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11 **Application of a dense genetic map for assessment of genomic responses to selection and**  
12 **inbreeding in *Heliothis virescens*.**

13

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19 **Abstract**

20           Adaptation of pest species to laboratory conditions and selection for resistance to toxins in the  
21 laboratory are expected to cause inbreeding and genetic bottlenecks that reduce genetic variation.  
22 *Heliothis virescens*, a major cotton pest, has been colonized in the laboratory many times, and a few  
23 laboratory colonies have been selected for Bt resistance. We developed 350 bp Double-Digest  
24 Restriction-site Associated DNA-sequencing (ddRAD-seq) molecular markers to examine and compare  
25 changes in genetic variation associated with laboratory adaptation, artificial selection, and inbreeding in  
26 this non-model insect species. We found that allelic and nucleotide diversity declined dramatically in  
27 laboratory-reared *H. virescens* as compared with field-collected populations. The declines were  
28 primarily due to the loss of low frequency alleles present in field-collected *H. virescens*. A further,  
29 albeit modest decline in genetic diversity was observed in a Bt-selected population. The greatest  
30 decline was seen in *H. virescens* that were sib-mated for 10 generations, where more than 80% of loci  
31 were fixed for a single allele. To determine which regions of the genome were resistant to fixation in  
32 our sib-mated line, we generated a dense intraspecific linkage map containing 3 PCR-based, and 659  
33 ddRAD-seq markers. Markers that retained polymorphism were observed in small clusters spread over  
34 multiple linkage groups, but this clustering was not statistically significant. Here, we confirmed and  
35 extended the general expectations for reduced genetic diversity in laboratory colonies, provided tools  
36 for further genomic analyses, and produced highly homozygous genomic DNA for future whole  
37 genome sequencing of *H. virescens*.

38 **Keywords:** *Heliothis virescens*, genetic variation, colonization, inbreeding, ddRAD-seq, linkage map

## 39 **Introduction**

40 Laboratory-reared insect colonies are important resources for many types of entomological  
41 experiments. They are used to quantify physiological or behavioral differences between insect  
42 populations or species (Dekker *et al.*, 2006; Dobzhansky & Spassky, 1954; Fritz *et al.*, 2015; Groot *et*  
43 *al.*, 2005; Shaw *et al.*, 2000; Sokolowski, 1980; Tomaru *et al.*, 2000), identify the genetic architecture  
44 of insect traits (Gahan *et al.*, 2010, Mackay *et al.*, 2012, Oppenheim *et al.*, 2012), develop insect  
45 populations that express desirable traits (Collins, 1984; Goldman *et al.*, 1986; Gould *et al.*, 1995; Hoy,  
46 1990; Pradeep *et al.*, 2005), and generate genetically modified species as a means of pest control (de  
47 Valdez *et al.*, 2011). A major concern for researchers maintaining insect colonies is the degree to which  
48 adaptation to the laboratory environment affects insect genotypic, and thereby phenotypic diversity  
49 (Boller, 1972; Huettel, 1976).

50 The phenotypic consequences of adaptation to the laboratory depend upon the trait of interest,  
51 and range from undetectable to severe (Baeshen *et al.*, 2014; Fox *et al.*, 2007; Gerloff *et al.*, 2003;  
52 Raulston, 1975; Roush, 1986). Observed phenotypic changes can be attributed to inadvertent selection  
53 for traits that are favorable in the laboratory environment (Roush, 1986), inbreeding depression (*i.e.*  
54 reduction in fitness caused by matings between related individuals; reviewed in Charlesworth & Willis,  
55 2009; Mackauer, 1976), or the interaction of the two. Indeed, the selection that occurs during colony  
56 establishment creates conditions conducive to inbreeding (Roush, 1986). Families with higher fitness  
57 under laboratory conditions contribute disproportionately to the reproductive pool, thereby increasing  
58 the probability of matings between related individuals. Where selection is very strong, as in the  
59 production of an insecticide resistant colony, measures must often be taken to minimize the effects of  
60 inbreeding and thereby inbreeding depression (Gould *et al.*, 1995). Overall, the expectation is that the  
61 selection and inbreeding that takes place during insect colonization results in an overall loss of genetic

62 diversity (Munstermann, 1994), and concomitant genome-wide increase in homozygosity (reviewed in  
63 Etzel & Legner, 1999).

64 Previous studies that have examined genetic differences between field-collected, laboratory-  
65 adapted, and inbred populations of non-model insects have primarily focused on Dipteran species and  
66 were limited to small numbers of molecular markers (Mukhopadhyay *et al.*, 1997; Munstermann, 1994;  
67 Norris *et al.*, 2001). Such small numbers of markers allow for estimation of the genome-wide average  
68 change in genetic variability across populations, but cannot be used to examine fine-scale patterns of  
69 genomic change. Examination of these patterns allows for identification of where and how genetic  
70 variation, the raw material necessary for environmental adaptation, is maintained (Dobzhansky &  
71 Spassky, 1954). The relatively recent development of high-throughput sequencing combined with  
72 reduced-representation DNA library preparation techniques allows for the discovery of hundreds to  
73 thousands of new molecular markers, even in species for which genomic data are absent (Davey *et al.*,  
74 2012). Here we used Double-Digest Restriction-Site Associated DNA Sequencing (ddRAD-seq;  
75 Peterson *et al.*, 2012), one type of reduced representation library preparation, for *de novo* construction  
76 of molecular markers in the non-model species, *Heliothis virescens*.

77 The tobacco budworm, *H. virescens*, is an historically important pest of cotton throughout much  
78 of the Southeastern United States (Blanco, 2012). This non-model Lepidopteran species has been  
79 colonized a number of times for investigations of mating and host-selection behaviors (Sheck & Gould,  
80 1995; Sheck & Gould, 1996; Sheck *et al.*, 2006), as well as detecting the underlying genetic basis for  
81 insecticide resistance (Gahan *et al.*, 2001; Gahan *et al.*, 2010; Taylor *et al.*, 1993). We used our newly  
82 developed ddRAD-seq markers to examine and compare the effects of colonization, selection, and sib-  
83 mating on *H. virescens* genome-wide measures of genetic diversity. To examine fine-scale patterns of  
84 change in genetic diversity, we also used our ddRAD-seq markers to generate a dense intraspecific

85 genetic map for *H. virescens*. This map consists of 659 high quality 350-bp markers which will serve  
86 as an important genomic resource to the entomological community.

87 Overall, our research aims to:

- 88 1. Quantify overall patterns of change in genomic diversity across field-collected, laboratory-  
89 reared (non-selected), Bt-selected, and sib-mated *H. virescens*.
- 90 2. Determine whether the observed degree of inbreeding in our sib-mated *H. virescens* calculated  
91 from our ddRAD-seq genotypic data matched theoretical expectations (Falconer & Mackay  
92 1996).
- 93 3. Use ddRAD-enabled linkage mapping to determine if specific genomic regions were resistant to  
94 fixation, even under intense inbreeding, by comparing genotypic data from long-term colony  
95 and sib-mated lines.

