



Supplementary Information for

A homeotic shift late in development drives mimetic color variation in a bumble bee

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SI Figures

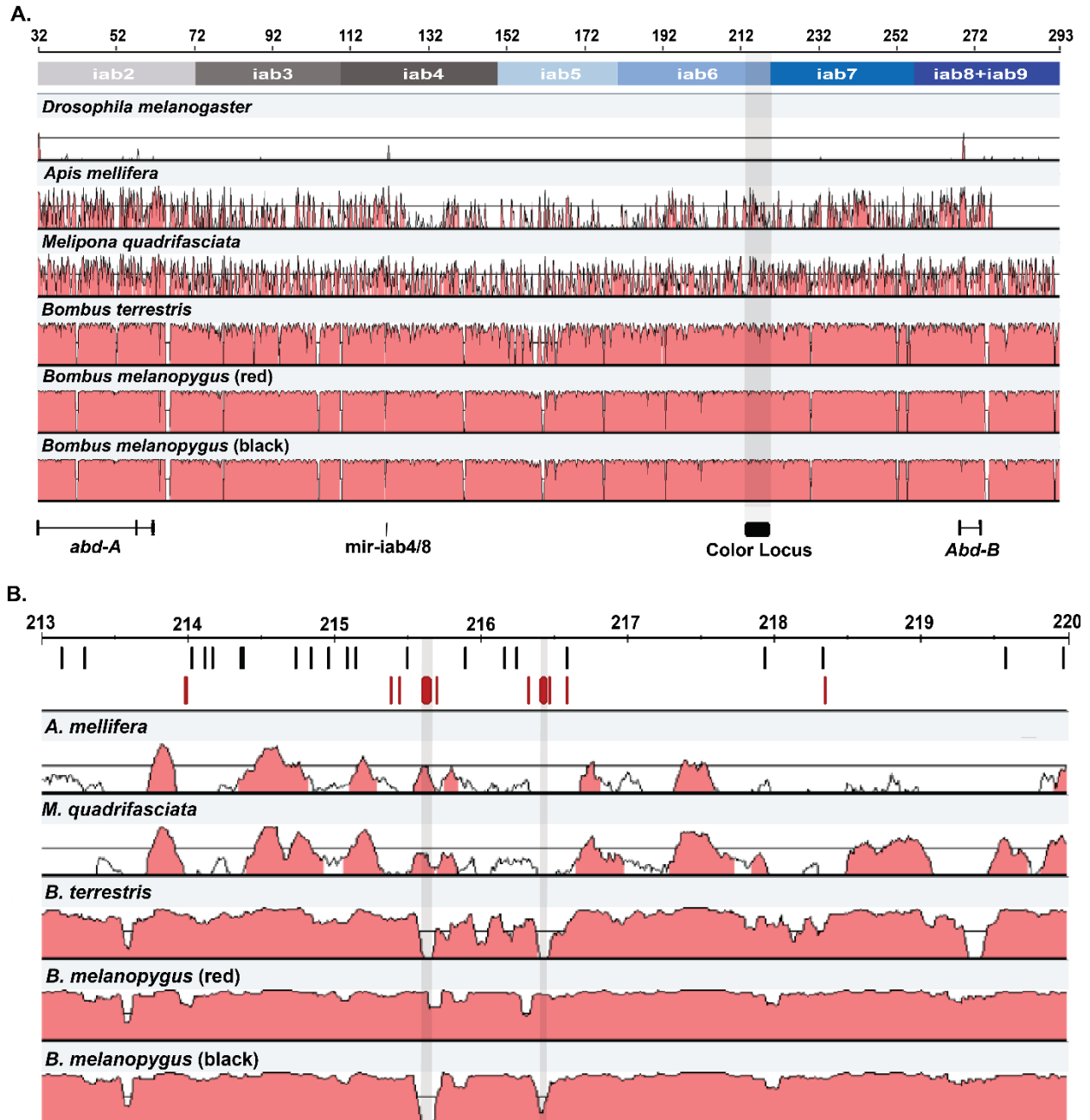


Fig. S1. Sequence conservation plot across the *abdominal-A/B* intergenic region (A) and in an ~7 kb region including the narrowed fixed color interval (B). Sequences are compared to reference contig BIMP 2.0 NT177935.1 from *Bombus impatiens* (1). The height of m-VISTA plots indicates the amount of sequence conservation which was calculated using a 100 bp sliding window (Minimum Y = 50%, Maximum Y= 100%, Minimum percent conservation identity = 70%, minimum width of a conservation region = 100bp, RankVISTA probability threshold set to 0.5). In (A), the position of the microRNA, *abd-A*, and *Abd-B* CDS are shown at the bottom, as

well as the approximated positions of *iab* domains at top (2), determined based on relative distances of these regions from these three aligned genes in *Drosophila melanogaster*. The position of the color locus relative to these is highlighted in gray. In (B), the positions of SNPs (top row, black) and indels (bottom row, red) relative to conserved regions are indicated. Genomic sequences used for the conservation plots were obtained from NCBI for *Drosophila melanogaster* (RefSeq assembly accession: GCF_000001215.4, RefSeq sequence accession: NT_033777.3), *Apis mellifera* (GenBank assembly accession: GCA_000002195.1, GenBank sequence accession: CM000069.5), *Melipona quadrifasciata* (GenBank assembly accession: GCA_001276565.1, GenBank sequence accession: KQ435720.1), *Bombus terrestris* (GenBank assembly accession: GCA_000214255.1, GenBank sequence accession: CM001186.1) and *Bombus impatiens* (RefSeq assembly accession: GCF_000188095.1, RefSeq sequence accession: NT_177925.1) which was used as the reference sequence. *Bombus melanopygus* sequences were *de novo* assembled from Bme004 (black form) and R007 (red form). Sequence files (in FASTA format) are provided in the Dryad Digital Repository (3).

