Table S1. Number of reads retained through quality control steps.

	Read Number			
	PE [d]	MP [d]	SR [d]	Total
Raw reads	290,684,913	405,178,215	NA	695,863,128
After trimming [a]	251,070,361	326,649,635	69,701,500	647,421,496
After PhiX removal [b]	241,504,981	318,932,115	68,367,821	628,804,917
After error correction [c]	239,540,116	299,172,619	87,719,032	626,431,767

[a] Trimming was carried out via Scythe/Sickle.

[b] Reads failing to map to the PhiX genome via bowtie2 were retained.

[c] Error correction was performed using Quake.

[d] PE = paired-end, MP = mate pair, SR = single-read.

Figure S1. Average base quality of raw (left) and processed (right) reads from the PE genome sequencing library. Figures were generated using FastQC, and show a slight improvement in sequencing quality following processing. The Read2 reads for the PE data, and both sets of reads from the MP data showed similar quality improvement following processing.

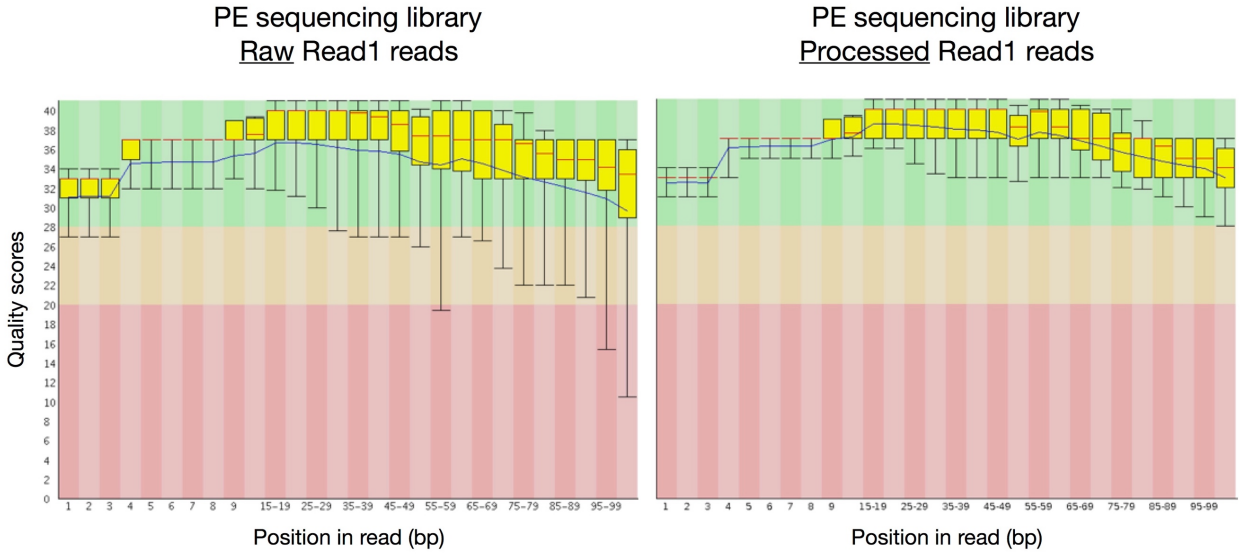

Table S2. Relationship between our set of 30 linkage groups and those identified by Gleason *et al.* (2016).

Present study linkage group IDs	Gleason *et al.* (2016) linkage group IDs [b]
Z [a]	1
1 [a]	8
2 [a]	6
3	7 [c], 17
4	–
5	11, 25
6 [a]	22
7 [a]	2
8	3 [d]
9 [a]	4
10 [a]	16
11 [a]	10
12	3 [d], 9
13	7 [c]
14 [a]	19
15	–
16 [a]	5
17	15 [e]
18 [a]	20
19 [a]	13
20	–
21 [a]	26
22 [a]	12
23 [a]	21
24	14, 15 [e]
25	–
26 [a]	18
27	–
28	–
29	–

[a] The 16 linkage groups that show an unambiguous one-to-one relationship between studies.

[b] When 2 linkage groups are listed for Gleason *et al.* (2016) this means 1 LG from our study was split into 2 in this previous work. When no linkage group is listed ("–") this means the LG from our study was not tagged by a marker in Gleason *et al.* (2016). Gleason *et al.* (2016) also identified 4 other linkage groups (23, 24, 27, 28), but markers from these could not be mapped to our scaffolds, or those scaffolds were not placed on our linkage groups.

[c] Gleason *et al.* LG7 is split into our LG3 and LG13.

[d] Gleason *et al.* LG3 is split into our LG8 and LG12.

[e] Gleason *et al.* LG15 is split into our LG17 and LG24.

Table S3. Number of markers placed on linkage groups in all 3 populations, and estimates of linkage group length (from Lep-MAP2) in the FL-BC and KS-BC backcross populations.