96

## 97 **Results**

98 We sequenced 204 *H. virescens* individuals from a total of 6 populations that were used in a  
99 population-level analysis of genomic change associated with laboratory colonization, artificial selection  
100 and inbreeding. These populations were comprised of 2 field-collected (LA, TX populations collected  
101 in 2012), 2 laboratory-reared (BENZ, YDK), and 1 Bt-selected population (YHD2), as well as  
102 specimens from a single inbred family following 10 generations of full-sibling mating (see Table 1 for  
103 information on population history, sample sizes, and read counts). Three of these populations, YDK,  
104 YHD2, and the inbred line were founded from a collection in Yadkin County, NC, in 1988 (Gould *et*  
105 *al.*, 1995), but were thereafter subjected to different rearing conditions, allowing us to make  
106 comparisons of population-genomic change within the same genetic background. In addition, 99  
107 individuals (1 BENZ parent, 1 BENZ-YHD2 hybrid parent and 97 progeny) were sequenced for

108 linkage analysis. This produced a total of 105,487,499 Illumina MiSeq reads (38,221,995 and  
109 67,265,504 for linkage- and population-level analyses, respectively) that passed quality filters (data  
110 available upon request).

### 111 *Genomic diversity among H. virescens populations*

112 On average, 338,892 sequencing reads (*s.d.* = 113,397) were produced per individual, and the  
113 variation in read count was spread uniformly across populations (Supplementary Figure 1). Ninety-  
114 four percent of individuals had read counts between 90,000 and 688,000, and these were fed into the  
115 Stacks pipeline (Catchen *et al.*, 2011; 2013) for *de novo* locus construction (Supplementary Figure 1).  
116 Loci constructed by Stacks had an average of 6× depth of coverage per individual. In total, 4,281  
117 polymorphic 350-bp ddRAD-seq markers (hereafter loci) were detected in at least one individual per  
118 population across all populations. Two well-documented challenges commonly encountered when  
119 working with moderate coverage reduced representation library data like ours are: 1) uneven  
120 distribution of missing data across sets of loci (Davey *et al.*, 2012; Xu *et al.*, 2014), and 2) under-  
121 sampling of heterozygotes (Li *et al.*, 2009; Nielsen *et al.*, 2011). Both reduce confidence in final  
122 genotypes called by genotyping-by-sequencing SNP calling algorithms, including the algorithm used in  
123 Stacks. To overcome these challenges, we examined several subsets of these 4,281 polymorphic loci  
124 for our downstream population genomic analyses. These subsets contained between 125-1231 loci, and  
125 were chosen based upon the overall proportion missing genotype calls in the subset. The smallest  
126 subset consisted of loci for which over 75% of individuals per population had genotypic data present  
127 and were therefore likely sequenced to greatest depth of coverage. Each larger subset allowed  
128 additional loci at the expense of coverage (*i.e.* more missing genotypic data were allowed;  
129 Supplementary Table 1). By using multiple datasets, we were able to examine whether the presence of  
130 missing genotype calls influenced overall estimates of genomic diversity across populations.

131 We first examined all subsets of loci and determined the mean and maximum numbers of alleles  
132 per locus (Supplementary Table 1). In our case, alleles were not analogous to SNPs, but rather the  
133 accumulation of SNPs per 350 bp locus per individual. For the total sequenced population ( $n = 192$   
134 total *H. virescens*), the mean numbers of unique alleles detected per locus ranged from 29 to 34  
135 depending upon the number of loci included in the analysis. As more loci were included, the average  
136 number of unique alleles detected per locus decreased. However, the maximum number of unique  
137 alleles detected in the total population increased from 86 in the smallest subset of loci to 94 in the 3  
138 larger subsets. We also examined the proportion of loci that were fixed (*i.e.* only a single allele  
139 present) across populations. Across subsets, few loci were fixed for a single allele in laboratory-reared  
140 (5.6-10.9%), Bt-selected (5.3-7.3%) and field-collected (0-2.4%) populations (Supplementary Table 1).  
141 Yet over 80% of loci were fixed in the inbred line following 10 generations of sib-mating  
142 (Supplementary Table 1). Of the 125 loci with the fewest missing genotype calls, 86% were fixed in  
143 the inbred line. Expanding the number of loci to include those with more missing genotypes ( $n = 378,$   
144 573, 1231) reduced the percentage of fixed loci in the inbred line by up to 5% (Supplementary Table 1).

145 We then determined the mean number of unique alleles present per locus for each subset of loci  
146 (Supplementary Figure 2). In general, we found no within population differences in the mean numbers  
147 of unique alleles detected among subsets of loci, and therefore we used a single, conservatively chosen  
148 subset of loci ( $n = 378$ ) where at least 10 individuals were genotyped per population per locus for  
149 further analysis. The mean numbers of unique alleles per locus were 2.1 for the inbred line, 5.3 for the  
150 Bt-selected population, 5.4 and 4.4 for the non-selected, laboratory-reared populations (YDK and  
151 BENZ, respectively), and 18.4 and 17 for the field-collected populations (LA and TX, respectively).  
152 However, our sample sizes (*i.e.* numbers of individuals sequenced; see Table 1) differed for each  
153 population, and it was unclear whether differences between the aforementioned means were caused by

154 sample size or population-level differences. For example, it is to be expected that as sample size  
155 increases there will be an increase in the probability of sampling additional, likely rare, alleles.  
156 Therefore, we randomly sub-sampled pools of alleles 6, 12, 18, and 24 times without replacement for  
157 each population. This allowed us to hold sample sizes constant across populations, and infer whether  
158 the mean numbers of unique alleles truly differed by population. As expected, we found that increasing  
159 the total number of alleles sampled led to an increase in the mean numbers of unique alleles per locus  
160 for all but the inbred line (Figure 1). Yet we also found strong population-level differences.  
161 Regardless of the number of alleles sampled, field populations always exhibited the greatest allelic  
162 diversity, followed by selected and non-selected colony populations. The lowest allelic diversity was  
163 observed in the inbred line.

164         When 18 alleles were randomly sampled per population per locus, we detected an average of  
165 just over 1 unique allele per locus in the inbred line, indicating that most loci were fixed for a single  
166 allele. For the inbred line, 52 of the 378 loci did not reach fixation. Of these, forty-seven had 2 unique  
167 alleles, four had 3 unique alleles, and one had 4 unique alleles when 18 were randomly sampled. On  
168 average, Bt-selected and non-selected colony strains each had *ca.* 3 unique alleles per locus, and field-  
169 collected populations had *ca.* 9 unique alleles per locus (Figure 1). The majority of unique alleles  
170 present in the field-collected populations (70.3% and 68.7% for LA and TX populations, respectively)  
171 were observed only once (of 18 alleles; Figure 2). Such low frequency alleles were less common in the  
172 Bt-selected and non-selected laboratory populations (35.1% and 21.5%, respectively) and rare (0.7%)  
173 in the sib-mated inbred line (Figure 2).