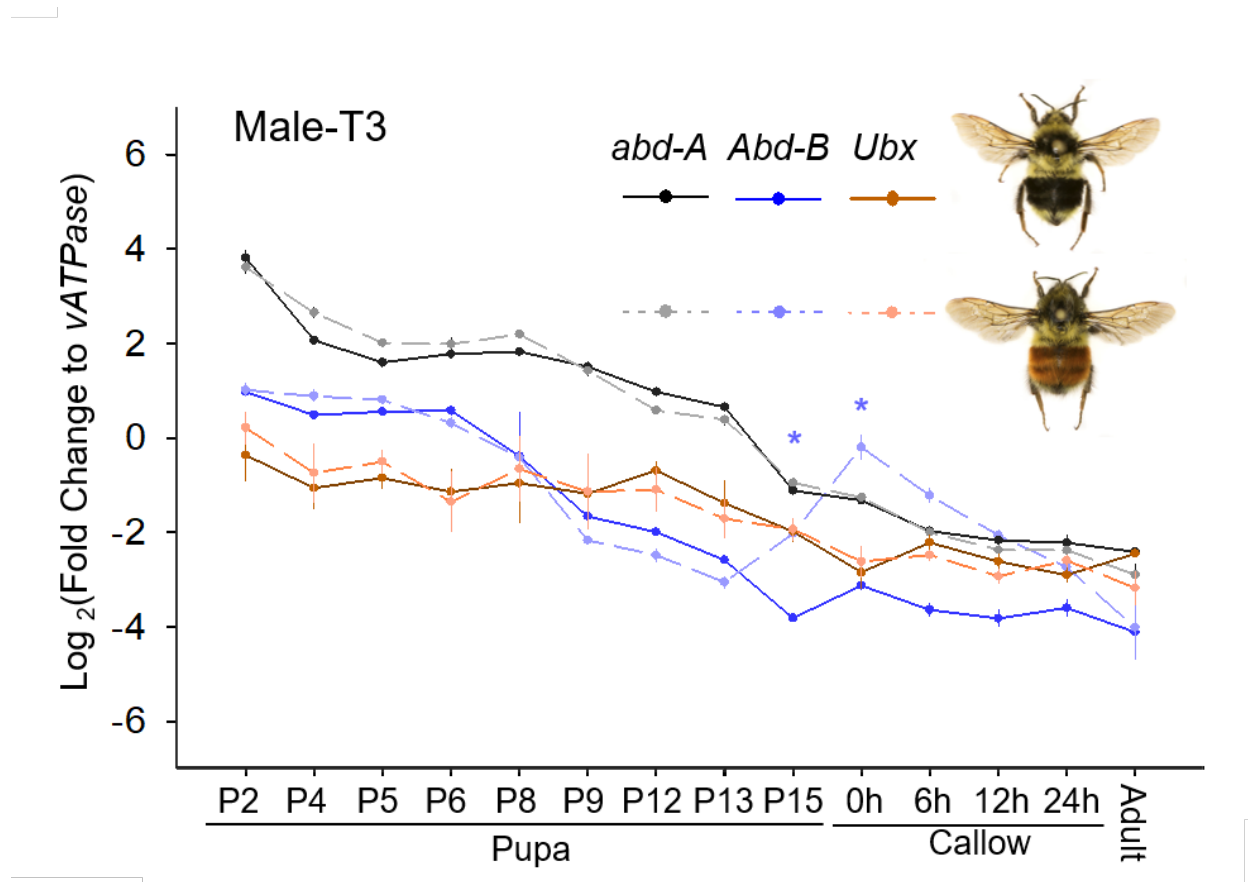


Fig. S2. BX-C expression differences in *B. melanopygus* males are consistent across polymorphic segments. A similar homeotic shift of *Abd-B* in the red form to that presented in Fig. 3C is also observed in metasomal tergite segment T3 of *B. melanopygus* males. * $p < 0.01$, Mann-Whitney *U* test.

