

Figure S1. Quantification of fluorescence in *Wolbachia*-infected *Nasonia* oocytes, Related to Figure 2. (A) Bars represent the average corrected total cell fluorescence of *Wolbachia* in *N. vitripennis* 12.1 and *N. giraulti* IntG oocytes from stage 3 egg chambers. A *Wolbachia*-cured *N. vitripennis* line serves as a negative control. (B) Bars represent the average corrected total cell fluorescence of *Wolbachia* in *N. vitripennis* 12.1 and *N. giraulti* IntG nurse cell cytoplasm. A *Wolbachia*-cured *N. vitripennis* line serves as a negative control. (C) Bars represent counts of *Wolbachia* indicated fluorescent puncta in *N. vitripennis* 12.1 and *N. giraulti* IntG nurse cell cytoplasm. A *Wolbachia*-cured *N. vitripennis* line served as a negative control. * $p < 0.05$, Kruskal Wallis test with Dunn's multiple correction. Error bars are mean \pm S.E.M.

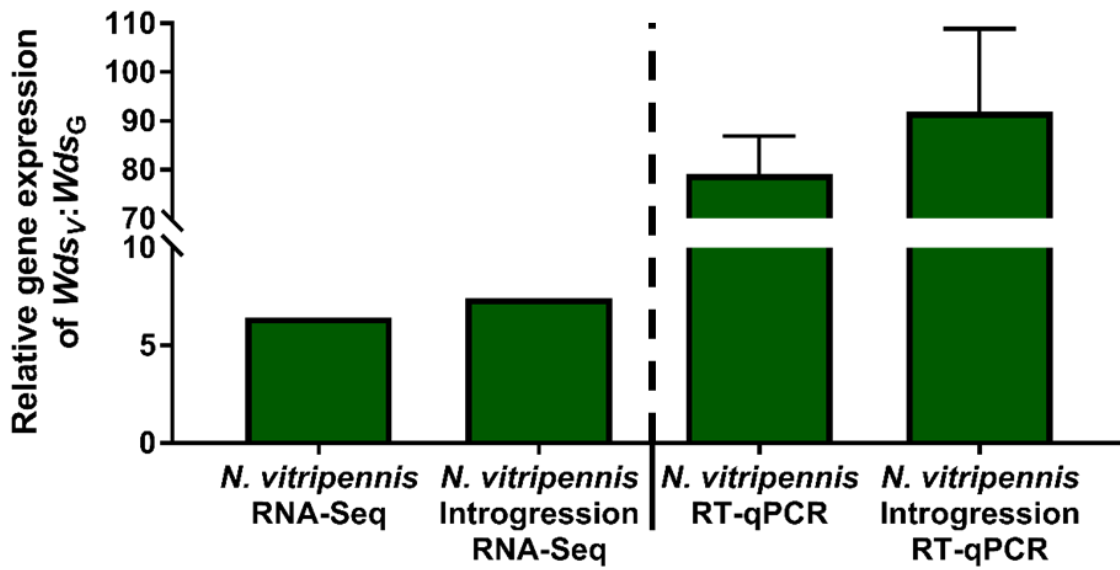


Figure S2. RT-qPCR validation of Wds_V expression in ovaries of *N. vitripennis* and the *N. vitripennis* introgression IntC3 compared to *N. giraulti*, Related to Figure 5. Bars represent the average fold change of *N. vitripennis* ovarian gene expression of Wds_V , compared to *N. giraulti* IntG Wds_G expression. Error bars are mean \pm S.E.M.