Linkage group ID	FL-BC		KS-BC		KS-SG
	N	cM	N	cM	N
Z	352	85.2	301	108.4	583
1	332	79.4	437	128.2	685
2	361	91	307	112.2	681
3	277	92.8	491	106	662
4	206	60.5	452	118.6	626
5	287	83.5	343	110	588
6	172	50.2	307	95.7	583
7	313	99.5	296	115.7	566
8	325	75.9	339	112.9	527
9	186	74.4	377	128.4	506
10	224	76.1	277	95.5	493
11	201	59.8	365	108	484
12	206	90.3	199	81	474
13	177	80.3	339	102.6	473
14	245	59.4	293	103.7	459
15	211	77.8	272	100	445
16	179	60.8	298	105.6	435
17	226	66.4	294	108.3	433
18	138	56	324	64.2	426
19	130	37.6	319	109.6	395
20	124	63.7	222	84.1	373
21	196	80.6	227	100.3	373
22	202	78.6	293	83	372
23	108	50.1	135	69	245
24	115	83	147	79.3	244
25	67	49.3	145	69	225
26	60	61.2	130	64.9	182
27	53	59.4	67	68.2	102
28	24	28.9	50	36.2	85
29	24	26.9	45	23.2	76
Total	5,721	2,038.5	8,091	2,791.6	12,801

Table S4. Homology between *Achroia grisella* linkage groups and chromosomes from sequenced lepidopterans.

Linkage group ID	*H. melpomene* Chromosome [a]	*B. mori* Chromosome [a]	Number *H. mel* hits [b]	Number *B. mori* hits [b]
Z	Z / 21	1	16	13
1	20	10	25	26
2	13	22	27	19
3	1	–	2	NA
	10	5	34	30
4	1	4	37	30
5	17	13	33	35
6	3	6	25	23
7	11	15	25	20
8	18	23	13	9
9	5	3	13	14
10	16	18	17	15
11	14	–	13	NA
12	7	11	16	11
13	–	7	NA	3
	–	18	NA	1
	8	25	17	15
14	4	21	21	16
15	6	9	16	22
16	12	8	22	15
17	–	17	NA	14
18	–	–	NA	NA
19	9	–	9	NA
20	–	–	NA	NA
21	19	12	18	12
22	2	–	8	NA
23	18	27	13	11
24	–	–	NA	NA
25	10	–	6	NA
	18	–	2	NA
26	11	–	1	NA
27	–	–	NA	NA
28	–	22	NA	2
29	–	–	NA	NA

[a] In all cases where we associate a specific *A. grisella* linkage group with an *H. melpomene* chromosome and a *B. mori* chromosome, the predicted homology between the chromosomes in these other two species is consistent with data provided in Table 28.2.1 in the original *H. melpomene* genome paper (Heliconius Genome Consortium 2012).

[b] The number of independent scaffolds associated with a particular linkage group in *A. grisella* that map to a chromosome in either *H. melpomene* or *B. mori*.

Table S5. Comparison of genotypes at markers from the present study to the EST-based markers used in Gleason *et al.* (2016)

Gleason *et al.* (2016) marker name [a]	Gleason *et al.* (2016) linkage group IDs [a]	Present study linkage group IDs [a]	Number of compared individuals [b]	Fraction identical genotypes (%)
C151	1	Z	272	97.06
C55	1	Z	310	95.81
C19	1	Z	233	95.71
C214	2	7	219	95.89
C88	2	7	240	97.08
1G12	2	7	329	97.87
C121	2	7	255	96.47
C110	2	7	189	89.42
1E10	2	7	330	97.27
C212	4	9	304	96.71
C7	4	9	234	94.02
C186	4	9	306	96.41
C90	4	9	283	98.23
C222	5	16	163	97.55
2H07	5	16	326	97.24
4B05	6	2	310	99.35
3E12	6	2	337	96.74
2H03	6	2	312	97.12
C22	8	1	161	96.89
2G06	8	1	195	96.92
C156	10	11	318	98.74
3C09	10	11	263	96.96
C145	12	22	359	98.61
1A12	13	19	219	96.35
C139	16	10	286	96.85
C49	16	10	273	97.80
3H02	18	26	225	96.89
C30	19	14	236	96.61
2G09	20	18	297	96.63
1A06	21	23	351	99.72
C126	22	6	334	98.50
C4	26	21	182	96.70

[a] These 32 markers are those that reside on linkage groups that match one-to-one between the present study and Gleason *et al.* (2016, see Table S2 above).

[b] The number of individuals that were both assigned a genotype call for the stated marker by Gleason *et al.* (2016), and either (i) assigned a call for the single genotyping-by-sequencing marker present on that scaffold in our study, or (ii) where the set of markers present on that scaffold yielded a single, consensus genotype call.

Figure S2. Fraction of heterozygous genotype calls per segregant individual per linkage group for the KS-SG population. Given the lack of crossing over, segregant males for this population should have both a Kansas Z chromosome and a Florida Z chromosome, and for each autosome should either be homozygous for the Kansas allele, or be heterozygous. Therefore, genotyping errors are evidenced by (1) any homozygous genotype calls on the Z, (2) any rare homozygous calls on an otherwise primarily heterozygous autosome, and (3) any rare heterozygous calls on an otherwise primarily homozygous autosome. Below we show a histogram of the fraction of called marker genotypes that are heterozygous for each individual/linkage group combination, only considering values for combinations where the genotyping call rate is at least 30%. As expected, nearly all Z-linked markers show a heterozygous genotype, and each autosome in any given individual has nearly all heterozygous or all homozygous genotype calls.