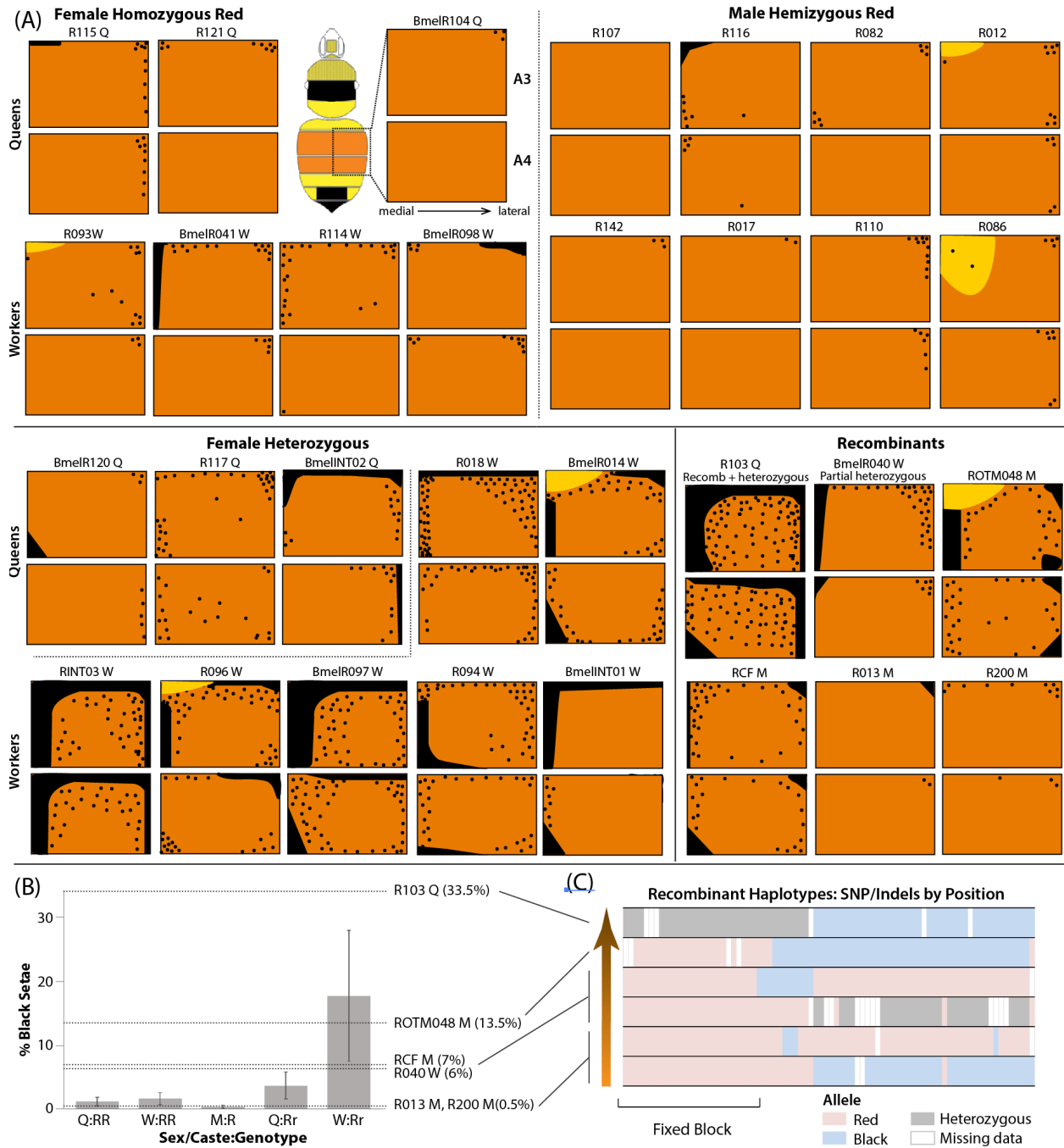


Fig. S3. Color phenotypes by genotype in the color locus. (A) Queens, workers, and males of homozygous/hemizygous red individual phenotypes are compared to those that are heterozygous at the color locus, with genotypes determined through sequencing of the color locus. Depictions show amount of black hairs on orange-red with dots representing individual hairs and blocks of black indicating regions with exclusively black hairs, assessed using drawings from microscope observations. Yellow indicates yellow hairs contained within an arch for some individuals. The 2nd and 3rd metasomal segment (T2, T3) for each individual is shown for one side of the body only, with medial regions on the left. Black patterning tended to be symmetrical. (B) Plot of the

average percent of black pile across both segments per condition, with standard error indicated. Dashed lines denote the percent of black hairs for each recombinant individual. Percentages were obtained using the Blur: Average function in Photoshop for the diagrams and obtaining relative brightness levels of the averaged color to the pure orange color. These values were averaged across both segments for each individual and converted to percent black using percent decrease in brightness. Means with the same letter are not significantly different (Kruskal-Wallis One-Way followed by Dunn's post-hoc test, $\alpha=0.05$). (C) Allelic designation for each recombinant individual from Part A and B. Each box represents a fixed SNP or indel between red and black form in the genomic dataset by location, with colors in boxes representing the color form the allele is assigned to in the genomic analysis.

Workers tend to have more mixing of black both in homozygous individuals and in the heterozygous form than males and queens, in line with the reduced expression in workers compared to males of *Abd-B* during periods of differential expression. Female heterozygotes display increased black above homozygotes (10-fold for workers, 3-fold for queens). Paired Mann-Whitney two sample tests indicated these differences to be significant in workers ($p=0.003$) but not queens ($p=0.385$), supporting incomplete dominance of the phenotype. These data also support the importance of SNPs outside of the fixed block for reinforcing the phenotype. Although all sampled individuals are still majority red, some recombinant individuals have substantial mixing of black similar to what is seen in heterozygous workers, suggesting these SNPs help to strengthen the expression phenotype. The region immediately adjacent to the fixed interval appears to be most important to the phenotype as seen in increased black with ROTM048 and RCF but not R013. The increased black in R040, which has heterozygosity in the non-fixed end of the interval only, upon what is typically found in a homozygous worker, suggests that some of the SNPs in this region are likely reinforcing the phenotype as well. R040 has an intermediate level between a homozygote and heterozygote. R103 is both recombinant and heterozygous and has the most black of any of the individuals, further supporting that SNPs across this interval are important for the phenotype.

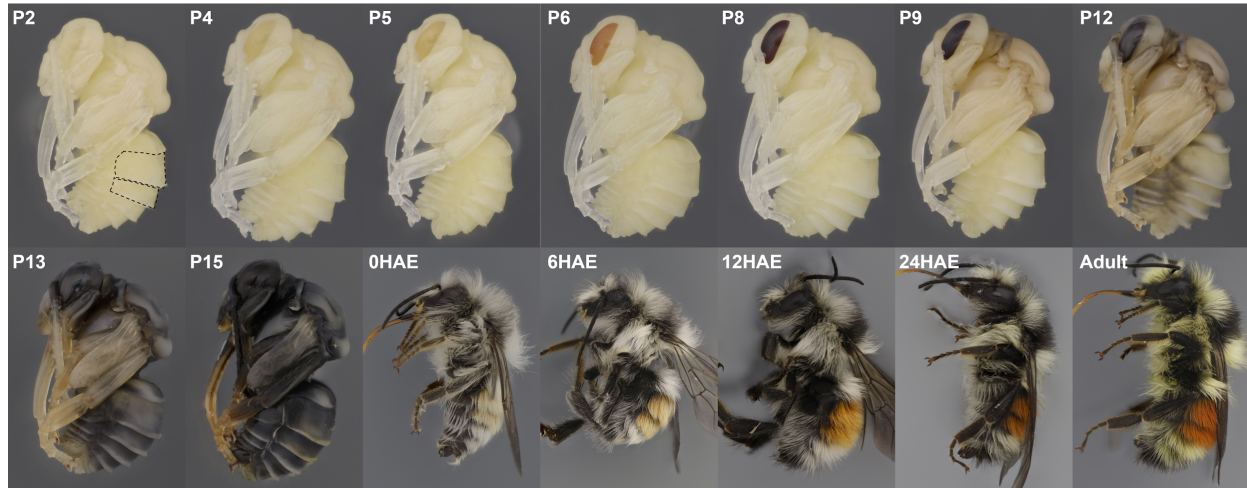


Fig. S4. *B. melanopygus* pupal and early adult stages used for gene expression analysis. Male bees are shown. For callow adults, only red form bees are shown. The polymorphic tergites of metasomal T2 and T3 (dashed line on P2) were dissected and used for qPCR. The average time between each pupal stage is 12-24 hours (4). HAE: Hours after eclosion from cocoon. Adult: 3-days after eclosion.