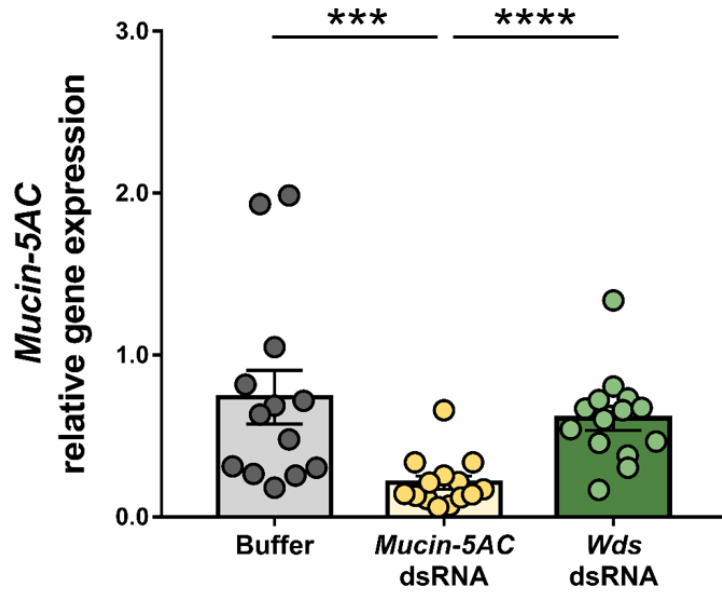


Figure S3. Relative gene expression of *Mucin-5AC* in late pupae of dsRNA-injected females, Related to Figure 5. Relative gene expression of *Mucin-5AC* in late pupae of *Mucin-5AC*-RNAi and *Wds*-RNAi females normalized to *Mucin-5AC* expression in buffer-injected females. *** $p < 0.001$, and **** $p < 0.0001$, Mann-Whitney U test. Error bars represent mean \pm S.E.M.

Table S1. Primers for *Nasonia* microsatellite markers, Related to Figure 3 and Figure 4.

cM locations based on genetic linkage map from [S1]. N/A = sequence is absent in *N. giraulti* so no PCR product is generated. Primers were used for quantitative trait loci mapping (QTL), fine mapping in segmental introgression lines (FM), or both. All primers were designed as part of this study with the following exceptions: NvC1-20, Nv-20, and NvC3-18 are from [S2]; Nv184 is from [S3].

Primer name	Chr	cM*	Primer Set (5' to 3')	Size <i>Nvit</i> (bp)	Size <i>Ngir</i> (bp)	Annealing Tm (°C)	Used For
MM1.12	1	31.4	F: GCGGTCCTGCTCCATTAACCGC R: CCAGACTCGCGCGGGTGTATTT	284	242	57	QTL
MM1.13	1	32.9	F: AGCTCCGAGAGCGCGAGTGA R: TCCCGTGCCGACGCATACAC	224	167	57	QTL
MM1.14	1	35.8	F: GCCGTCGAGAGACGAGCGAG R: GCGCGGCTGGAGGATGCTTT	219	266	57	QTL
MM1.L521	1	38.7	F: ACACGTCCCAGTCCCTTCTTTGAC R: GCGCCTCACTTGTTGTGCAT	118	160	54	QTL
MM1.16	1	40.9	F: ACGCGACTCCTTTCTCCGCA R: GCGGAAATCGAATGCGCGGC	233	199	56	QTL
MM1.17	1	43.8	F: TGCCTCGCGAGAGCGCAAAA R: ACTGCTCTCGTCAAGGCCGC	177	217	57	QTL
NvC1-21	1	46.7	F: GTAACAGTGAGATAAATGTG R: TAGCAACGATAGTCCACG	148	N/A	45	QTL
MM1.057	1	49.6	F: CTACCACATCTTTTCGCCAGTTT R: TCGAGTGATTAGAGATCGACGTT	180	206	51	QTL
MM1.L3567	1	53.3	F: CGCTCTGTCTACCTGTCCCT R: CGGCCACAAAGCAAATAGGC	154	184	52	QTL
MM1.31	1	56.2	F: CGCATCATCAACCCCGACCA R: TCCGCGGCATAACCACTTGCT	266	297	57	QTL
MM1.32	1	57.7	F: ACCGGGACGACTTGAGCGTA R: ACAATGGGCGAATTTTTCTGCCG	183	220	55	QTL
MM2.13	2	19	F: AAGACGAGAGCCGACGTTGC R: GGCCTGCACGAGTGTGTATAGGG	240	206	55	QTL
MM2.15	2	21.9	F: TGGCAGATGACTCACGGAAATTAACAG R: CAGTTTTAGATGAGTTTATGAACTGTGT C	87	154	52	QTL
MM2.17	2	24.8	F: CGCCGACGTCGTTGCTGCTT R: AGCTCCACAACGGCGGCATC	143	99	58	QTL
MM2.20	2	29.2	F: TCTCCGTTAATTTCCAGCGCGT R: TCTTCCAATCCACGGGAAAAGTGGT	207	168	55	QTL
Nv-20	2	30.7	F: TGACGAAGTATCCGAGAAG R: TCGAAAAACGATATTGCTCG	105	87	48	QTL
MM2.26	2	32.9	F: GCATCGCGTATGCTAATCTGCCG R: GGCGGAGTGAGAGAGCGTTTCA	220	172	56	QTL
MM2.L5335	2	36.5	F: CGCACGCGGTAATTGGCTTT R: TGTCCACGGCTGCGATTTGT	202	168	54	QTL
MM2.28	2	38	F: ACGCTTACACGCTGGTGAATGAA	256	287	55	QTL

