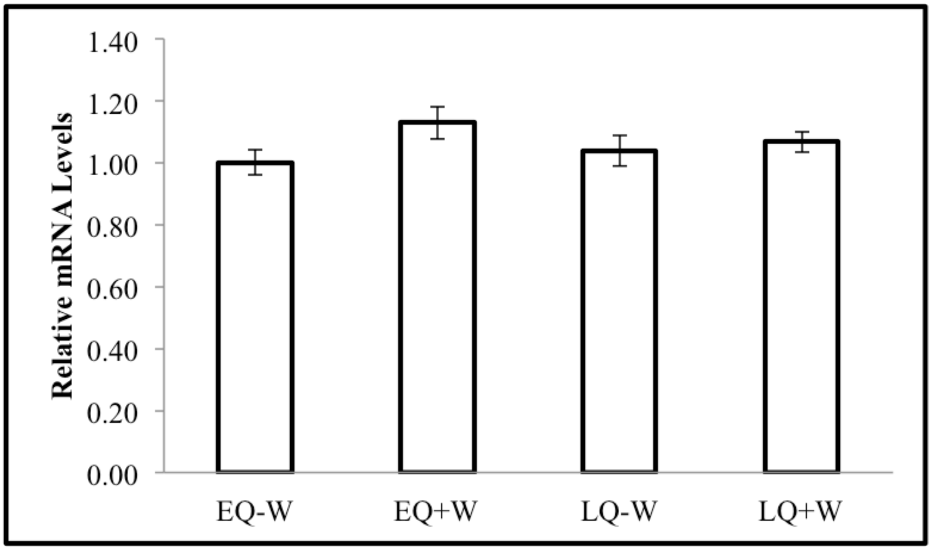
Figure S1. qRT-PCR results for *arrestin-2*.



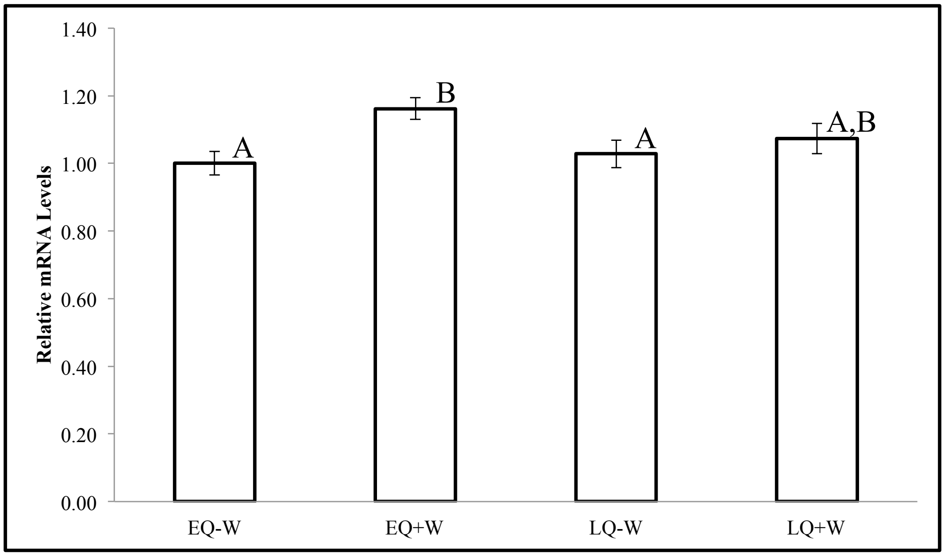
Bars represent means $^{+}/_{-}$ SE. qRT-PCR results: ANOVA, $P_{\text{stage(df=1)}} > 0.05$, $P_{\text{workers(df=1)}} < 0.01$, $P_{\text{stageXworkers(df=1)}} > 0.05$; pairwise tests, significant ($P < 0.01$) in late queens with vs. without workers. Microarray results: ANOVA, significant (FDR-corrected $P < 0.05$) for main effect of workers; pairwise tests: significant (FDR-corrected $P < 0.05$) in late queens with vs. without workers (other tests N.S.).

Figure S2. qRT-PCR results for *TBC1 domain family, member 10b*.