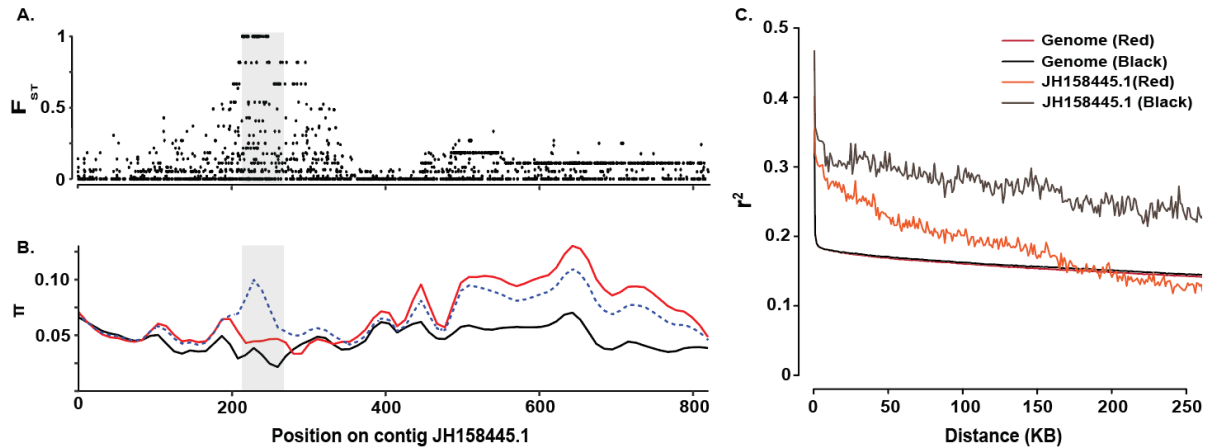


Fig. S5. Population genetic statistics in the interval and genome. (A). Genetic differentiation (F_{ST}) and (B) per site nucleotide diversity (π) in the color locus contig JH158445.1 calculated from genomic sequences of red and black phenotypes. The highlighted areas (light grayscale box) represent the location of the genomic fixed block. In (B), black (black solid line) and red (red solid line) forms exhibit reduced nucleotide diversity in the color interval within populations but high nucleotide diversity in combination (blue dashed line), as may be expected given their nucleotide divergence. (C). Linkage Disequilibrium (LD) decay in the color contig and the whole genome minus the color locus contig for red and black phenotypes where r^2 measurements of Linkage Disequilibrium values were plotted against the genetic distances (kilobases) between SNPs. While black phenotypes exhibit higher LD than red ones in the color locus contig JH158445.1, both forms exhibit very similar trends of LD in the rest of the genome. Raw datasets and details of these analyses are provided in the Dryad Digital Repository (3).

SI Tables

Table S1. A summary of whole-genome sequencing datasets utilized in the Genome-wide Association Analysis (GWAS)*.

Sample ID	NCBI BioSample ID	Sequencing Depth (Coverage)	Breadth at 1X (% Genome covered)	Total number of raw reads (M)	Total number of post-QC reads (M)	% reads mapped
Bme004	SAMN11091098	17.51	94.32	53.10	36.27	96.85
Bme012	SAMN11091099	16.25	94.02	50.87	36.85	96.36
Bme15	SAMN11091100	14.41	93.85	38.52	30.95	96.25
Bme19	SAMN11091101	16.62	93.96	44.90	36.26	95.99
Bme7	SAMN11091102	6.75	91.29	17.34	14.00	96.06
BmeIB-003	SAMN11091104	7.14	90.63	54.60	16.72	94.27
BmeIB006	SAMN11091105	6.70	89.89	51.53	15.53	94.82
BmeIB1	SAMN11091106	5.51	87.09	25.31	11.19	95.92
BmeIB4	SAMN11091107	5.81	88.47	27.10	12.05	96.81
BmeIR003	SAMN11091108	7.61	90.65	56.03	17.88	94.91
BmeIR004	SAMN11091109	7.24	90.73	52.29	16.85	95.49
BmeIR2	SAMN11091110	7.29	91.12	36.24	15.94	96.20
BmeIR5	SAMN11091111	4.49	81.49	19.76	8.60	96.07
Bme-SIENF01_28_S13	SAMN11091103	15.72	93.90	40.50	33.14	95.92
Bmm035	SAMN11091112	16.16	93.94	49.07	34.06	95.52
Bmm13	SAMN11091113	7.41	91.98	19.73	15.58	95.52
Bmm1	SAMN11091114	14.81	93.88	41.01	32.76	95.82
Bmm37	SAMN11091115	12.62	93.60	34.62	27.21	96.45
Bmm_WA001	SAMN11091116	13.24	89.90	49.77	43.13	95.30
R001	SAMN11091117	20.53	94.12	62.78	43.20	97.00
R007	SAMN11091118	20.47	94.14	63.23	43.17	96.33

*Sequencing depth = average depth of coverage per base of the *Bombus impatiens* genome (249185056 bp, GenBank Assembly GCA_000188105.2, BIMP_2.0; (1)), Breadth at 1X = percent of the *B. impatiens* genome with reads mapped. Total number of raw reads = number of paired end reads sequenced (in millions, M). Total number of post-QC reads = number of paired end reads after quality trimming (in millions, M), and % reads mapped = total percent of those reads that mapped to the *B. impatiens* genome. Multi-sample variant calling performed in GATK Unified Genotyper version 3.6 (5) (-ploidy 1 -glm SNP -stand_call_conf 25.0) resulted in 6,275,931 SNP calls. SNP filtration in VCFtools 0.1.15 (6) using parameters to filter sites with substantial amount of missing data (--max-missing 0.75) and low variability (max-non-ref-af 0.95) resulted in the 1,574,240 SNPs utilized in genotype-phenotype association analysis.

Table S2. Specimens sampled for whole genomic sequencing (WGS) and genotyping (GENO) experiments. Specimens are housed in the laboratory of H. M. Hines (Pennsylvania State University, University Park, PA).