174         We used the same conservative subset of 378 loci, and calculated sample-size corrected  $S_K$   
175 (Charlesworth & Charlesworth, 2010), and  $\pi$  (Nei 1978). These two measures are complementary:  $\pi$  is  
176 calculated as the proportion of nucleotides that differ per two randomly chosen DNA sequences,



177 averaged across all pairwise comparisons per marker per population, and  $S_K$  is calculated as the number  
178 of unique single nucleotide variants in a population at a single locus. When averaged across all  
179 markers ( $n = 378$ ), the number of variant sites ( $S_K$ ) per 350 bp marker was 0.15 for the inbred line, and  
180 the maximum  $S_K$  was 2.95. Bt-selected and non-selected laboratory populations had, on average, just  
181 over 1 nucleotide variant per 350 bp locus, with a maximum of *ca.* 6. Field-collected populations had  
182 the greatest number of variant sites per 350 bp locus, where the genome-wide average was just over 5  
183 nucleotide variants per locus, with a maximum of *ca.* 15. Similar trends were observed for genome-  
184 wide and maximum nucleotide diversity ( $\pi$ ) values. Relative to the laboratory-reared populations,  
185 genome-wide estimates of  $\pi$  were nearly an order of magnitude lower for the inbred line. The genome-  
186 wide  $\pi$  estimate for laboratory-reared populations ranged from  $4.0 \times 10^{-3}$  (Bt-selected population) to  
187  $6.7 \times 10^{-3}$  (non-selected, YDK population), and  $6.2 \times 10^{-4}$  for the inbred line. Field-collected populations  
188 exhibited genome-wide  $\pi$  estimates of  $9.4 \times 10^{-3}$  and  $9.2 \times 10^{-3}$  for the LA and TX populations,  
189 respectively. Genome-wide and maximum  $\pi$  and  $S_K$  estimates, along with their corresponding 95%  
190 non-parametric bootstrapped confidence intervals ( $N = 5000$ ) are reported in Table 2.

191 To further quantify and compare genetic diversity by population, we also examined mean  
192 observed heterozygosity in the total population ( $n = 192$  total *H. virescens*), as well as within each sub-  
193 population. Observed heterozygosity for the total population ( $n = 192$ ) was 0.27 when averaged across  
194 the 378 loci, and considerable variation in heterozygosity existed between populations. Mean observed  
195 heterozygosities ranged from 0.06 in the inbred line to 0.40-0.46 in field-collected populations. For  
196 laboratory-reared populations, mean observed heterozygosity estimates were intermediate to those of  
197 the inbred line and field-collected populations, and ranged from 0.15 in the Bt-selected population to  
198 0.22-0.25 in the non-selected laboratory populations (Table 2).

199

200 *Genomic divergence among populations*

201       To examine the degree to which the above genetic diversity could be attributed to between  
202 population differences, we calculated pairwise estimates of  $F_{ST}$  (Table 3) according to Weir and  
203 Cockerham (1984). Despite the *ca.* 400 km distance between collection locations for the field  
204 populations, very little (0.4%) of the genetic diversity observed in these populations could be attributed  
205 to differences between populations. When field populations were compared with non-selected  
206 laboratory-reared populations, 16-25% of the genetic variation could be attributed to differences  
207 between populations. Additional inbreeding and selection further exacerbated these differences,  
208 increasing the percentage of variation attributable to between population differences to over 30%. Of  
209 particular interest was the comparison between the ancestral non-selected YDK population, with the  
210 more derived Bt-selected (YHD2) and inbred populations. Despite their shared ancestry, a comparison  
211 of YDK to YHD2 and the inbred line revealed that 28% and 33% of the existing genetic variation could  
212 be attributed to between population differences, respectively.

213

214 *Inbreeding among laboratory-reared H. virescens populations*

215       To determine whether the observed degree of heterozygosity in our inbred line was consistent  
216 with that which would be expected following 10 generations of sib-mating, we compared the  
217 inbreeding coefficient  $F$ , as calculated according to pedigree- (Falconer & Mackay, 1996) and DNA  
218 marker-based information (Keller & Waller, 2002; Kim *et al.*, 2007). The expected inbreeding  
219 coefficient ( $F_e$ ), following 10 generations of sib-mating was 0.89. This expected value fell within the  
220 bootstrapped 95% confidence intervals for marker-based inbreeding coefficients ( $F_{IT}$ ) calculated from  
221 all subsets of markers. This indicated that there was no significant difference between the expected  
222 inbreeding coefficient and the observed inbreeding coefficient calculated using ddRAD-seq marker

223 data. The genome-wide  $F_{IT}$  values (95% CIs) were 0.92 (0.88, 0.96), 0.89 (0.86, 0.92), 0.89 (0.87,  
224 0.92), and 0.88 (0.86, 0.89), for the inbred line as calculated from 125, 378, 573, and 1231 ddRAD-seq  
225 loci, respectively.

226

### 227 *Linkage mapping*

228 Few genomic resources are available for *H. virescens*. Therefore, we determined the genomic  
229 location of loci which were resistant to fixation by generating a dense linkage map. The map was  
230 produced via ddRAD-sequencing of the parents and progeny from a male informative cross (reviewed  
231 in Baxter *et al.*, 2009). We generated an average of 381,096 sequencing reads (*s.d.* 165,334) per  
232 progeny, as well as 493,537 and 762,171 reads per the male and female parents, respectively  
233 (Supplementary Figure 3). From this, we produced a linkage map comprised of 659 informative  
234 ddRAD-seq loci, plus 3 partial gene sequences of *ABCC2*, *HevCaLP*, and *Desat1*. Adding these partial  
235 gene sequences to our linkage map, all with known locations in the *B. mori* genome allowed us to  
236 validate marker groupings for our linkage map. All informative ddRAD-seq loci were grouped into 33  
237 linkage groups, two more than the expected 30 *H. virescens* autosomes, and one segregating Z  
238 chromosome from the hybrid male parent used in our cross. Linkage groups ranged in size from 7cM  
239 to 110cM (Figure 3), and yielded a total map length of 1919.5 cM. On average, there were 20  
240 ddRAD-seq loci per linkage group, and the average spacing was one locus per 3.5 cM. The smallest  
241 and largest linkage groups contained 3 and 53 loci, respectively. The *HevCaLP*, *Desat1*, and *ABCC2*  
242 genes were grouped with linkage groups 15, 16, and 22, respectively. These linkage groups  
243 corresponded to *B. mori* chromosomes 6, 23, and 15 (Table 4), where these candidate genes are known  
244 to reside (Gahan *et al.*, 2001; Gahan *et al.*, 2010; Mita *et al.*, 2004).

245 In total, 99 of the 659 mapped ddRAD-seq loci could be aligned uniquely to a single locus in

246 the *B. mori* genome. Twenty-two linkage groups contained ddRAD-seq loci that could be aligned to a  
247 single *B. mori* chromosome (Table 4), while 8 linkage groups did not contain any that could be aligned.  
248 Linkage groups 19, 25, and 33 contained ddRAD-seq loci that aligned uniquely to more than one *B.*  
249 *mori* chromosome. This was unlikely caused by spurious associations between ddRAD-seq loci;  
250 increasing the LOD score to 8 failed to break up associations for those three linkage groups.