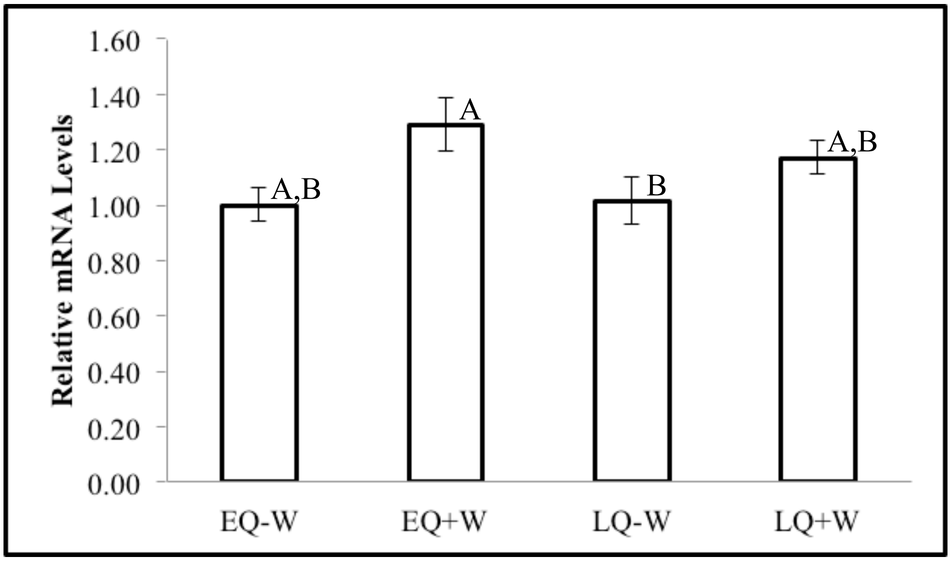
Bars represent means $^{+}/_{-}$ SE. qRT-PCR results: ANOVA, $P_{\text{stage(df=1)}} > 0.05$, $P_{\text{workers(df=1)}} = 0.07$, $P_{\text{stageXworkers(df=1)}} > 0.05$; pairwise tests, N.S. Microarray results: ANOVA, significant (FDR-corrected $P < 0.05$) for main effects of stage of queen, workers, and interaction; pairwise tests, significant (FDR-corrected $P < 0.05$) in late queens with vs. without workers and in queens without workers, late vs. early stage (other tests N.S.).

Figure S3. qRT-PCR results for *inositol 1,4,5,-tris-phosphate receptor*



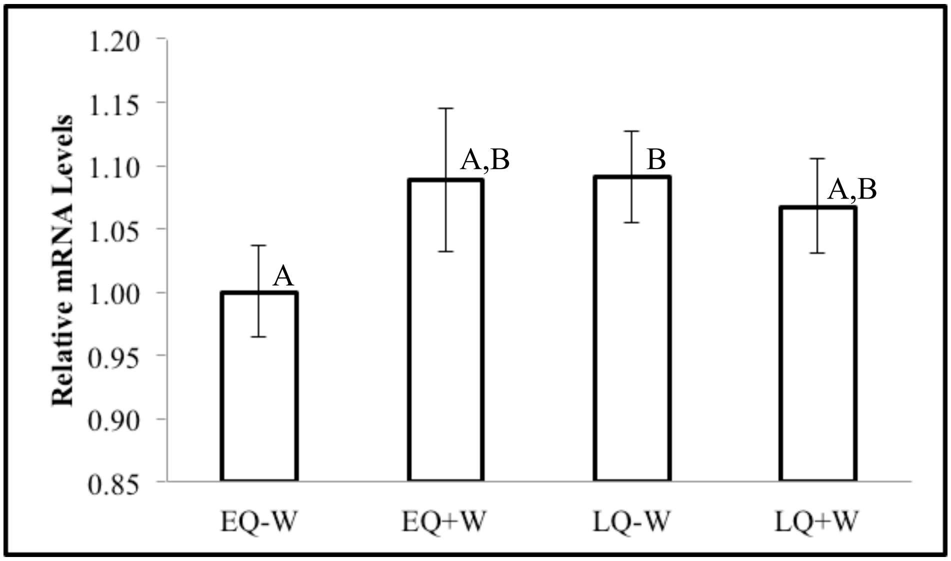
Bars represent means $^{+}/_{-}$ SE. qRT-PCR results: ANOVA, $P_{\text{stage(df=1)}} > 0.05$, $P_{\text{workers(df=1)}} < 0.01$, $P_{\text{stageXworkers(df=1)}} > 0.05$; pairwise tests, significant ($P < 0.01$) in early queens, with vs. without workers. Microarray results, ANOVA: significant (FDR-corrected $P < 0.05$) for main effect of stage of queen; microarray results, pairwise tests: significant (FDR-corrected $P < 0.05$) in queens without workers, late vs. early stage (other tests N.S.).

Figure S4. qRT-PCR results for *CG8369*.