			R: ACACCGTAATGCAATTTCCCGCT				
MM2.30	2	40.9	F: TGGATGCGAGCGGGTTAT R: CCCATCGCTGATCCACGTTCTT	135	172	55	QTL
MM2.L6870	2	43.8	F: GCTCTACACGGCGAAGGTCA R: CGCGCTTCTCTTTATGCCCG	140	191	54	QTL
MM2.33	2	46	F: ACGAAACTCTGTACTGTATACTCCGGT R: CGGCGAGTCCTCGAGAGCAG	204	250	55	QTL
MM2.36	2	49.6	F: GCCGTTGGAGAAATGTGCGGGA R: TCGCGTATATTTCCGTAGTCACGC	178	139	55	QTL
MM2.39	2	52.6	F: ACCGTTACAAAGCGAGCGAGAAT R: GCCGCCGCATAGCTCGATGA	161	207	55	QTL
MM2.40	2	54.8	F: TCCGTTTATCGCGCTTCGGACG R: CATCGGGCTGACCTTGCCCG	179	211	57	QTL
MM2.L7336	2	57.7	F: CATTCATCGCTCGTGTGCGC R: ACACATCTCTCCGAACGGCG	118	85	54	QTL
MM2.44	2	60.6	F: TCGACGGAAGCGAGGACGAG R: CTGGGCCGCAACGGTAAGCA	203	172	56	QTL
MM2.49	2	68.6	F: ACTGTTGCAGATGATGATGGTAATTT R: TCTGAAACATGCAACAATCAGGT	146	92	51	QTL
MM3.14	3	17.5	F: CTCTCGAAGCCGCGCGTGAA R: AGCCAGCTTTGCTTTCGACCG	231	206	56	QTL
MM3.15	3	20.4	F: ACACACGTTGTGCGGGGGTG R: GGTCGAAAATTTCTGCGCAGCCT	106	152	56	QTL
MM3.17	3	23.4	F: TGC GCGATGGCTGCTGTGAT R: TCGAGCGCAATAAACGCCCGC	126	170	57	QTL
MM3.19	3	26.3	F: GCGGAAATTCTCGCCCCTGC R: TCCCATCATCAAAACGAAAAAGTCGC	177	220	55	QTL, FM
MM3.22	3	29.2	F: TCTCCTCCTGCTTCGGCCCC R: TCGTTCATCGTTCGTCATCGCA	116	146	55	QTL, FM
MM3.L8514	3	32.1	F3: GCGGCGAGAAGAACAGAAGCGA R3: ACGTTGTCGCTGTTCTTCTTGCACT	488	279	57	FM
MM3.23	3	32.9	F: TTGAAGGGCTCATGGTCGCA R: CGCGAAACAGCGCACACG	183	219	55	QTL, FM
MM3.L8610	3	34.3	F1: CGCGTATGCTTGATGTCGGC R1: AACACAGAGGAATATGCGGGA	172	129	51	FM
MM3.L8623	3	34.3	F: TTGGAGTTTCGCACAAGAGC R: CTACCGCCGAGAAAAGTGC	185	136	54	FM
MM3.L8651	3	35.0	F: AGATGAGAAGAAAGAGGAAGCCC R: ATGGCGATTCTTCATGTCGC	164	117	54	FM
MM3.L8678	3	35.0	F: GCAGCCAGGGAGTGATATGCT R: AAAGGCCGACGACGAGAGAC	186	138	54	QTL, FM
MM3.L8716	3	36.5	F: TGCCCATCAAAGGTGAGAGG R: CGGACTCACTGTTTGCCAG	237	208	54	FM
MM3.L8724	3	36.5	F: CCTCTGTCTGTGCTTTTACGG R: TTCCCGTAAACACGATTGCC	105	82	54	FM
MM3.L8756	3	36.5	F: CGCGTGTCGTGTGGACGTAA R: TCAAACATCCGCGAGAGTCGA	115	157	54	FM