STUDY	SAMPLE ID	SPECIES	T2-T3 COLOR	LATITUDE	LONGITUDE	COUNTRY: STATE	DATE COLLECTED	SEX/ CASTE
WGS	BmelB1	<i>melanopygus</i>	Black	42.1065111	-124.3466306	USA: Oregon	2013-06-18	male
WGS	BmelB4	<i>melanopygus</i>	Black	42.3289	-124.4258611	USA: Oregon	2013-06-18	male
WGS	BmelB-003	<i>melanopygus</i>	Black	42.1065111	-124.3466306	USA: Oregon	2013-06-18	male
WGS	BmelB006	<i>melanopygus</i>	Black	42.3289	-124.4258611	USA: Oregon	2013-06-19	male
WGS	Bme004	<i>melanopygus</i>	Black	43.9561167	-124.1374444	USA: Oregon	2014-06-01	male
WGS	Bme012	<i>melanopygus</i>	Black	42.3288278	-124.4265417	USA: Oregon	2014-06-04	male
WGS	Bme15	<i>melanopygus</i>	Black	43.9563833	-124.1371056	USA: Oregon	2014-06-09	male
WGS	Bme19	<i>melanopygus</i>	Black	44.0518139	-124.1095361	USA: Oregon	2014-06-10	male
WGS	BmelR2	<i>melanopygus</i>	Red	42.3289	-124.4258611	USA: Oregon	2013-06-18	male
WGS	BmelR5	<i>melanopygus</i>	Red	42.1065111	-124.3466306	USA: Oregon	2013-06-18	male
WGS	BmelR003	<i>melanopygus</i>	Red	42.3289	-124.4258611	USA: Oregon	2013-06-18	male
WGS	BmelR004	<i>melanopygus</i>	Red	42.1065111	-124.3466306	USA: Oregon	2013-06-18	male
WGS	Bmm035	<i>melanopygus</i>	Red	43.9565444	-124.1168472	USA: Oregon	2014-06-09	male
WGS	R001	<i>melanopygus</i>	Red	42.4648389	-124.3487806	USA: Oregon	2013-06-19	male
WGS	R007	<i>melanopygus</i>	Red	43.8121667	-124.1589972	USA: Oregon	2013-06-15	male
WGS	Bmm1	<i>melanopygus</i>	Red	43.9561167	-124.1374444	USA: Oregon	2014-06-01	male
WGS	Bmm13	<i>melanopygus</i>	Red	43.6625278	-124.1988528	USA: Oregon	2014-06-02	male
WGS	Bmm37	<i>melanopygus</i>	Red	44.0518139	-124.1095361	USA: Oregon	2014-06-09	male
WGS	Bmm_WA001	<i>melanopygus</i>	Red	47.017	-122.9710000	USA: Washington	2013	male
WGS	Bme7	<i>melanopygus</i>	Black	42.1043972	-124.3469500	USA: Oregon	2014-06-03	male
WGS	Bmel-SIENF01 _28_S13	<i>melanopygus</i>	Black	37.4877024	-119.6235133	USA: California	2017-06-10	male
GENO	BmelB002	<i>melanopygus</i>	Black	42.1065111	-124.3466306	USA: Oregon	2013-06-18	male
GENO	BmelB005	<i>melanopygus</i>	Black	42.3289	-124.4258611	USA: Oregon	2013-06-18	male
GENO	BmelB020	<i>melanopygus</i>	Black	42.08	-122.7200000	USA: Oregon	2015-06-06	male
GENO	BmelB021	<i>melanopygus</i>	Black	42	-122.7700000	USA: California/ Oregon	2015-07-15	male
GENO	BmelB022	<i>melanopygus</i>	Black	43.209836	-124.3104600	USA: Oregon	2015-06-07	male
GENO	BmelB025	<i>melanopygus</i>	Black	43.9563833	-124.1371056	USA: Oregon	2015-03-02	queen
GENO	BmelB026	<i>melanopygus</i>	Black	43.209836	-124.3104600	USA: Oregon	2015-06-07	male
GENO	BmelB027	<i>melanopygus</i>	Black	42.0735861	-122.6048889	USA: Oregon	2014-06-07	worker
GENO	BmelB028	<i>melanopygus</i>	Black	44.0518139	-124.1095361	USA: Oregon	2014-06-10	male
GENO	BmelB029	<i>melanopygus</i>	Black	43.9563833	-124.1371056	USA: Oregon	2014-06-09	male
GENO	BmelB030	<i>melanopygus</i>	Black	42.1063056	-124.3464917	USA: Oregon	2014-06-03	male
GENO	BmelB031	<i>melanopygus</i>	Black	43.9563833	-124.1371056	USA: Oregon	2014-06-01	male
GENO	BmelB032	<i>melanopygus</i>	Black	44.0518139	-124.1095361	USA: Oregon	2014-06-10	male

STUDY	SAMPLE ID	SPECIES	T2-T3		LONGITUDE	COUNTRY:	DATE	SEX/
			COLOR	LATITUDE				
GENO	BmelB034	<i>melanopygus</i>	Black	43.956923	-124.1411510	USA: Oregon	2015-03-02	queen
GENO	BmelB036	<i>melanopygus</i>	Black	42.3289	-124.4258611	USA: Oregon	2013-06-19	male
GENO	BmelB037	<i>melanopygus</i>	Black	42.1065111	-124.3466306	USA: Oregon	2013-06-18	male
GENO	BmelB038	<i>melanopygus</i>	Black	40.756594	-124.1646333	USA: California	2015-03-02	queen
GENO	BmelB051	<i>melanopygus</i>	Black	41.797607	-124.0818450	USA: Oregon	2015-06-06	male
GENO	BmelB053	<i>melanopygus</i>	Black	43.9563833	-124.1371056	USA: Oregon	2014-06-09	male
GENO	BmelB054	<i>melanopygus</i>	Black	43.9563833	-124.1371056	USA: Oregon	2014-06-01	male
GENO	BmelB055	<i>melanopygus</i>	Black	42.1043306	-124.3465667	USA: Oregon	2014-06-03	male
GENO	BmelB056	<i>melanopygus</i>	Black	42.1065111	-124.3466306	USA: Oregon	2013-06-19	male
GENO	BmelB057	<i>melanopygus</i>	Black	42.1065111	-124.3466306	USA: Oregon	2013-06-19	male
GENO	BmelB058	<i>melanopygus</i>	Black	43.9563833	-124.1371056	USA: Oregon	2014-06-01	male
GENO	BmelB059	<i>melanopygus</i>	Black	42.1041917	-124.3457361	USA: Oregon	2014-06-04	male
GENO	BmelB060	<i>melanopygus</i>	Black	43.9563833	-124.1371056	USA: Oregon	2014-06-09	male
GENO	BmelB061	<i>melanopygus</i>	Black	44.0518139	-124.1095361	USA: Oregon	2014-06-10	male
GENO	BmelB062	<i>melanopygus</i>	Black	41.34678	-122.2414300	USA: California	2015-07-15	male
GENO	BmelB063	<i>melanopygus</i>	Black	40.3273	-123.9254680	USA: California	2015-06-05	male
GENO	BmelB064	<i>melanopygus</i>	Black	41.769369	-124.1551440	USA: California	2015-06-06	worker
GENO	BmelB066	<i>melanopygus</i>	Black	42.1065111	-124.3466306	USA: Oregon	2013-06-18	male
GENO	BmelB067	<i>melanopygus</i>	Black	42.1065111	-124.3466306	USA: Oregon	2013-06-18	male
GENO	BmelB068	<i>melanopygus</i>	Black	40.3273	-123.9254680	USA: California	2015-06-05	male
GENO	BmelB069	<i>melanopygus</i>	Black	43.9563833	-124.1371056	USA: Oregon	2014-06-01	male
GENO	BmelB070	<i>melanopygus</i>	Black	42.1045972	-124.3467667	USA: Oregon	2014-06-03	male
GENO	BmelB071	<i>melanopygus</i>	Black	42.1043972	-124.3469500	USA: Oregon	2014-06-03	male
GENO	BmelB072	<i>melanopygus</i>	Black	43.9563833	-124.1371056	USA: Oregon	2014-06-01	male
GENO	BmelB073	<i>melanopygus</i>	Black	42.1065111	-124.3466306	USA: Oregon	2013-06-18	male
GENO	BmelB074	<i>melanopygus</i>	Black	41.34678	-122.2414300	USA: California	2015-07-15	male
GENO	BmelB100	<i>melanopygus</i>	Black	42.081689	-122.706948	USA: Oregon	2016-05-28	male
GENO	BmelB101	<i>melanopygus</i>	Black	43.036851	-124.071454	USA: Oregon	2016-05-29	male
GENO	BmelB102	<i>melanopygus</i>	Black	42.687131	-124.437234	USA: Oregon	2016-05-25	male
GENO	BmelB103	<i>melanopygus</i>	Black	41.796994	-124.0816120	USA: California	2016-05-27	male
GENO	BmelB202	<i>melanopygus</i>	Black	37.3972864	-119.5954027	USA: California	2017-06-10	worker
GENO	BmelB203	<i>melanopygus</i>	Black	36.1363782	-118.5407347	USA: California	2017-06-24	male
GENO	BmelBOTM043	<i>melanopygus</i>	Black	42.0649176	-124.3026239	USA: Oregon	2016-05-27	male
GENO	BmelBOTM045	<i>melanopygus</i>	Black	41.1782051	-124.07747	USA: California	2016-05-26	male
GENO	BmelBOTM047	<i>melanopygus</i>	Black	41.2215212	-124.0931031	USA: California	2016-05-26	male