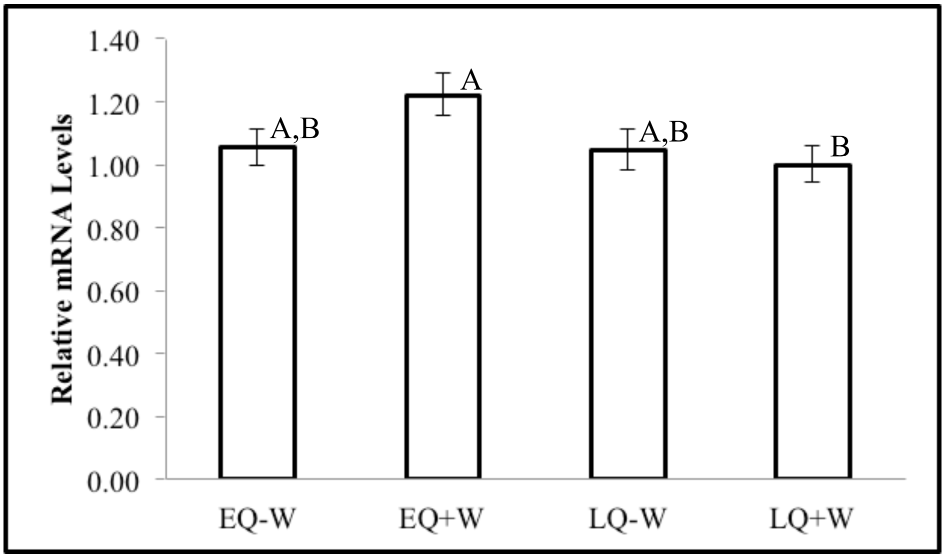
Bars represent means $^{+}/_{-}$ SE. qRT-PCR results: ANOVA, $P_{\text{stage(df=1)}} > 0.05$, $P_{\text{workers(df=1)}} < 0.01$, $P_{\text{stageXworkers(df=1)}} > 0.05$; pairwise tests, significant ($P < 0.05$) in early queens, with vs. without workers. Microarray results: ANOVA, significant (FDR-corrected $P < 0.05$) for main effects of stage of queen, workers, and interaction; pairwise tests, significant (FDR-corrected $P < 0.05$) in late queens with vs. without workers and in queens without workers, late vs. early stage (other tests N.S.).

Figure S5. qRT-PCR results for *nejire*.



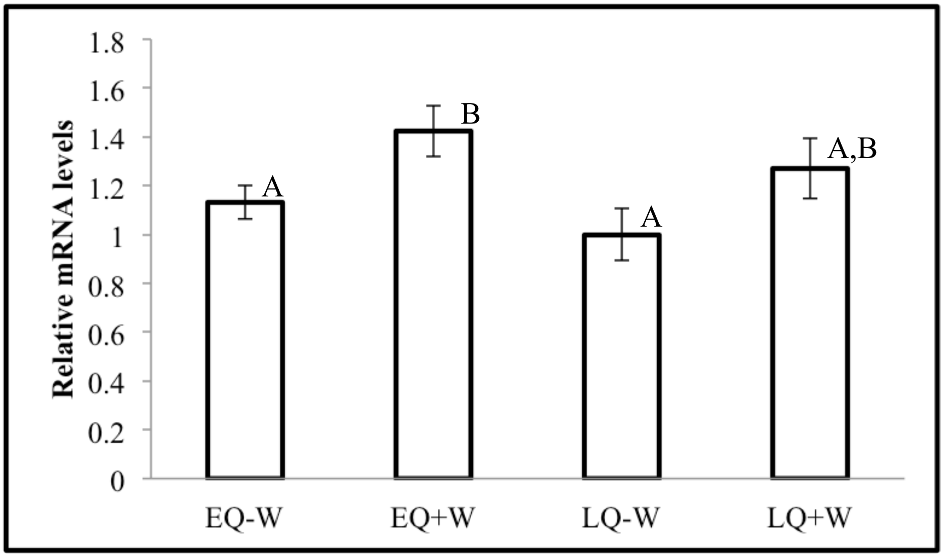
Bars represent means $^{+}/_{-}$ SE. qRT-PCR results: ANOVA, $P_{\text{stage(df=1)}} > 0.05$, $P_{\text{workers(df=1)}} > 0.05$, $P_{\text{stageXworkers(df=1)}} > 0.05$; pairwise tests, not significant. Microarray results: ANOVA, significant (FDR-corrected $P < 0.05$) for main effect of stage; pairwise tests, significant (FDR-corrected $P < 0.05$) in queens without workers, late vs. early stage (other tests N.S.).

Figure S6. qRT-PCR results for *calcium/calmodulin-dependent protein kinase*.