MM3.L8780	3	37.2	F1: TTAACCGAATAGCACCGCCG R1: AAATCCCAGATCCCAGACTAG	185	140	51	FM
MM3.L8790	2	37.2	F1: ATTTACCGACGCGCAACAGC R1: AGGGCGGAGAGATTAGATTTCCC	159	195	53	FM
MM3.L8813	3	37.2	F: CCGAGTGTGGGAGGTTTGACA R: TGTCAGCCGAGAATAGGCCG	177	148	54	FM
MM3.L8850	3	37.2	F: TGGTTGAGAGATCCACGCGA R: TCCGCGTTTACAACCAACATGG	159	206	53	FM
NvC3-18	3	38	F: GCCCAAATCATGCTTTTCG R: GTTGTCTTAAATGTGTATTCC	104	N/A	48	QTL
MM3.29	3	39.4	F: GGCCGATTTTCTCGACAGACC R: GCGAGGGAGAGCGAACGTC	241	285	53	QTL, FM
MM3.L10131	3	40.2	F: TGATGCGTTCTCGCCTTTCC R: CGACCGCAGAGCAACGATCA	155	204	53	FM
MM3.L10212	3	41.6	F: CCTCCCAAATCACTTCCGCGT R: TCAGCGCAATCGTTACCCTT	108	135	52	QTL, FM
Nv184	3	44.5	F: GCGTCATCGATGCATTTCTT R: TCTCGGGAGAGATTCAGTACG	209	141	49	QTL
MM3.L10340	3	45.3	F: CGAAACACCATTTCGCAACGAGT R: TGTCGCATCGAGAACTGCA	194	167	52	FM
MM3.29.7M	3	45.3	F: CCAGTTGGATAATTCTTGAGGTCTTTC R: ACTTTGCTTGGCCCGACGAT	148	118	52	FM
MM3.35	3	46.7	F: GTACGTGAACCGGAAGTGTTC R: GACGGCTGCTACCGGCTATA	111	161	51	QTL, FM
MM3.36	3	48.2	F: ATTCGCGCCGCGGCTAATGG R: TTCCATACGTGTGGCAGGCG	150	197	55	FM
MM3.37	3	50.4	F: ACAAGCTTCGCACACACCGCA R: CGGTCAAGAAGCGTCGCACA	185	157	58	QTL, FM
MM3.L10502	3	54.8	F: GCGCGAAACGACGAGGAATT R: CGAGCGTCGTGTGCTCTTCT	63	94	54	QTL
MM3.41	3	58.4	F: ACCGTGGGTCCGTGCAAC R: GGTTTGTACTTCATCGTGAGGCAATCG	186	142	55	QTL, FM
MM3.L10553	3	63.5	F: GCGCTTAATTGCGTCGTGTT R: CCGGTGCGGTTTCTTCTCCT	196	234	52	QTL
MM3.43	3	65.7	F: CGGCTGTTTATATTCCTCACCTGACGC R: GCAGCGACGAATCAGGAAATGCG	138	158	57	QTL
MM3.45	3	69.4	F: CGATTATGCAAACGACGCGA R: TTCCGATCACGATTCTCTCCTT	222	168	51	QTL
MM3.L10661	3	73	F: CCCTCCGATTATAGATGCAAGTGTCA R: GGCAGTAGTGGCTCTCTTTGCT	159	181	54	QTL

Table S2. Mapping statistics for RNA-seq of *Nasonia* ovaries, Related to Figure 5.