251

### 252 *Identification of genomic regions resistant to fixation*

253 To determine where genetic diversity was being maintained in the genome, we examined  
254 observed heterozygosity and nucleotide diversity at the 659 mapped ddRAD-seq loci for one field-  
255 collected (LA), the Bt-selected (YHD2), one non-selected (YDK), and inbred lines. Of these 659  
256 mapped loci, 302 (46%) were previously included in our population-level analyses, and 357 had not  
257 been previously analyzed. For each mapped locus, observed heterozygosity and nucleotide diversity  
258 ( $\pi$ ) values were only calculated if at least 3 individuals were genotyped per population. Therefore only  
259 441 loci were examined for the inbred line, 658 loci were examined for the non-selected (YDK)  
260 population, 659 loci were examined for the Bt-selected population, and 546 loci were examined for the  
261 field-collected population. In total, 13% ( $n = 60$ ) of mapped loci retained polymorphism in the inbred  
262 line, whereas 98% ( $n = 645$ ), 86% ( $n = 583$ ), and 99% ( $n = 543$ ) of mapped loci retained  
263 polymorphism in the Bt-selected, non-selected (YDK), and field-collected (LA) populations,  
264 respectively. For each of these populations, levels of observed heterozygosity and nucleotide diversity  
265 across the genome are compared in Figure 4.

266 Within the inbred line, mapped loci that retained polymorphism were spread over 16 linkage  
267 groups (Figure 4), and often appeared in clusters within a linkage group (Supplementary Figure 4).  
268 Linkage groups 7 and 11 had particularly high numbers of polymorphic markers relative to other

269 linkage groups. Therefore, we examined whether clustering of polymorphic markers in our inbred line  
270 was greater than could be expected due to random chance. Such clustering may point to the presence  
271 of large chromosomal inversions as observed in other insect species (Turissini et al. 2014). A replicated  
272 G-test of independence (Sokal and Rohlf, 1995) demonstrated that 8 linkage groups contained  
273 significantly more polymorphic loci than would be expected following 10 generations of inbreeding  
274 (Supplementary Table 2). Yet based upon the non-significant heterogeneity g-value ( $G = 23.87$ ,  $df =$   
275  $23$ ,  $p$ -value = 0.41) we could not reject the null hypothesis that the distribution of polymorphic loci was  
276 homogeneous across linkage groups, and the clustering observed within some linkage groups did not  
277 significantly differ from that which could be observed by chance.

278 We also examined whether balancing selection, perhaps due to balanced lethal systems, may be  
279 responsible for the residual polymorphism observed in the inbred line. To this end, we calculated and  
280 compared Tajima's D values at each polymorphic locus for the inbred line and its ancestral population  
281 (YDK). Tajima's D is a statistical test which identifies departures from the neutral model of molecular  
282 evolution. A positive Tajima's D value at a locus indicates an excess of intermediate frequency alleles,  
283 thereby signifying either a recent population contraction, or balancing selection at that locus. We  
284 reasoned that positive Tajima's D values in the inbred line would be much less likely to signify a recent  
285 population contraction, and therefore more likely to indicate balancing selection, if these same regions  
286 were also significant in their ancestral population (YDK). Indeed, YDK had not undergone any  
287 obvious population contractions around the time of this separation 10 generations prior. Therefore, we  
288 examined which, if any, of these polymorphic loci in the inbred line shared strongly positive Tajima's D  
289 values with YDK. When we calculated Tajima's D values for the 60 polymorphic sites remaining in the  
290 inbred line, 21 were significantly positive ( $\alpha = 0.05$ ). Following a Benjamini-Hochberg correction for  
291 multiple comparisons (Benjamini and Hochberg, 1995), only two loci remained statistically significant.

292 Furthermore, neither of these two polymorphic loci from the inbred line overlapped with loci showing  
293 significantly positive Tajima's D values in YDK. Thus we found little evidence of ongoing balancing  
294 selection in our inbred line.

295

## 296 **Discussion**

297 Here, we examined the degree to which colonization, artificial selection, and intense inbreeding  
298 influence genome-wide and fine-scale patterns of diversity. In the absence of a publicly available *H.*  
299 *virescens* reference genome, we used ddRAD-seq *de novo* locus construction to identify multiple  
300 subsets of polymorphic loci ranging in size from 125 to 1231 markers. Genome-wide measures of  
301 allelic diversity,  $F_{IT}$  values, and the degree of homozygosity were either unaffected (Supplementary  
302 Figure 2), or minimally affected (Supplementary Table 1) by inclusion of markers with high levels of  
303 missing genotypic data. Therefore, any biased genotype calls made by the Stacks SNP calling  
304 algorithm due to our moderate depth of sequencing coverage had little impact on our overall genome-  
305 wide estimates of diversity. Our results demonstrate that moderate coverage ddRAD-seq data can be  
306 used with confidence when conducting population genomic comparisons of genome-wide means.

307 We observed a precipitous decline in nucleotide and allelic diversity following long-term  
308 laboratory colonization, selection, and inbreeding for *H. virescens*. Despite the decline in genomic  
309 diversity for non-selected and Bt-selected laboratory-reared populations, fewer than 10% of loci were  
310 fixed. While our Bt-selected population did not retain the level of genetic diversity that their ancestral  
311 (YDK) laboratory-reared population did, they consistently had higher measures of genomic diversity  
312 than did the non-selected (BENZ) population. Retention of higher levels of polymorphism in our Bt-  
313 selected line was likely due to the measures taken during its generation to ensure genomic diversity was  
314 maintained in the face of strong selection (Gould, 1995). Alternatively, strong genetic bottlenecks in

315 the non-selected (BENZ) population prior to their use in our study could explain why our Bt-selected  
316 line was more genetically diverse than the BENZ non-selected line. Overall, differences among Bt-  
317 selected and non-selected laboratory-reared populations were modest; when 18 total alleles were  
318 sampled, laboratory-reared populations retained *ca.* 3 alleles per 350-bp locus relative to 9 alleles per  
319 350-bp locus present in field-collected *H. virescens*. However, few low frequency alleles remained in  
320 the laboratory-reared populations relative to the field-collected populations (Figure 2), which has been  
321 observed elsewhere (Munstermann, 1994). For this reason, laboratory-reared populations are generally  
322 considered inbred (Roush, 1986). In the case of *H. virescens*, our results clearly show that a great deal  
323 of genomic diversity is retained, even following decades in colony. Few genome assembly algorithms  
324 accommodate polymorphism well (Kajitani *et al.*, 2014), and it is clear that the reductions in  
325 heterozygosity in our inbred line will be useful for production of a high quality *H. virescens* reference  
326 genome assembly.