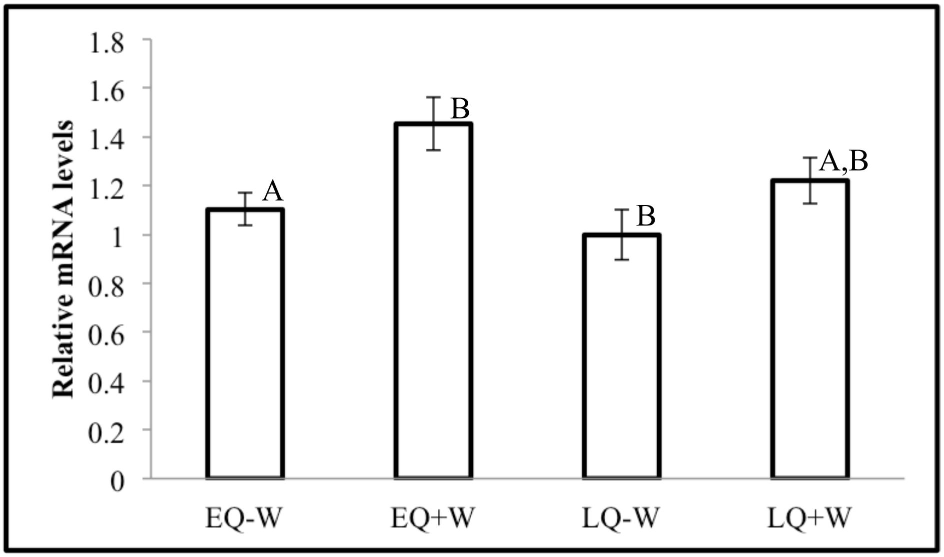
Bars represent means $^{+}/_{-}$ SE. qRT-PCR results: ANOVA, $P_{\text{stage(df=1)}} = 0.08$, $P_{\text{workers(df=1)}} > 0.05$, $P_{\text{stageXworkers(df=1)}} > 0.05$; pairwise tests, significant ($P < 0.05$) in queens with workers, early vs. late stage. Microarray results: ANOVA, significant (FDR-corrected $P < 0.05$) for main effect of stage of queen; microarray results; pairwise tests, significant (FDR-corrected $P < 0.05$) in queens without workers, late vs. early stage (other tests N.S.).

Figure S7. qRT-PCR results for *phosphopantothenoylcysteine decarboxylase*.



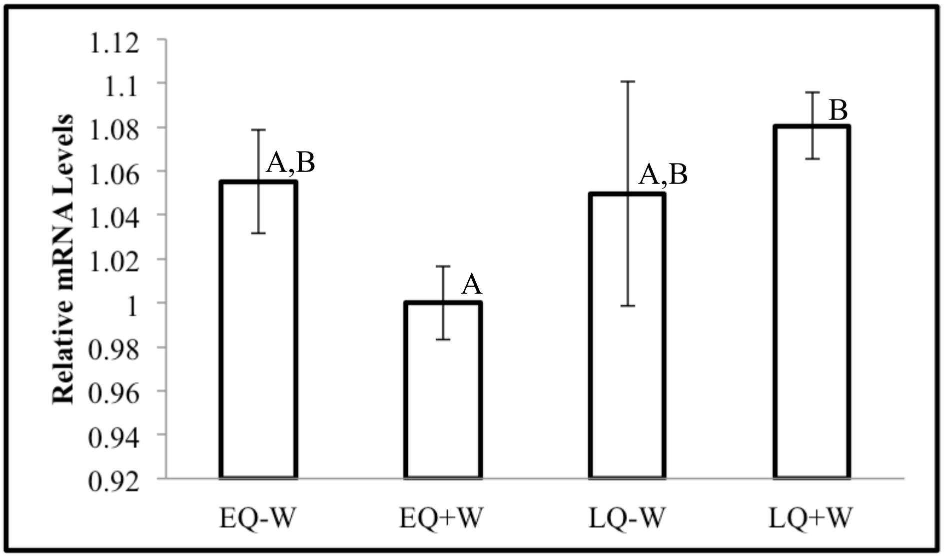
Bars represent means $^{+}/_{-}$ SE. ANOVA: $P_{\text{stage(df=1)}} > 0.05$, $P_{\text{workers(df=1)}} < 0.01$, $P_{\text{stageXworkers(df=1)}} > 0.05$. Microarray results, ANOVA and pairwise tests: not significant (FDR-corrected $P < 0.05$).

Figure S8. qRT-PCR results for *unchar LOC551381*.



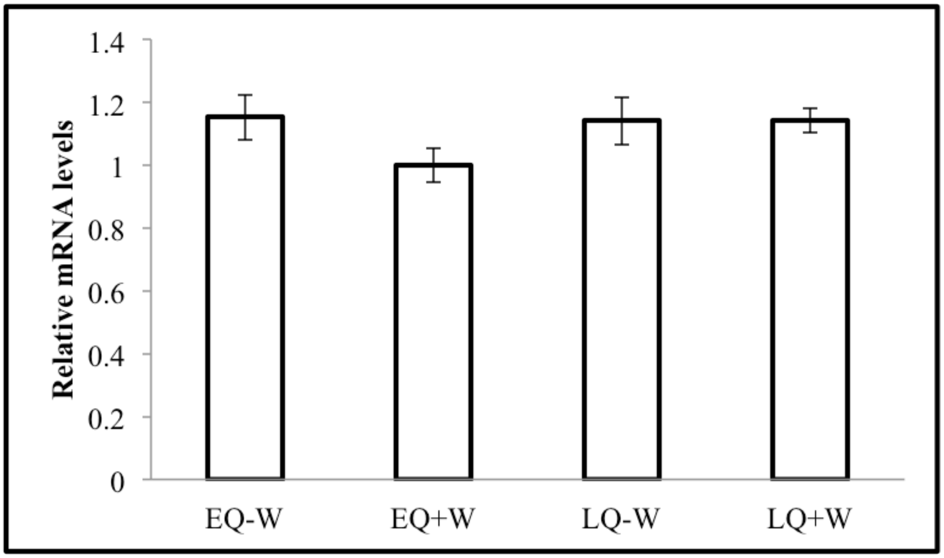
Bars represent means $^{+}/_{-}$ SE. ANOVA: $P_{\text{stage(df=1)}} > 0.05$, $P_{\text{workers(df=1)}} < 0.01$, $P_{\text{stageXworkers(df=1)}} > 0.05$. Microarray results, ANOVA and pairwise tests: not significant (FDR-corrected $P < 0.05$).