Nvit: *N. vitripennis* strain 12.1; IntG: *N. giraulti* strain IntG; IntC3: *N. giraulti* strain IntG introgressed with a chromosome 3 region from *N. vitripennis* strain 12.1. Analysis of the RNA-seq paired-end reads was performed in CLC Genomics Workbench 8.

Sample	# of Reads after QC	# of Mapped Paired Reads	% of Total Paired Reads Mapped	# of Mapped Intergenic Gene Reads	# of Mapped Gene Reads	# of Uniquely Mapped Gene Reads
Nvit-1	30,390,144	22,202,334	73.06	1,030,126	10,071,041	9,635,225
Nvit-2	34,665,838	25,567,636	73.75	1,253,382	11,530,436	11,022,442
Nvit-3	30,525,996	23,230,600	76.10	1,130,430	10,484,870	10,028,047
Nvit-4	26,369,828	19,186,084	72.76	1,046,799	8,546,243	8,152,609
IntC3-1	44,261,706	33,917,290	76.63	1,293,427	15,665,218	15,064,507
IntC3-2	49,692,100	35,712,884	71.84	1,658,981	16,197,461	15,517,397
IntC3-3	34,959,996	25,770,826	73.72	1,180,170	11,705,243	11,202,164
IntC3-4	42,765,380	30,299,978	70.85	1,327,688	13,822,301	13,261,676
IntG-1	36,821,016	27,095,510	73.59	1,051,834	12,495,921	12,020,977
IntG-2	35,451,744	26,505,936	74.77	1,114,883	12,138,085	11,647,781
IntG-3	40,863,430	31,432,456	76.92	1,234,399	14,481,829	13,913,515
IntG-4	38,523,210	25,325,636	65.74	1,744,894	10,917,924	10,470,342
IntG-5	38,697,378	27,021,558	69.83	1,201,004	12,309,775	11,765,553

Table S3. Significant differentially expressed genes in R6-3 candidate region, Related to Figure 5.

Mean number of reads for each gene was calculated by dividing the total number of reads from all replicates that mapped to the gene by the number of replicates (N = 4-5 for each *Nasonia* strain). Positive fold change = upregulation in *N. vitripennis* 12.1 or introgression line IntC3, while negative fold change = upregulation in *N. giraulti* IntG. Fold change and p-values were calculated using EdgeR in CLC Genomics.

NCBI Gene ID	NCBI Annotated Gene Name	Mean Reads for Nvit	Mean Reads for IntC3	Mean Reads for IntG	Fold Change (Nvit/IntG)	p-value (FDR-corrected)	Fold Change (IntC3/IntG)	p-value (FDR-corrected)
LOC100679092	Uncharacterized (<i>Wds</i>)	38.8	66.3	7.60	6.44	3.09E-10	7.42	2.07E-14
LOC100118928	Dentin Sialophosphoprotein-like	23.5	18	11.2	2.67	2.98E-3	1.40	1
LOC100118450	Abnormal long morphology protein 1-like	81.3	117	234	-2.26	2.29E-8	-2.30	5.32E-7
LOC100679834	Myb-like protein	147.5	380	546	-2.89	2.76E-5	-1.63	3.35E-3
LOC100114497	Girdin	640.5	1281	2508	-3.05	1.46E-40	-2.24	7.42E-19
LOC103317608	Interaptin-like	144	140	517	-2.82	3.48E-16	-4.25	1.28E-27
LOC100678491	Uncharacterized	26	46.3	152	-4.49	5.25E-15	-3.76	2.43E-13

Table S4. Blastp homology to Wdsv protein sequence, Related to Figure 6.