STUDY	SAMPLE ID	SPECIES	T2-T3		LONGITUDE	COUNTRY:	DATE	SEX/
			COLOR	LATITUDE				
GENO	BmelBOTM049	<i>melanopygus</i>	Black	42.100447	-124.3470295	USA: Oregon	2016-05-27	male
GENO	BmelBOTM050	<i>melanopygus</i>	Black	42.687131	-124.437234	USA: Oregon	2016-05-25	male
GENO	BmelBOTM051	<i>melanopygus</i>	Black	42.2847724	-124.4070977	USA: Oregon	2016-05-25	male
GENO	BmelBOTM061	<i>melanopygus</i>	Black	42.687131	-124.437234	USA: Oregon	2016-05-25	male
GENO	BmelBOTM111	<i>melanopygus</i>	Red	42.081689	-122.706948	USA: Oregon	2016-05-28	male
GENO	BmelBOTM115	<i>melanopygus</i>	Black	42.081689	-122.706948	USA: Oregon	2016-05-28	male
GENO	Bmel_RCF	<i>melanopygus</i>	Red	42.1065111	-124.3466306	USA: Oregon	2016-05-25	male
GENO	Bmel_RCP	<i>melanopygus</i>	Red	44.1726266	-122.9500286	USA: Oregon	2016-05-24	male
GENO	Bmel_RPRL	<i>melanopygus</i>	Red	42.2847724	-124.4070977	USA: Oregon	2016-05-25	male
GENO	BmelB023	<i>melanopygus</i>	Red	43.9563833	-124.1371056	USA: Oregon	2014-06-08	male
GENO	BmelB033	<i>melanopygus</i>	Red	44.0518139	-124.1095361	USA: Oregon	2014-06-10	male
GENO	BmelR010	<i>melanopygus</i>	Red	45.30289	-121.7936800	USA: Oregon	2015-07-18	male
GENO	BmelR011	<i>melanopygus</i>	Red	44.79286	-121.8378600	USA: Oregon	2015-07-17	male
GENO	BmelR012	<i>melanopygus</i>	Red	43.209836	-124.3104600	USA: Oregon	2015-06-07	male
GENO	BmelR013	<i>melanopygus</i>	Red	42.3289	-124.4258611	USA: Oregon	2013-06-19	male
GENO	BmelR014	<i>melanopygus</i>	Red	42.0735861	-122.6048889	USA: Oregon	2014-06-07	worker
GENO	BmelR015	<i>melanopygus</i>	Red	44.0435583	-124.0991028	USA: Oregon	2014-06-08	male
GENO	BmelR016	<i>melanopygus</i>	Red	42.3286083	-124.4280056	USA: Oregon	2014-06-04	male
GENO	BmelR017	<i>melanopygus</i>	Red	43.6582111	-124.1886111	USA: Oregon	2013-06-19	male
GENO	BmelR018	<i>melanopygus</i>	Red	42.1043306	-124.3465667	USA: Oregon	2014-06-03	worker
GENO	BmelR019	<i>melanopygus</i>	Red	43.9561167	-124.1374444	USA: Oregon	2014-06-01	male
GENO	BmelR040	<i>melanopygus</i>	Red	44.504979	-123.5510790	USA: Oregon	2015-06-10	worker
GENO	BmelR041	<i>melanopygus</i>	Red	43.797869	-124.1479150	USA: Oregon	2015-06-07	worker
GENO	BmelR042	<i>melanopygus</i>	Red	42.3289	-124.4258611	USA: Oregon	2013-06-19	male
GENO	BmelR043	<i>melanopygus</i>	Red	42.1065111	-124.3466306	USA: Oregon	2013-06-17	male
GENO	BmelR044	<i>melanopygus</i>	Red	42.1043306	-124.3465667	USA: Oregon	2014-06-03	male
GENO	BmelR045	<i>melanopygus</i>	Red	42.3288028	-124.4284667	USA: Oregon	2014-06-04	male
GENO	BmelR080	<i>melanopygus</i>	Red	43.9561167	-124.1374444	USA: Oregon	2014-06-01	male
GENO	BmelR081	<i>melanopygus</i>	Red	43.9561167	-124.1374444	USA: Oregon	2014-06-01	male
GENO	BmelR082	<i>melanopygus</i>	Red	43.9563833	-124.1371056	USA: Oregon	2014-06-01	male
GENO	BmelR083	<i>melanopygus</i>	Red	43.9563833	-124.1371056	USA: Oregon	2014-06-01	male
GENO	BmelR084	<i>melanopygus</i>	Red	42.1065556	-124.3464944	USA: Oregon	2014-06-03	male
GENO	BmelR085	<i>melanopygus</i>	Red	44.0435583	-124.0991028	USA: Oregon	2014-06-08	male
GENO	BmelR086	<i>melanopygus</i>	Red	44.0435583	-124.0991028	USA: Oregon	2014-06-08	male
GENO	BmelR087	<i>melanopygus</i>	Red	44.0518139	-124.1095361	USA: Oregon	2014-06-09	male
GENO	BmelR088	<i>melanopygus</i>	Red	43.9563833	-124.1371056	USA: Oregon	2014-06-09	male
GENO	BmelR089	<i>melanopygus</i>	Red	43.8121667	-124.1589972	USA: Oregon	2013-06-15	male
GENO	BmelR090	<i>melanopygus</i>	Red	43.6741	-124.1676194	USA: Oregon	2013-06-15	male
GENO	BmelR091	<i>melanopygus</i>	Red	42.1065111	-124.3466306	USA: Oregon	2013-06-17	male