327         To determine the degree to which the genomic diversity detected above could be attributed to  
328 differences between populations, we calculated pairwise  $F_{ST}$  values according to Weir and Cockerham  
329 (Table 3). An  $F_{ST}$  value of 0.0004 demonstrated that most of the existing genetic diversity occurred  
330 within, as opposed to between, these two field-collected populations of *H. virescens*. This result is  
331 similar to that of Groot *et al.* (2011), which described over 98% of the genetic variation detected across  
332 North American *H. virescens* to be found within populations. Both studies suggest that extensive gene  
333 flow occurs naturally among geographically disparate *H. virescens* populations. Although we did not  
334 sample field-collected populations from Yadkin County, NC, nor from Stoneville, MS, the original  
335 collection sites of our laboratory-adapted YDK and BENZ populations, our results along with those of  
336 Groot *et al.* (2011), suggest that genomic divergence among all four sites would have been low. To  
337 examine how decades of laboratory rearing could influence the structure of genomic diversity in

338 laboratory-adapted populations, we compared our YDK and BENZ populations to our field-collected  
339 populations. As would be expected, we observed an increase (16-25%) in the percentage of genetic  
340 variation that could be attributed to between population differences. This is likely due to the purging  
341 of rare alleles (as shown in Figure 2) and the fixation of others as caused by the random process of  
342 genetic drift. Further manipulations, like Bt-selection and full-sibling mating increased that percentage  
343 of between population genetic variation to well over 30% relative to the field-collected populations.  
344 Additional comparisons between ancestral YDK and the more derived Bt-selected and inbred  
345 populations further underscore the affects that these population-level manipulations can have on insect  
346 colonies in a laboratory setting. Although they were derived from YDK, following Bt-selection and  
347 over 2 decades of separation in the laboratory, 28% of the total genetic variability could be attributable  
348 to between-population differences. Likewise, 10 generations of full-sibling mating resulted in over  
349 32% of the total genetic variability existing between YDK and the inbred line.

350       Following 10 generations of inbreeding, over 80% of markers went to fixation in our sib-mated  
351 *H. virescens* population. Indeed, our inbreeding coefficient  $F_{IT}$ , as observed from our ddRAD-seq data,  
352 met theoretical expectations for all subsets of loci. Our *H. virescens* laboratory population was more  
353 amenable to inbreeding than other insect species (Munstermann, 1994; Rumball *et al.*, 1994; Turissini  
354 *et al.*, 2014; You *et al.*, 2013), despite their relatively high levels of genomic diversity (Figure 1) and  
355 genetic load (Supplementary Figure 5). Higher than expected allelic diversity has been observed in  
356 several other insect species following experimental inbreeding attempts (Munstermann, 1994; Rumball  
357 *et al.*, 1994; Turissini *et al.*, 2014; You *et al.*, 2013). As one example, only 57% of the *An. gambiae*  
358 genome went to fixation, as observed according to SNP markers, following 10 generations of  
359 inbreeding (Turissini *et al.*, 2014). Observed differences between our *H. virescens* population and  
360 other insects could be species specific, but is more likely related to the proportion of the genome



361 containing balanced lethal systems (Falconer & Mackay, 1996).

362 To determine where heterozygosity was being maintained in our inbred line, we developed a  
363 high density genetic linkage map for *H. virescens*. Our map contained 659 newly developed 350 bp  
364 ddRAD-seq markers that are long enough for future primer design and direct sequencing. This map  
365 represents a new tool for an historically important pest species that lacks genomic resources. No  
366 ddRAD-seq markers remained unlinked following mapping, which indirectly speaks to the quality of  
367 our linkage map. However, the number of groups in our linkage map was 2 more than expected  
368 ( $n = 31$ ). This is likely due to the relatively small mapping population size used in this work; other  
369 mapping studies that analyzed segregating populations of a similar size have also reported genetic maps  
370 with excess numbers of linkage groups (Pootakham *et al.*, 2015; Singh *et al.*, 2009). Additional  
371 explanations for the disparity between our observed and expected number of linkage groups include the  
372 uneven distribution of markers over the chromosomes (Paterson, 1996), or recombination ‘hotspots’,  
373 which make it difficult to reduce the number of linkage groups to 31.

374 When we applied our linkage map to examine fine-scale patterns of genomic change following  
375 10 generations of sib-mating, we found that several linkage groups seemed to contain clusters of loci  
376 that retained polymorphism (Figure 4). However, results from a replicated G-test of independence  
377 demonstrated that clustering of these polymorphic loci was not significantly different from what could  
378 be expected due to chance. This does not preclude the possibility that chromosomal inversions exist  
379 among populations of *H. virescens*. Rather it suggests that there were no large inversions responsible  
380 for maintaining polymorphisms in our inbred line.

381 Furthermore, only 2 polymorphic loci had significantly positive Tajima's D values, and neither  
382 overlapped with those loci that had high D values in YDK. This made it impossible to rule out  
383 population contraction as a contributor to the positive Tajima's D values. Unlike the findings for other

384 insects (Mackay et al. 2012, Turissini et al. 2014), the combined results of our  $F_{IT}$  test, replicated G-  
385 test, and Tajima's D tests suggest that neither balanced lethals nor large chromosomal inversions appear  
386 to play a major role in retention of polymorphism for this particular *H. virescens* inbred line.

387

## 388 **Conclusions**

389 This work serves as one of the most thorough attempts to quantify the effects of genomic  
390 responses to selection and inbreeding in a non-model insect species. We demonstrated that laboratory-  
391 reared *H. virescens* have reduced allelic and nucleotide diversity relative to field-collected populations,  
392 and that inbreeding further diminishes genetic diversity. Although we identified several loci that did  
393 not go to fixation in *H. virescens* following 10 generations of inbreeding, our ddRAD-seq marker-based  
394  $F_{IT}$  values met theoretical expectations. This work demonstrates the difficulty involved in producing  
395 fully homozygous insect strains, which are currently critical to producing high-quality, complete  
396 reference genomes.

397

## 398 **Methods**

### 399 *Field-collected H. virescens*

400 Adult male moths were collected from Bossier Parish, Louisiana, and College Station, Texas  
401 using pheromone-baited live traps. Collections took place in LA from May through September, 2012,  
402 and in TX from May through October, 2012. Moths from each collection date were immediately  
403 placed in bottles of 95% ethanol for long-term storage. All bottles were held at -20 °C until DNA  
404 isolations took place.

405

### 406 *H.virescens colonies*

407 *H. virescens* were collected from Yadkin County, NC in 1988 (Gould *et al.*, 1995). This original  
408 population founded two of the colonies used in this study, each of which had been reared in the  
409 laboratory for *ca.* 290 generations. YHD2 was selected for high levels of Bt resistance for 4 years (up  
410 to 48 generations) on MVP- treated (0.864 mg/mL diet; Mycogen, San Diego, CA) corn-soy diet  
411 (Gould *et al.*, 1995), whereas a non-selected population (YDK) was reared on corn-soy diet alone. A  
412 third population (BENZ) originating from Stoneville, MS, was acquired from Benz Research  
413 Incorporated (Carlisle, PA) and had been reared in the laboratory for over 10 years (120 generations).  
414 BENZ *H. virescens* were acquired in their pupal stage, and newly eclosed adults were used for  
415 population-level comparisons. To produce an inbred population, single pair matings (SPMs) were set  
416 up between YDK siblings for 10 generations. An initial 37 SPMs were used to establish 29 lineages in  
417 filial generation one (8 single pair matings did not produce progeny). When SPMs failed to produce  
418 offspring, likely due to inbreeding depression, surviving lineages were expanded (Supplementary  
419 Figure 5). This was done to extend inbreeding for as many generations as possible, thus promoting as  
420 complete a reduction in heterozygosity as possible. Adult males from each laboratory-reared  
421 population were killed by freezing (-20 °C), and stored at -80 °C until DNA isolation.