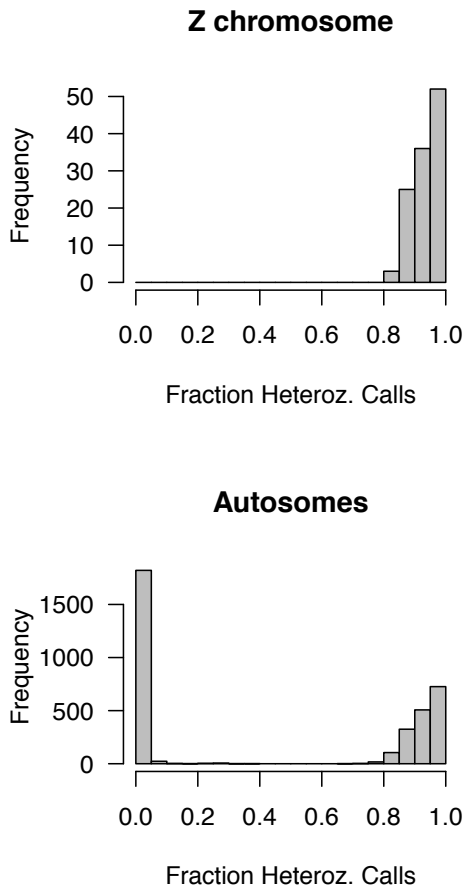

Table S6. QTL mapped for song traits corrected for body weight variation.

Popn	Weight-corrected phenotype [a]	Linkage group	LOD	Threshold (α)	Variance Expl (%) [c]	Effect [d]	Power [e]
FL-BC	Peak amplitude	8 [b]	2.47	0.05	2.59	6.45	0.94
KS-BC	Pulse-pair rate	11	2.79	0.05	2.83	–2.78	0.90
		13	2.22	0.05	2.28	–2.73	0.89
KS-SG	Peak amplitude	11	1.98	0.1	4.80	10.32	0.79

[a] For each population the song phenotype was regressed on body weight using the R *glm* function, and the residuals from the model used for mapping.

[b] This QTL is novel to this analysis, and was not identified using the raw peak amplitude data. The other three QTL in the table were identifed using raw phenotypes (see Table 3).

[c] Calculated via the R/qtl *fitqtl* function.

[d] Calculated via the R/qtl *fitqtl* function. Describes the phenotypic effect of substituting a FL-derived allele for a KS-derived allele.

[e] The statistical power to detect a QTL of the stated effect using our experimental design (see "Materials and Methods").

Table S7. QTL mapped by Gleason *et al.* (2016), annotated based on their relationship to the present study.

Data taken directly from Gleason *et al.* (2016, their Table 5)								Current study	
Phenotype	QTL num	Linkage group	Homologous chromosome in *Bombyx mori*	Genetic position (cM)	Likelihood ratio	Additive effect	Variance Explained (%)	QTL identified?	Linkage group
Development time	1	2	15	4.72	56.68	0.61	9.17	Yes (KS-BC)	7
	2 [a]	5	8	0.01	12.89	0.28	2.00	no	16
Body weight	1	2	15	2.67	33.34	–0.47	5.42	Yes (KS-BC)	7
	2	7	–	0.01	11.58	–0.27	1.83	no	3,13 [b]
Pulse-pair rate	1	1	Z	0.75	14.54	–1.77	2.42	no	Z
	2	7	–	0.01	11.68	1.58	1.93	no	3,13 [b]
Peak amplitude	1	2	15	5.08	18.94	–0.37	3.35	no	7
	2 [a]	9	11	6.55	10.63	–0.28	1.86	no	12

[a] Gleason *et al.* (2016) employed composite interval mapping, varying the "window size" used to select marker covariates to add to the mapping model. This pair of QTL was only identified when a specific window size was employed, while the remaining 6 QTL were robust to window size.

[b] This pair of QTL was resolved to a single linkage group that, in the current genetic map, is split into two different linkage groups. We were unable to recapitulate these QTL in our study, perhaps because of their small effects, but perhaps also because the Gleason *et al.* (2016) genetic map may have contained some inaccuracies due to the relatively small number of markers used to generate it.

Table S8. Number of MAKER2 annotated genes associated with each linkage group.

Linkage group IDs	Annotated Genes
Z	500
1	548
2	477
3	576
4	534
5	408
6	460
7	572
8	308
9	398
10	360
11	473
12	419
13	435
14	313
15	369
16	381
17	382
18	326
19	332
20	240
21	323
22	379
23	185
24	247
25	173
26	129
27	127
28	100
29	102
–	5,272 [a]
TOTAL	15,848

[a] This set of genes were annotated on scaffolds that could not be tied to linkage groups via our marker set.