BLASTp analysis performed on uncharacterized protein LOC100679092 (NCBI accession number XP_008213336.1) using the non-redundant protein sequences (nr) database.

BLASTp Result Organisms	Accession	BLASTp E-value	Query coverage	% identity	Reciprocal BLASTp E-value to LOC100679092
Hymenopterans					
Uncharacterized protein LOC100679092 [<i>Nasonia vitripennis</i>]	XP_008213336.1	1.00E-143	100%	100%	1.00E-143
hypothetical protein TSAR_005991 [<i>Trichomalopsis sarcophagae</i>]	OXU27029.1	3.00E-99	100%	95%	1.00E-101
Uncharacterized protein LOC106659966 [<i>Trichogramma pretiosum</i>]	XP_014238261.1	4.00E-24	74%	42%	1.00E-21
Uncharacterized protein LOC106636480 [<i>Copidosoma floridanum</i>]	XP_014204363.1	5.00E-18	98%	35%	2.00E-17
Uncharacterized protein LOC106793189 [<i>Polistes canadensis</i>]	XP_014615392.1	2.00E-09	78%	29%	5.00E-07
Uncharacterized protein LOC100878703 [<i>Megachile rotundata</i>]	XP_003702181.1	6.00E-08	56%	32%	4.00E-05
Uncharacterized protein LOC107264954 isoform X2 [<i>Cephus cinctus</i>]	XP_015589287.1	5.00E-08	30%	55%	3.00E-08
Uncharacterized protein LOC107186552 [<i>Dufourea novaeangliae</i>]	XP_015429933.1	3.00E-07	56%	31%	2.00E-03
Uncharacterized protein LOC105276036 [<i>Ooceraea biroii</i>]	XP_011331664.1	4.00E-07	91%	31%	5.10E-02
Uncharacterized protein LOC108574276 [<i>Habropoda laboriosa</i>]	XP_017792325.1	2.00E-06	75%	26%	1.00E-03
Uncharacterized protein LOC100650078 [<i>Bombus terrestris</i>]	XP_003396525.1	2.00E-06	56%	31%	8.00E-05
Uncharacterized protein LOC107963970 [<i>Apis mellifera</i>]	XP_001121053.1	2.00E-05	56%	31%	1.00E-02
Uncharacterized protein LOC109860277 [<i>Pseudomyrmex gracilis</i>]	XP_020294850.1	3.00E-04	68%	28%	4.00E-03
Dipterans					
Uncharacterized protein LOC109418023 [<i>Aedes albopictus</i>]	XP_019547726.1	1.50E-02	32%	38%	1.60E+00
GL10758 [<i>Drosophila persimilis</i>]	XP_002015939.1	1.90E-02	32%	36%	8.90E-02
Uncharacterized protein Dpse_GA24472 [<i>Drosophila pseudoobscura</i>]	XP_002138301.1	7.60E-02	32%	34%	8.60E-02

Table S5. Primers for *Nasonia* gene sequencing and allelic genotyping, Related to Figure 4.

cM locations based on genetic linkage map from [S1].