STUDY	SAMPLE ID	SPECIES	T2-T3		LONGITUDE	COUNTRY:	DATE	SEX/
			COLOR	LATITUDE				
GENO	BmelR092	<i>melanopygus</i>	Red	43.6582111	-124.1886111	USA: Oregon	2013-06-19	male
GENO	BmelR093	<i>melanopygus</i>	Red	43.209836	-124.3104600	USA: Oregon	2015-06-07	worker
GENO	BmelR094	<i>melanopygus</i>	Red	44.504979	-123.5510790	USA: Oregon	2015-06-10	worker
GENO	BmelR095	<i>melanopygus</i>	Red	45.503753	-122.6842700	USA: Oregon	2015-06-11	male
GENO	BmelR096	<i>melanopygus</i>	Red	44.504979	-123.5510790	USA: Oregon	2015-06-10	worker
GENO	BmelR097	<i>melanopygus</i>	Red	44.504979	-123.5510790	USA: Oregon	2015-06-10	worker
GENO	BmelR098	<i>melanopygus</i>	Red	47.322326	-122.3972930	USA: Washington	2015-06-12	worker
GENO	BmelR099	<i>melanopygus</i>	Red	45.30289	-121.7936800	USA: Oregon	2015-07-18	male
GENO	BmelR100	<i>melanopygus</i>	Red	44.0518139	-124.1095361	USA: Oregon	2014-06-10	male
GENO	BmelR101	<i>melanopygus</i>	Red	43.9563833	-124.1371056	USA: Oregon	2014-06-09	male
GENO	BmelR102	<i>melanopygus</i>	Red	43.6582111	-124.1886111	USA: Oregon	2013-06-15	male
GENO	BmelR103	<i>melanopygus</i>	Red	43.9556444	-124.1387944	USA: Oregon	2014-03-04	queen
GENO	BmelR104	<i>melanopygus</i>	Red	43.9556444	-124.1387944	USA: Oregon	2014-03-04	queen
GENO	BmelR105	<i>melanopygus</i>	Red	42.1065111	-124.3466306	USA: Oregon	2013-06-17	male
GENO	BmelR107	<i>melanopygus</i>	Red	43.6582111	-124.1886111	USA: Oregon	2013-06-19	male
GENO	BmelR108	<i>melanopygus</i>	Red	43.095433	-124.4302800	USA: Oregon	2015-06-07	male
GENO	BmelR109	<i>melanopygus</i>	Red	43.6582111	-124.1886111	USA: Oregon	2013-06-19	male
GENO	BmelR110	<i>melanopygus</i>	Red	42.1065111	-124.3466306	USA: Oregon	2013-06-17	male
GENO	BmelR111	<i>melanopygus</i>	Red	43.6741	-124.1676194	USA: Oregon	2013-06-15	male
GENO	BmelR112	<i>melanopygus</i>	Red	43.6582111	-124.1886111	USA: Oregon	2013-06-15	male
GENO	BmelR113	<i>melanopygus</i>	Red	43.812164	-124.1589970	USA: Oregon	2013-06-16	male
GENO	BmelR114	<i>melanopygus</i>	Red	44.504979	-123.5510790	USA: Oregon	2015-06-10	worker
GENO	BmelR115	<i>melanopygus</i>	Red	43.9556444	-124.1387944	USA: Oregon	2014-03-04	queen
GENO	BmelR116	<i>melanopygus</i>	Red	44.0518139	-124.1095361	USA: Oregon	2014-06-10	male
GENO	BmelR117	<i>melanopygus</i>	Red	43.956923	-124.1411510	USA: Oregon	2015-02-19	queen
GENO	BmelR118	<i>melanopygus</i>	Red	43.9561167	-124.1374444	USA: Oregon	2014-06-09	male
GENO	BmelR119	<i>melanopygus</i>	Red	43.6582111	-124.1886111	USA: Oregon	2013-06-19	male
GENO	BmelR120	<i>melanopygus</i>	Red	43.956923	-124.1411510	USA: Oregon	2015-02-19	queen
GENO	BmelR121	<i>melanopygus</i>	Red	43.956923	-124.1411510	USA: Oregon	2015-02-19	queen
GENO	BmelR140	<i>melanopygus</i>	Red	42.081689	-122.706948	USA: Oregon	2016-05-28	male
GENO	BmelR141	<i>melanopygus</i>	Red	43.035102	-124.0774220	USA: Oregon	2016-05-29	male
GENO	BmelR142	<i>melanopygus</i>	Red	42.687131	-124.437234	USA: Oregon	2016-05-25	male
GENO	BmelR143	<i>melanopygus</i>	Red	41.796994	-124.0816120	California	2016-05-27	male
GENO	BmelR200	<i>melanopygus</i>	Red	43.01821	-109.75566	USA: Wyoming	2016-08-10	male
GENO	BmelR201	<i>melanopygus</i>	Red	40.84828	-109.645073	USA: Utah	2016-08-10	male
GENO	BmelRInt01	<i>melanopygus</i>	Red	42.081689	-122.706948	USA: Oregon	2016-05-28	worker
GENO	BmelRInt02	<i>melanopygus</i>	Red	43.036851	-124.071454	USA: Oregon	2016-05-29	queen
GENO	BmelRInt03	<i>melanopygus</i>	Red	42.687131	-124.437234	USA: Oregon	2016-05-25	worker