422

#### 423 *Mapping cross*

424 A non-selected female from the BENZ population was crossed to a Bt-selected (YHD2) male in  
425 a single pair mating. One hybrid male offspring was then back-crossed to a Bt-selected (YHD2)  
426 female, and their progeny were reared to adulthood on untreated corn-soy diet according to Joyner and  
427 Gould (1985). Of the 120 progeny, 97 reached adulthood. Parents and their 97 adult progeny were  
428 killed by freezing and stored until DNA isolation as described above.

429

430 *Genomic DNA library preparation*

431 All DNA was isolated from the adult thorax using a Qiagen Dneasy Blood and Tissue Kit  
432 (Qiagen, Inc., Valencia, CA, U.S.A.). Genomic DNA samples were prepared for Illumina sequencing  
433 according to the Poland *et al.*, (2012) protocol with minor modifications. Two-hundred ng of DNA per  
434 individual were digested with EcoRI and MspI. For each individual, the overhang sites were ligated to  
435 standard Truseq Universal adapters (Illumina, Inc. San Diego, CA). Adapters ligated to EcoRI  
436 overhang sites contained one of 48 unique barcodes (Elshire *et al.*, 2011; Supplementary Table 3).  
437 DNA fragments from each individual were assigned a unique barcode, and individuals were combined  
438 into pools of no more than 48 individuals. A Pippin Prep (Sage Science, Inc., Beverly, MA) was used  
439 to select adapter-ligated DNA fragments ranging from 450-650 bp from each pool. Size-selected DNA  
440 fragments were amplified in a Peltier PTC200 thermalcycler (here and throughout) using Illumina  
441 primers (Supplementary Table 4) under the following conditions: 72 °C for 5min, 18 cycles of 98 °C  
442 for 30 sec, 65 °C for 20 sec, 72 °C for 30 sec, followed by 72 °C for 5 min. For each pool, 1 of 4  
443 Illumina indices was added via PCR to the MspI adapter. Therefore, sequences from each individual  
444 could be identified by the unique combination of barcode and index. A complete list of barcodes and  
445 indices used in this study can be found in the Supplementary Tables 3 and 4, respectively. Amplified  
446 libraries were pooled, cleaned with a Qiaquick PCR Purification Kit (Qiagen, Inc., Valencia, CA,  
447 U.S.A.), and diluted to 4nM prior to sequencing. Prepared genomic DNA libraries constructed from  
448 303 *H. virescens* individuals were spread across 9 full and partial Illumina MiSeq runs. The MiSeq  
449 reagent kit v. 2 was used for initial preparation of the mapping family and the inbred line. All  
450 subsequent preparations, including re-runs of the mapping family and inbred line were prepared with  
451 the MiSeq reagent kit v. 3.

452

453 *De novo marker formation*

454 Overlapping paired-end reads were merged with FLASH (Magoc & Salzberg, 2011), and Stacks  
455 v. 1.09 (Catchen *et al.*, 2011; 2013) was used for demultiplexing and *de novo* formation of loci.  
456 Merged paired-end reads were filtered for quality using the process\_radtags script. Further quality  
457 filtering entailed removal of reads when: 1) they did not have an intact EcoRI cut site, 2) had a quality  
458 score < 30, or 3) were smaller than 350 bp. We did not allow process\_radtags to rescue reads where  
459 barcode sequences contained an error. All remaining merged reads were truncated at a length of 350  
460 bp, and fed into the Stacks pipeline.

461

462 *Stacks parameter settings*

463 Reads from all individuals were run through ustacks with the following parameter settings: -m  
464 3, -M 14 (allowing for 5% nucleotide mismatch rate between alleles per individual), -max\_locus\_stacks  
465 2, --alpha 0.05. A consensus catalog of loci was first formed using the parents of the mapping cross  
466 with cstacks, where the -n 14 parameter allowed for a 5% between individual nucleotide mismatch rate.  
467 For the mapping family, genotype calls were made using sstacks prior to field- and colony-strain alleles  
468 being added to the catalog. Progeny genotypes were automatically corrected using the Stacks  
469 genotypes script. Twenty-four individuals of each colony and field-collected strain collected in 2012  
470 were later added to the catalog, and all field-collected and laboratory-reared populations were also  
471 genotyped using sstacks.

472

473 *Data analyses*

474 All population genomic and linkage analysis were conducted in R version 3.1.2 (R core team,  
475 2014).

476

477 *Genomic diversity among H. virescens populations*

478 In total, we sequenced the 13 surviving males from an inbred line subjected to 10 generations of  
479 sib-mating, 42-46 males per colony strain, and 30 males per field-collected population (Table 1). Prior  
480 to running sequence data through the Stacks pipeline, we checked individual read counts across  
481 populations to ensure uniformity (Supplementary Figure 1). Twelve of the 204 individuals sequenced  
482 had too few (< 90,000) or too many reads (> 688,000) and were removed from the dataset prior to  
483 analysis, following Bi *et al.* (2013). From our Stacks output, we constructed 4 different sets of  
484 consensus loci present across populations. These subsets, containing a core overlapping set of 125 loci,  
485 and increasing in size from 125 to 1231, consisted of marker sets with varying percentages of missing  
486 genotype calls (range = 11.2-29.5%) (Supplementary Table 1). We used these 4 different subsets to  
487 examine and compare changes in genomic diversity across populations.

488 We estimated the mean number of unique alleles present per locus, and corresponding 95% non-  
489 parametric bootstrapped confidence intervals (N = 5000) across populations using a custom-written R  
490 script. Each allele represented the accumulation of SNPs within a 350bp locus, analogous to a  
491 haplotype. At a given locus, and within each population of size N (see Table 1 for sample sizes), alleles  
492 (total = 2N) were randomly sampled without replacement either 6, 12, 18, or 24 times. Then the  
493 number of unique alleles were counted for each sampling regime. Due to their small sample size  
494 resulting from intensive inbreeding, we only sampled 6, 12, and 18 alleles per locus for the inbred line.  
495 Our analysis focused primarily on the subset of loci containing 378 consensus loci because genotype  
496 calls were present for at least 10 individuals per population. We also calculated two measures of  
497 nucleotide diversity per 350 bp locus using the R package, pegas (v. 0.6; Paradis, 2010):  $\pi$  (Nei, 1987)  
498 and  $S_K$  corrected for sample size (Charlesworth & Charlesworth, 2010; Watterson, 1975). We then

499 generated population-level genome-wide means and 95% non-parametric bootstrapped (N = 5000)  
500 confidence intervals for each metric (Table 2).

501

### 502 *Genetic divergence between populations*

503 To determine the degree of genetic diversity accounted for by differences between our field-  
504 collected, laboratory-reared, Bt-selected, and inbred populations, we calculated Weir and Cockerham's  
505  $F_{ST}$  (Weir and Cockerham, 1984) along with corresponding 95% bootstrapped confidence intervals (N =  
506 5000). Calculations were carried out using the R package, *diveRsity* (v. 1.9.89; Keenan *et al.*, 2013).