Figure S9. qRT-PCR results for *rcp1*.



Bars represent means $^{+}/_{-}$ SE. ANOVA, $P_{\text{stage(df=1)}} > 0.05$, $P_{\text{workers(df=1)}} > 0.05$, $P_{\text{stageXworkers(df=1)}} > 0.05$; pairwise tests, significant ($P < 0.01$) in queens withworkers, late vs. early stage (other tests N.S.).

Figure S10. qRT-PCR results for *S8*.



Bars represent means $^{+}/_{-}$ SE. ANOVA, $P_{\text{stage(df=1)}} > 0.05$, $P_{\text{workers(df=1)}} > 0.05$, $P_{\text{stageXworkers(df=1)}} > 0.05$; pairwise tests, not significant.

Figure S11. Comparison of gene lists from Pairwise and Rank Product Tests.

Up in Early-Stage Queens with Workers (*vs.* Without)		**Rank Product Test** (*n* = 8)	
		Yes	No
Pairwise Test (*n* = 0)	Yes	0	0
	No	8	

Up in Queens with Workers, Early-Stage (*vs.* Late)		**Rank Product Test** (*n* = 28)	
		Yes	No
Pairwise Test (*n* = 0)	Yes	0	0
	No	28	

Down in Early Stage Queens with Workers (*vs.* Without)		**Rank Product Test** (*n* = 0)	
		Yes	No
Pairwise Test (*n* = 0)	Yes	0	0
	No	0	

Down in Queens with Workers, Early-Stage (*vs.* Late)		**Rank Product Test** (*n* = 18)	
		Yes	No
Pairwise Test (*n* = 0)	Yes	0	0
	No	18	

Up in Late-Stage Queens with Workers (*vs.* Without)		**Rank Product Test** (*n* = 30)	
		Yes	No
Pairwise Test (*n* = 303)	Yes	30	0
	No	273	

Up in Queens without Workers, Early Stage (*vs.* Late)		**Rank Product Test** (*n* = 453)	
		Yes	No
Pairwise Test (*n* = 464)	Yes	373	91
	No	80	

Down in Late-Stage Queens with Workers (*vs.* Without)		**Rank Product Test** (*n* = 132)	
		Yes	No
Pairwise Test (*n* = 7)	Yes	7	0
	No	125	

Down in Queens without Workers, Early Stage (*vs.* Late)		**Rank Product Test** (*n* = 566)	
		Yes	No
Pairwise Test (*n* = 441)	Yes	407	34
	No	159	

“Yes” = gene(s) present in list; “No” = gene(s) not present in list.

Table S1. Functional terms enriched in the lists of differentially expressed genes.

	TERM	GO ID	ENRICH.	***P*** **- VALUE**
Main Effect of Stage of Queen (ANOVA)				
Biological Processes	chitin metabolic process	GO:0006030	5.55	0.00891
	photoreceptor cell differentiation	GO:0046530	2.56	0.02020
	chromosome segregation	GO:0007059	2.71	0.02326
	cellular ion homeostasis	GO:0006873	4.07	0.02848
	cognition	GO:0050890	2.13	0.02849
	compound eye photoreceptor cell differentiation	GO:0001751	2.57	0.03063
	negative regulation of nitrogen compound metabolic process	GO:0051172	2.18	0.03432
	negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	GO:0045934	2.18	0.03432
	behavior	GO:0007610	1.84	0.03524
	ion homeostasis	GO:0050801	3.82	0.03561
	eye photoreceptor cell differentiation	GO:0001754	2.44	0.03943
	negative regulation of RNA metabolic process	GO:0051253	2.24	0.04133
	cellular chemical homeostasis	GO:0055082	3.59	0.04370
	neurological system process	GO:0050877	1.59	0.04536
	negative regulation of transcription	GO:0016481	2.20	0.04590
Molecular Functions	chitin binding	GO:0008061	6.28	0.00515
	solute:cation symporter activity	GO:0015294	3.23	0.03180
	solute:sodium symporter activity	GO:0015370	3.77	0.03665
	adenyl nucleotide binding	GO:0030554	1.32	0.04459
	symporter activity	GO:0015293	2.95	0.04556
	polysaccharide binding	GO:0030247	3.54	0.04559
	pattern binding	GO:0001871	3.54	0.04559
	purine nucleoside binding	GO:0001883	1.32	0.04827
Upregulated in LQW- vs. EQW- (Pairwise Tests)				
Biological Processes	chromatin organization	GO:0006325	1.93	0.01395
	chromosome organization	GO:0051276	1.71	0.01555
	translation	GO:0006412	1.53	0.01584
	DNA packaging	GO:0006323	2.71	0.02148
	regulation of Rab GTPase activity	GO:0032313	3.31	0.02709
	regulation of Rab protein signal transduction	GO:0032483	3.31	0.02709
	cellular macromolecular complex subunit organization	GO:0034621	1.67	0.03372
	regulation of hydrolase activity	GO:0051336	2.27	0.03698
	regulation of Ras GTPase activity	GO:0032318	2.68	0.03769