STUDY	SAMPLE ID	SPECIES	T2-T3		LONGITUDE	COUNTRY:	DATE	SEX/
			COLOR	LATITUDE				
GENO	BmelRInt04	<i>melanopygus</i>	Red	41.796994	-124.0816120	USA: California	2016-05-27	male
GENO	BmelROTM042	<i>melanopygus</i>	Red	42.0649176	-124.3026239	USA: Oregon	2016-05-27	male
GENO	BmelROTM048	<i>melanopygus</i>	Red	42.100447	-124.3470295	USA: Oregon	2016-05-27	male
GENO	BmelROTM052	<i>melanopygus</i>	Red	42.2847724	-124.4070977	USA: Oregon	2016-05-25	male
GENO	BmelROTM052	<i>melanopygus</i>	Red	42.687131	-124.437234	USA: Oregon	2016-05-25	male
GENO	BmelROTM053	<i>melanopygus</i>	Red	44.1726266	-122.9500286	USA: Oregon	2016-05-24	male
GENO	BmelROTM105	<i>melanopygus</i>	Red	42.081689	-122.706948	USA: Oregon	2016-05-29	male
GENO	BmelB050	<i>mixtus</i>	Black	44.0518139	-124.1095361	USA: Oregon	2014-06-10	male
GENO	BmelB065	<i>mixtus</i>	Black	45.268	-121.738	USA: Oregon	2015-06-11	worker
GENO	sylB	<i>sylvicola</i>	Black	46.76846	-121.73096	USA: Washington	2015-07-19	male
GENO	sylR	<i>sylvicola</i>	Red	46.76846	-121.73096	USA: Washington	2015-07-19	male
GENO	tern	<i>ternarius</i>	Red	40.7705481	-77.7574485	USA: Pennsylvania	2015-06-29	worker
GENO	vos	<i>vosnesenskii</i>	Black	41.34678	-122.2414300	USA: California	2015-07-15	male
GENO	BIFA95R05	<i>bifarius</i>	Red	40.72609	-109.46854	USA: Utah	2016-08-10	male
GENO	BIF019	<i>bifarius</i> <i>nearcticus</i>	Black	42.2567	-122.469	USA: Oregon	2016-04-12	queen
GENO	BIF209	<i>bifarius</i> <i>nearcticus</i>	Red	41.789995	-111.7734059	USA: Utah	2016-04	queen
GENO	BIFA95R04	<i>bifarius</i> <i>nearcticus</i>	Red	40.81215	-109.46517	USA: Utah	2016-08-10	male
GENO	Bimac	<i>bimaculatus</i>	Black	40.730995	-77.883116	USA: Pennsylvania	2017-08-01	male
GENO	EPHIP66	<i>ephippiatus</i>	Black	16.7332939	-92.6722174	Mexico: Chiapas	2011-05-09	male
GENO	EPHIP68	<i>ephippiatus</i>	Red	16.7332939	-92.6722174	Mexico: Chiapas	2011-05-09	male
GENO	hunt	<i>huntii</i>	Red			USA: Utah	2016	worker

Table S3. Primers used for genotyping[†].

Primer Name	Forward (5'-3')	Reverse (5'-3')	~Size (bp)
Pre-1	CCAGTCTTCCGTTTCTTCGT	CGCTTTTGCAATTTTGTTGT	340
AbdB1	CACGAACTCGCGATAGACAA	CGCCACGTCGGTAATAAAGT	1440
1to2_2	CCGTAGCTGTTACCGAACAA	GGGCTGACTTTCAACCAAAT	1440
1to2_3	TTCACGCGATAATCATCCAA	AAGATCGCGCGAAAAAGTTA	640
1to2_6	CCGAAACATCTTCCTCCTCT	AAAATCTCCGATTGCTTGGT	655
AbdB2	CGAAGCGATTGCAAACAGTA	GATGGAACGGTTGTCTTCGT	710
1to2_K	ACGGGATCAGGAAACAAAAG	CGATGAAAGTCTGCTTCTCG	1505
1to2_L	GATTCCGTCGTTTTTCTGGT	CGCTTGTAAGTTCGCGATAA	310
2to3_4	CACACGGTCCAATTCCTTA	GCCAATTAACCCTTTGTTCC	710

AbdB3	CCCGATCTTCTCGGATGTAA	TAAGTGCATAGGCACGCAAC	770
3to4_1	AAGTTAACGTTGCGTGCCTA	TGGCCTTTTCAGTTTCTGTG	840
3to4_2	GGTCGATGTCGCAATTAATA	TCTCTTTCGCGATGTTTCAT	400
AbdB4	ACGCGAGTCAGACTTTCCAT	GCAAAAATTCTTCCGCACAT	740
4to5_C	CAACCCCTTCGCTATTTTGT	ATCGCGTAGTACACCATCCA	1240
4to5_5	AAGAACACTCGTTGCCGATA	GGAACGAGACGAGGAACTT	1020
4to5_D	GCTGGTTCGCGTGAAATA	TTGCACGAGTTCCTCTGT	740
45DRinv_	ACAGAGAGGAACTCGTGCAA	TTGTACGTGTGTGGGTTTCGT	600
4to5_EH	ACGAACCCACACACGTACAA	TAACGTTTTCCACGGAGGAG	1410
4to5_FI	CGTTTTTATCGCGTCGATTT	GAGTGGCAACATGCGTCTTA	1880
4to5_G	TCGGTAAAGTAGGATGCGGTA	CCATCGGTGGCTTACAGTTT	1505
45GRinv_	AAACTGTAAGCCACCGATGG	TTGAACATTCGCGTGCTGAC	430
AbdB5	GTCAGCACGCGAATGTTCAA	TGTTTCGAGAGTGGGGAACG	695
5to6_J	TCGTCGTCGGATGACTGTAT	TGGAGATAGCGACATTGAGC	475
AbdB6	AGGATGATCTCGCGTTCAGG	CAACCTCAAACGCCTCTGC	160

†All primers were run at (51-52°C, 30 sec for annealing, 72°C, 30-60 sec for elongation).

Table S4. Primers used for quantitative PCR[‡].

Primer name	Forward (5'-3')	Reverse (5'-3')
α-tubulin	GGAGGAGGTGATGACAGTTT	AACCTCGTCGACTACTGTTG
Rps-5	GTCCCATAGTTGAACGCCTTAC	GCCGTCACAAGAACCTGTAAA
ef1-α	CCGACAAGGCTCTTCGTTTA	ATGCCTGGCTTCAGAATACC
GADPH	GCTGGAGCTGAATATGTTGTAGA	CCACCTTCAAATGAGCAGA
v-ATPase	GGCAATCGTATCAACAGTCTCA	TGCATCTCGAACACTAGCATC
abd-A	GACATCATGCCAACCACTACCA	TTCGGCCCGCACACGAC
Abd-B	CCACCTCCAGTGATGTGG	CAGAGGGTTCGAGCTTCC
Ubx	CGGTAGCGGTAACCATAACAA	CCAGGAGTGTCATGTCAAGTAG

‡Primers below were designed for this study by comparing *B. impatiens* (GenBank Assembly GCA_000188105.2, BIMP_2.0) (1) and *B. melanopygus* genomic sequences and selecting primers that were conserved across both species. qPCR reaction: 1µl of first strand cDNA diluted 5X from 20µl of initial reaction volume, 0.3µmol of each primer, 5ul PowerSYBR[®] Green PCR master mix (Applied Biosystems), and nuclease-free water to 10µl run in a 384-well plate amplified for 40 cycles on an ABI 7900 real-time PCR detection system (50°C for 2 min, 95°C for 10 min, followed by 40 cycles of two step PCR reaction with denaturation at 95°C for 15 s and annealing extension at 60°C for 1 min).

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