507

### 508 *Estimating the inbreeding coefficient*

509 To estimate our marker-based inbreeding coefficient, we examined multiple sets of loci  
510 (Supplementary Table 1) and found that trends across all datasets were similar (data not shown).  
511 However, we reported  $F_{IT}$  values from a set of 378 loci because this reduced dataset contained few  
512 missing genotypes per population, while still making inferences from several hundred loci. We  
513 calculated  $F_{IT}$  for the inbred line relative to the non-selected (YDK) population after Keller and Waller  
514 (2002), where  $(1-F_{IS})(1-F_{ST}) = 1-F_{IT}$ .  $F_{IS}$  was the level of inbreeding within the inbred line, calculated  
515 as  $F_{IS} = 1-(H_O/H_E)$ , where  $H_O$  and  $H_E$  were calculated for each locus using the R package *adegenet* (v.  
516 1.4-2; Jombart, 2008).  $F_{ST}$  was the accumulated effect of inbreeding over time, calculated as 1-  
517  $(H_E(\text{inbred line})/H_E(\text{YDK}))$ .

518

### 519 *Amplification and genotyping of PCR-based markers for the mapping family*

520 Progeny from the mapping family were genotyped at three additional loci, and these loci were  
521 mapped alongside our ddRAD-seq markers to validate our linkage groupings. We targeted the

522 previously described *H. virescens* genes *DesatI*, *ABCC2*, and *HevCaLP* via PCR followed by gel  
523 electrophoresis, or direct sequencing. Amplification and genotyping protocols were as follows.

524 A 468 bp fragment from *DesatI* was amplified in a 30  $\mu$ L reaction with forward and reverse  
525 primers [5'-TGAGGGACCATCGTCTCCAT-3'] and [5'-CACTGCTACATTTTGGGCAG-3'],  
526 respectively (Ward, 2009). Each reaction contained 6  $\mu$ L of 5 $\times$  GoTaq buffer (ProMega), 29  $\mu$ M per  
527 dNTP, 92 ng per primer, 0.75 U GoTaq polymerase, and *ca.* 1  $\mu$ g genomic DNA. Sample DNA was  
528 amplified alongside a negative control (here and throughout), where pcr-grade H<sub>2</sub>O was substituted for  
529 genomic DNA. Reactions were incubated at 95°C for 1 min followed by 35 cycles of 95°C for 1min,  
530 52°C for 1min, and 72°C for 2 min. PCR products were purified using a standard ethanol precipitation,  
531 and directly sequenced on an ABI3730xl (Applied Biosystems, San Francisco, CA). A single  
532 nucleotide polymorphism (cytosine to tyrosine substitution) at bp 36 was found in the YHD2 parent of  
533 the mapping cross. Offspring were genotyped at this locus using PolyPhred (Nickerson *et al.*, 1997),  
534 and genotype calls were visually confirmed using consed (Gordon *et al.*, 1998).

535 An intronic region of the *ABCC2* gene previously described by Gahan *et al.* (2010) was  
536 amplified using primers Hs-ABC2dU02-F1 [5' – TGGTTACAAGAAATAGAAAATGCAAC-3'] and  
537 Hs-ABC2eU03-R2 [5' – CTTTCAAACCTGAACCGCATCAC – '3]. Each 30  $\mu$ L reaction volume  
538 consisted of 6  $\mu$ L of 5 $\times$  GoTaq buffer, 29  $\mu$ M per dNTP, 73 ng per primer, 0.75 U GoTaq polymerase,  
539 and 1  $\mu$ g genomic DNA. Reactions were held at 95°C for 2 min followed by 30 cycles of 95°C for 30  
540 sec, 58°C for 30 sec, and 72°C for 40 sec, and the resulting products were cleaned via ethanol  
541 precipitation. Following sequencing on an ABI3730xl, chromatogram files were visualized using  
542 FinchTV (version 1.3.1, PerkinElmer, Inc., Seattle, WA). As described by Gahan *et al.* (2010), the  
543 YHD2 parent was homozygous for a 22 bp deletion, whereas the F<sub>1</sub> parent was heterozygous for this  
544 deletion. Therefore, we examined the segregation of this deletion in the mapping family offspring,



545 which was detectable by the presence of a TAT sequence near amplicon bp 40.

546 Finally, the *HevCaLP* locus described by Gahan *et al.* (2007) was amplified in a multiplexed  
547 reaction using three primers: the universal reverse primer [5'-  
548 ATACGAGCTGACGACACGCTGGGAGA-3'], one forward primer that targets a retrotransposon  
549 insertion conferring resistance to *Bacillus thuringiensis* [5' –  
550 CGCAACGCGCGATCTACTCTTGTCACC – 3'], and another forward primer that targets wild-type  
551 sequence [5' – AAGTGTCCCAGTCGATGCTGAA – 3']. An initial 20- $\mu$ l reaction contained 4  $\mu$ l 5 $\times$   
552 GoTaq buffer, 29  $\mu$ M per dNTP, 56 ng per primer, 0.5 U GoTaq polymerase, and 1  $\mu$ g genomic DNA.  
553 Reactions were incubated at 95°C for 2 min, followed by 30 cycles of 95°C for 30 sec, 58°C for 20 sec,  
554 and 72°C for 40 sec. A reconditioning reaction, aimed at reducing heteroduplex formation, was set up  
555 as above, but incubated for 3 rather than 30 cycles. These reactions were capable of producing two  
556 amplicons, which differed in length by 76 bp. The YHD2 parent was homozygous for the long  
557 amplicon (*ca.* 800 bp) containing the insertion that confers resistance to *Bacillus thuringiensis*, whereas  
558 the F<sub>1</sub> parent was heterozygous for a long and short amplicon. PCR products from mapping family  
559 offspring were run on a 2% agarose gel alongside Hyperladder I (Bioline, Taunton, MA) for  
560 visualization and genotype scoring.

561

### 562 *Linkage mapping*

563 Double-digest RAD-seq markers present in fewer than 75% of the mapping family offspring  
564 were filtered out, and the remainder were checked using a chi-square test for Mendelian segregation ( $\alpha$   
565 = 0.01). PCR-based markers, as well as those ddRAD-seq markers that segregated in a mendelian  
566 fashion were assigned to linkage groups (LOD = 5, maximum recombination fraction = 0.3) using the  
567 onemap package (Margarido *et al.*, 2007) in R. We validated groupings by aligning all markers to the

568 *Bombyx mori* genome using Blastn in Kaikobase version 3.2.2 (<http://sgp.dna.affrc.go.jp/KAIKObase/>).  
569 Furthermore, we confirmed that the locations of the *DesatI*, *ABCC2*, and *HevCaLP* pcr-based markers,  
570 as well as ddRAD-seq markers found in their respective linkage groups aligned to the same *B. mori*  
571 chromosomes (Table 4). Markers on each linkage group were ordered using the recombination  
572 counting and ordering algorithm (RECORD; Van Os *et al.*, 2005). RECORD was chosen based upon  
573 previous studies demonstrating the reliability of its performance (Collard *et al.*, 2009, Mollinari *et al.*,  
574 2009). Recombination fractions were converted to centiMorgan distances using the Kosambi mapping  
575 function (Kosambi, 1944). The final linkage map was drawn using Genetic Mapper version 0.5.