	nucleosome organization	GO:0034728	2.68	0.03769
	cellular cation homeostasis	GO:0030003	3.67	0.03781
	chromosome segregation	GO:0007059	2.20	0.04307
	regulation of Ras protein signal transduction	GO:0046578	2.15	0.04980
Molecular Functions	metal ion binding	GO:0046872	1.34	0.00128
	ion binding	GO:0043167	1.32	0.00213
	cation binding	GO:0043169	1.31	0.00264
	adenyl nucleotide binding	GO:0030554	1.41	0.00412
	transition metal ion binding	GO:0046914	1.35	0.00461
	purine nucleoside binding	GO:0001883	1.40	0.00470
	nucleoside binding	GO:0001882	1.39	0.00572
	ATP binding	GO:0005524	1.40	0.00620
	adenyl ribonucleotide binding	GO:0032559	1.39	0.00662
	purine nucleotide binding	GO:0017076	1.29	0.01472
	purine ribonucleotide binding	GO:0032555	1.28	0.02010
	ribonucleotide binding	GO:0032553	1.28	0.02010
	zinc ion binding	GO:0008270	1.31	0.02782
	nucleotide binding	GO:0000166	1.23	0.02829
	aminopeptidase activity	GO:0004177	3.83	0.03206
	DNA binding	GO:0003677	1.35	0.04143
Downregulated in LQW- vs. EQW- (Pairwise Tests)				
Biological Processes	ion transport	GO:0006811	2.06	0.00137
	anion transport	GO:0006820	3.12	0.03512
	carbohydrate binding	GO:0030246	2.88	0.00903
	anion transmembrane transporter activity	GO:0008509	2.70	0.01374
	transition metal ion transmembrane transporter activity	GO:0046915	4.64	0.01609
	polysaccharide binding	GO:0030247	3.48	0.02207
	pattern binding	GO:0001871	3.48	0.02207
	di-, tri-valent inorganic cation transmembrane transporter activity	GO:0015082	4.22	0.02319
	symporter activity	GO:0015293	2.83	0.03009
	structural molecule activity	GO:0005198	1.52	0.03670
Upregulated in LQW+ vs. LQW- (Rank Product Tests)				
Biological Processes	ion transport	GO:0006811	2.35	0.00040
	photoreceptor cell differentiation	GO:0046530	2.91	0.00356
	regulation of Ras protein signal transduction	GO:0046578	3.30	0.00467
	adult behavior	GO:0030534	3.30	0.00467
	regulation of Rho protein signal transduction	GO:0035023	6.21	0.00644
	regulation of small GTPase mediated signal transduction	GO:0051056	2.90	0.01029
	photoreceptor cell fate commitment	GO:0046552	4.03	0.01372