Primer Name	Chr	cM*	Primer Set (5' to 3')	Annealing Temp (°C)	Product Size (bp)
LOC100119494	3	35.0	F: CGCACTCGCACAAACATTCCC R: GCCTTGCTCTTCTCCTTCTCCG	57	617
Mucin-5AC seq	3	35.0	F: TCGGCAAGAAGATGGGCGTC R: GCGTCGTTGTGGTGGTTGTG	57	747
LOC100679324	3	36.5	F: TATTGCCCTCGCCCCATTG R: CTGGTTCATAGCGTTGTTTGACATC	56	715
LOC100119358	3	36.5	F: CATCATCGCCCTCGTCTCTTC R: ACCGCTGCGTCACTTCCTG	56	682
LOC100119295	3	36.5	F: CCAGGACCCAGACCAGGATTAG R: AACCCACTTCTACCAGCCCC	55	670
LOC100119259	3	36.5	F: TTGACCACACCGACAACAAC R: GCATTTCATAAGTTCCGCCAGAG	53	670
LOC100679834	3	36.5	F: GCCGATTACTGGACCGACAG R: GTTGGGGTTGCGGATAGTTTCG	54	607
LOC103317434	3	36.5	F: ACGGGTATTTTCAGCCTTCGCC R: ACAACTCACACCTTCCCACCG	57	723
<i>Wds</i> seq	3	36.5	F: GTTCCTGATACTGCTCGCCG R: ACTTTGCTTGGCCCGACGAT	55	250
LOC100118928	3	36.5	F: CGAGCGAAGCACCGAGTTAC R: GCAGGCGACAGTTCTCAACG	55	677
LOC100679277	3	36.5	F: TTCGGGTCTTTTGTATTGCGAG R: TATCCTCCGTGTCTCCGTG	53	616
LOC100118712	3	36.5	F: TAGACCACGAACGCAACCTCG R: GCTTCCAAGAACCCATCCC	57	632
LOC100118529	3	36.5	F: GTCTCGGCGGGTTTGTATGG R: GCGTCCTTGGTGGCTGTTG	55	685
LOC100118450	3	36.5	F: ATGGAAAAGGCATCGGTAAGCG R: GCAACTCAGAAATCGTCCTGCG	55	630
LOC100114497	3	36.5	F: ACGAGTCATCTTCTATGGTTTTGGC R: TGTGGCAGGCGTTTGTGATATC	54	375
LOC100678491	3	36.5	F: GGATCACAACCAAAAGTTCCTG R: GGTACGGCCTAAACACGG	54	540

Table S6: Primers for dsRNA constructs and RT-qPCR of *Nasonia* genes. Related to Figure 5.

NCBI Gene ID	Gene Name	Name of Primer Set	Primer sequences (5' to 3')	Product Size (bp)
LOC100679092 (<i>Nasonia</i>)	Uncharacterized	Unchar RNAi	F: CGTTCCTGATACTGCTCGCC R: CCACCTGTTGCCTGTAGACG	438
		Unchar1 qPCR	F: ACCTACTGCTGACATCGTTCC R: AGCCCGTCTCTTGTTTCACG	165
LOC100679394 (<i>Nasonia</i>)	Mucin-5AC	Mucin5ac RNAi	F: TCGGCAAGAAGATGGGCGTC R: GCGTCGTTGTGGTGGTTGTG	627
		Mucin5ac qPCR	F: AAGGCTCGTGGAAGACTGCG R: TGGCGGCGTCCTGTTGTATC	145
malE (<i>E. coli</i>)	Maltose transporter subunit	MalE RNAi	F: ATTGCTGCTGACGGGGGTTAT R: ATGTTTCGGCATGATTTACCTTT	495
LOC100115795 (<i>Nasonia</i>)	60S Ribosomal protein L32	RP49 qPCR	F: CAAGCGTAACTGGAGGAAGC R: CTGCTAACTCCATGGGCAAT	221

Supplemental References

- S1. Desjardins, C.A., Gadau, J., Lopez, J.A., Niehuis, O., Avery, A.R., Loehlin, D.W., Richards, S., Colbourne, J.K., and Werren, J.H. (2013). Fine-scale mapping of the *Nasonia* genome to chromosomes using a high-density genotyping microarray. *G3* 3, 205-215.
- S2. Rutten, K.B., Pietsch, C., Olek, K., Neusser, M., Beukeboom, L.W., and Gadau, J. (2004). Chromosomal anchoring of linkage groups and identification of wing size QTL using markers and FISH probes derived from microdissected chromosomes in *Nasonia* (Pteromalidae: Hymenoptera). *Cytogenet Genome Res* 105, 126-133.
- S3. Beukeboom, L.W., Niehuis, O., Pannebakker, B.A., Koevoets, T., Gibson, J.D., Shuker, D.M., van de Zande, L., and Gadau, J. (2010). A comparison of recombination frequencies in intraspecific versus interspecific mapping populations of *Nasonia*. *Heredity (Edinb)* 104, 302-309.