	sodium ion transport	GO:0006814	5.04	0.01417
	neuron fate commitment	GO:0048663	3.87	0.01631
	cation transport	GO:0006812	2.08	0.01982
	tube development	GO:0035295	2.69	0.02585
	regulation of cell morphogenesis	GO:0022604	2.31	0.02620
	regulation of cell shape	GO:0008360	2.46	0.02649
	adult locomotory behavior	GO:0008344	3.23	0.03412
	neuron differentiation	GO:0030182	1.63	0.03809
	compound eye photoreceptor fate commitment	GO:0001752	3.67	0.04267
	alcohol catabolic process	GO:0046164	3.67	0.04267
	eye photoreceptor cell fate commitment	GO:0042706	3.67	0.04267
	metal ion transport	GO:0030001	2.23	0.04408
	respiratory system development	GO:0060541	2.10	0.04495
	open tracheal system development	GO:0007424	2.10	0.04495
	tube morphogenesis	GO:0035239	2.63	0.04591
	cellular carbohydrate catabolic process	GO:0044275	3.51	0.04925
	monovalent inorganic cation transport	GO:0015672	2.35	0.04969
	compound eye photoreceptor cell differentiation	GO:0001751	2.35	0.04969
Molecular Function	hexose transmembrane transporter activity	GO:0015149	7.81	0.00248
	glucose transmembrane transporter activity	GO:0005355	7.81	0.00248
	monosaccharide transmembrane transporter activity	GO:0015145	7.10	0.00370
	Ras guanyl-nucleotide exchange factor activity	GO:0005088	6.01	0.00725
	cofactor binding	GO:0048037	2.23	0.00838
	NAD or NADH binding	GO:0051287	4.46	0.00879
	sugar transmembrane transporter activity	GO:0051119	5.58	0.00966
	small GTPase regulator activity	GO:0005083	2.52	0.01536
	anion transmembrane transporter activity	GO:0008509	2.66	0.02739
	GTPase regulator activity	GO:0030695	2.05	0.03882
	coenzyme binding	GO:0050662	2.14	0.04020
	nucleoside-triphosphatase regulator activity	GO:0060589	1.97	0.04769
Downregulated in LQW+ vs. LQW- (Rank Product Tests)				
Biological Processes	translation	GO:0006412	2.20	0.00847
	cell cycle phase	GO:0022403	2.21	0.01633
	mitotic spindle elongation	GO:0000022	3.91	0.01668
	cell cycle process	GO:0022402	2.10	0.01705
	spindle elongation	GO:0051231	3.84	0.01790
	M phase	GO:0000279	2.10	0.03146
	cell cycle	GO:0007049	1.82	0.04596
Molecular Functions	structural constituent of ribosome	GO:0003735	2.74	0.00910
	structural molecule activity	GO:0005198	2.01	0.04095
Upregulated in LQW- vs. EQW- (Rank Product Tests)				

Biological Processes	chromosome organization	GO:0051276	1.98	0.00096
	chromatin organization	GO:0006325	2.21	0.00166
	chromosome segregation	GO:0007059	2.70	0.00344
	M phase	GO:0000279	1.70	0.00414
	cell cycle phase	GO:0022403	1.64	0.00698
	spindle organization	GO:0007051	1.80	0.00831
	mitotic spindle elongation	GO:0000022	2.31	0.01165
	dsRNA transport	GO:0033227	4.05	0.01204
	chromosome condensation	GO:0030261	4.05	0.01204
	cell cycle	GO:0007049	1.49	0.01266
	chromatin modification	GO:0016568	2.10	0.01291
	spindle elongation	GO:0051231	2.27	0.01328
	regulation of hydrolase activity	GO:0051336	2.51	0.01450
	mitotic cell cycle	GO:0000278	1.58	0.01635
	mitotic spindle organization	GO:0007052	1.77	0.01799
	microtubule cytoskeleton organization	GO:0000226	1.60	0.01957
	regulation of Rab protein signal transduction	GO:0032483	3.60	0.02027
	regulation of Rab GTPase activity	GO:0032313	3.60	0.02027
	programmed cell death	GO:0012501	2.03	0.02199
	microtubule-based process	GO:0007017	1.50	0.02228
	regulation of Ras GTPase activity	GO:0032318	3.02	0.02303
	cell cycle process	GO:0022402	1.48	0.02339
	DNA packaging	GO:0006323	2.70	0.02373
	death	GO:0016265	2.00	0.02442
	cell death	GO:0008219	2.00	0.02442
	apoptosis	GO:0006915	2.62	0.02782
	chromatin remodeling	GO:0006338	2.62	0.02782
	cellular amino acid biosynthetic process	GO:0008652	3.24	0.03149
	regulation of Ras protein signal transduction	GO:0046578	2.21	0.04490
Molecular Functions	ATP binding	GO:0005524	1.47	0.00110
	adenyl ribonucleotide binding	GO:0032559	1.47	0.00116
	ribonucleotide binding	GO:0032553	1.41	0.00125
	purine ribonucleotide binding	GO:0032555	1.41	0.00125
	adenyl nucleotide binding	GO:0030554	1.44	0.00131
	purine nucleoside binding	GO:0001883	1.44	0.00146
	purine nucleotide binding	GO:0017076	1.38	0.00166
	nucleoside binding	GO:0001882	1.43	0.00173
	nucleotide binding	GO:0000166	1.33	0.00216
	exopeptidase activity	GO:0008238	2.61	0.02799
	Rab GTPase activator activity	GO:0005097	3.13	0.03578
	aminopeptidase activity	GO:0004177	3.73	0.03768
	metal ion binding	GO:0046872	1.20	0.04242

Downregulated in LQW- vs. EQW- (Rank Product Tests)

Biological Processes	ion transport	GO:0006811	2.01	0.00010
	sodium ion transport	GO:0006814	3.84	0.00608
	long-term memory	GO:0007616	4.39	0.00737
	positive regulation of macromolecule biosynthetic process	GO:0010557	2.20	0.00824
	positive regulation of transcription	GO:0045941	2.24	0.00982
	positive regulation of cellular biosynthetic process	GO:0031328	2.06	0.01069
	positive regulation of biosynthetic process	GO:0009891	2.06	0.01069
	positive regulation of gene expression	GO:0010628	2.20	0.01150
	positive regulation of nitrogen compound metabolic process	GO:0051173	2.15	0.01339
	positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	GO:0045935	2.15	0.01339
	photoreceptor cell fate commitment	GO:0046552	2.93	0.01424
	cation transport	GO:0006812	1.74	0.01681
	neuron fate commitment	GO:0048663	2.81	0.01790
	memory	GO:0007613	3.07	0.01988
	positive regulation of macromolecule metabolic process	GO:0010604	1.89	0.02792
	biological adhesion	GO:0022610	1.78	0.02942
	cell adhesion	GO:0007155	1.78	0.02942
	compound eye photoreceptor fate commitment	GO:0001752	2.80	0.03150
	eye photoreceptor cell fate commitment	GO:0042706	2.80	0.03150
	monovalent inorganic cation transport	GO:0015672	1.92	0.04138
	wing disc development	GO:0035220	1.55	0.04394
	ion transport	GO:0006811	2.01	0.00010
	sodium ion transport	GO:0006814	3.84	0.00608
	long-term memory	GO:0007616	4.39	0.00737
	positive regulation of macromolecule biosynthetic process	GO:0010557	2.20	0.00824
	positive regulation of transcription	GO:0045941	2.24	0.00982
	positive regulation of cellular biosynthetic process	GO:0031328	2.06	0.01069
	positive regulation of biosynthetic process	GO:0009891	2.06	0.01069
	positive regulation of gene expression	GO:0010628	2.20	0.01150
	positive regulation of nitrogen compound metabolic process	GO:0051173	2.15	0.01339
	positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	GO:0045935	2.15	0.01339
	photoreceptor cell fate commitment	GO:0046552	2.93	0.01424
	cation transport	GO:0006812	1.74	0.01681

	neuron fate commitment	GO:0048663	2.81	0.01790
	memory	GO:0007613	3.07	0.01988
	positive regulation of macromolecule metabolic process	GO:0010604	1.89	0.02792
	biological adhesion	GO:0022610	1.78	0.02942
	cell adhesion	GO:0007155	1.78	0.02942
	compound eye photoreceptor fate commitment	GO:0001752	2.80	0.03150
	eye photoreceptor cell fate commitment	GO:0042706	2.80	0.03150
	monovalent inorganic cation transport	GO:0015672	1.92	0.04138
	wing disc development	GO:0035220	1.55	0.04394
Molecular Functions	L-amino acid transmembrane transporter activity	GO:0015179	5.35	0.00867
	symporter activity	GO:0015293	2.59	0.01080
	solute:sodium symporter activity	GO:0015370	3.15	0.01739
	hexose transmembrane transporter activity	GO:0015149	4.28	0.02151
	glucose transmembrane transporter activity	GO:0005355	4.28	0.02151
	solute:cation symporter activity	GO:0015294	2.48	0.02222
	alkali metal ion binding	GO:0031420	3.42	0.02334
	structural molecule activity	GO:0005198	1.44	0.02490
	vitamin B6 binding	GO:0070279	2.85	0.02845
	pyridoxal phosphate binding	GO:0030170	2.85	0.02845
	monosaccharide transmembrane transporter activity	GO:0015145	3.89	0.03075
	anion transmembrane transporter activity	GO:0008509	2.00	0.04062

"GO ID"=Gene Ontology ID; "Enrich."=fold enrichment. Gene lists used for enrichment analysis significant at FDR-corrected $P<0.05$. Terms with<5 genes are not included. List size for main effect of workers (ANOVA) was too small for enrichment analysis. For Pairwise Tests, the following lists were also too small to perform enrichment analysis: LQW+ *vs*. EQW+, EQW+ *vs*. EQW-, LQW+ *vs*. LQW-; for Rank Product Tests, the following lists were too small for GO enrichment analysis: Upregulated in LQW+ *vs*. EQW+, Downregulated in LQW+ *vs*. EQW+, Upregulated in EQW+ *vs*. EQW-, Downregulated in EQW+ *vs*. EQW-.

Table S2. Summary of qRT-PCR results for validation of microarrays.

Gene	**Significant for ‘Workers’**		**Significant for ‘Stage of Queen’**	
	Microarray	qRT-PCR	Microarray	qRT-PCR
arrestin-2	*	*		
TBC1 domain family, member 10b	*		*	
inositol 1,4,5,-tris-phosphate receptor		*	*	
CG8369	*	*	*	
nejire			*	
calcium/calmodulin-dependent protein kinase			*	
phosphopantothenoylcysteine decarboxylase		*		
unchar LOC551381		*		