576

#### 577 *Assessment of fine-scale differences in nucleotide diversity across laboratory-reared populations*

578 Mapped markers were examined for observed heterozygosity ( $H_o$ ) and nucleotide diversity ( $\pi$ ),  
579 as above, for the inbred line, the Bt-selected line, one non-selected line (YDK), and one field-collected  
580 population (LA), and these values were displayed for visual comparison in Figure 4. For each  
581 population, at least 3 individuals must have been genotyped for a marker to be included in the analysis.  
582 Then, we examined the distribution of mapped markers that retained polymorphism in the inbred line to  
583 determine whether heterogeneity, or significant clustering, could be observed. Using a replicated G-  
584 test of independence (Sokal and Rohlf, 1995), the distribution of polymorphic loci across all linkage  
585 groups that contained five or more markers (24 of the 33 total linkage groups) was examined. Under  
586 assumptions of homogeneity, we expected that the ratio of polymorphic to fixed loci across linkage  
587 groups in the inbred line would follow Hartl and Clark's (2007):

$$588 H_T = H_o(1 - 1/2^{*N_e})^T, \text{ where}$$

589  $H_o$  = initial observed heterozygosity in the population, which we set equal to the observed

590 heterozygosity of its ancestral YDK population.

591  $N_e$  = the number of breeding adults in each generation, which we set equal to 2 according to our sib-  
592 mating design.

593  $T$  = the number of generations

594 According to this equation, the expected frequency of polymorphic loci was 0.015 within a linkage  
595 group following 10 generations of sib-mating. Each linkage group was first examined for deviation  
596 from this expected frequency of polymorphic loci using a G-test of independence with a Bonferroni-  
597 adjusted alpha value ( $\alpha = 0.002$ ) to account for multiple comparisons. Individual uncorrected G-values  
598 produced for each of the 24 linkage groups were added together to generate a “pooled” G-value, and a  
599 “total” G-value was calculated according to Sokal and Rohlf (1995). Finally, a heterogeneity G-value,  
600 which tested the hypothesis that polymorphic loci were significantly more clustered within linkage  
601 groups than due would be due to random chance, was calculated by taking the difference between total  
602 and pooled G-values, then comparing it to a  $X^2$  distribution with 23 degrees of freedom and an a priori  
603 alpha value of 0.05.

604 Each marker that retained polymorphism in the inbred line was then examined for an excess of  
605 intermediate frequency alleles, which could indicate either a population contraction or ongoing  
606 balancing selection near that locus. For this, we used a Tajima's D test as calculated by the R package,  
607 pegas (v. 0.6; Paradis, 2010). To tease apart the effects of population demographic changes and forces  
608 of natural selection, we compared the Tajima's D values at each locus in the inbred line to those of their  
609 ancestral population (YDK). We reasoned that significantly positive Tajima's D values that overlapped  
610 between the two lines would be less likely due to the population demographic changes created by sib-  
611 mating, and more likely due to selection. A Benjamini-Hochberg adjustment was applied to all  
612 Tajima's D p-values to account for multiple hypothesis tests using the R package fdrtool (v.1.2.15;  
613 Strimmer 2008).

614

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622

623

624

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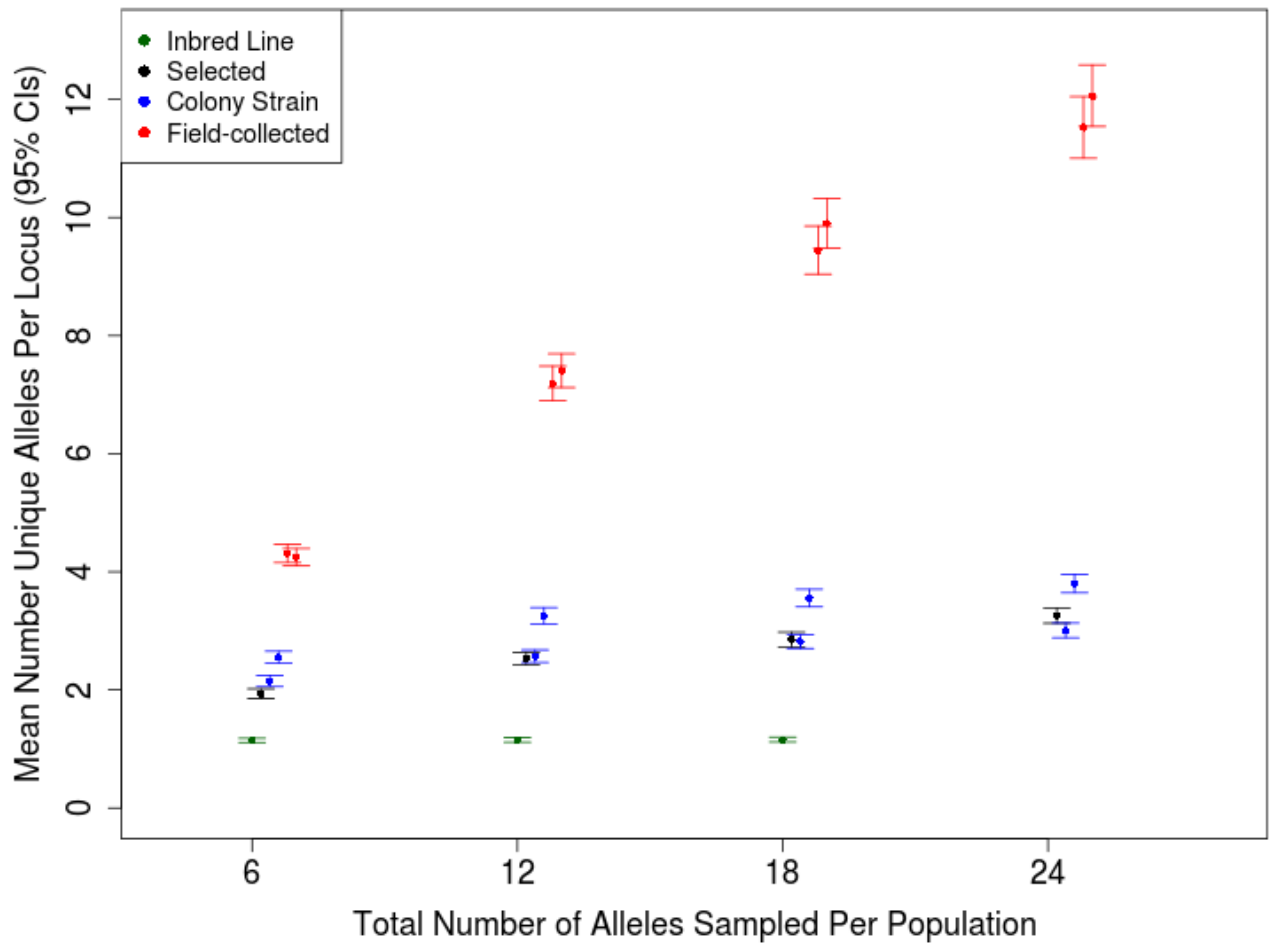
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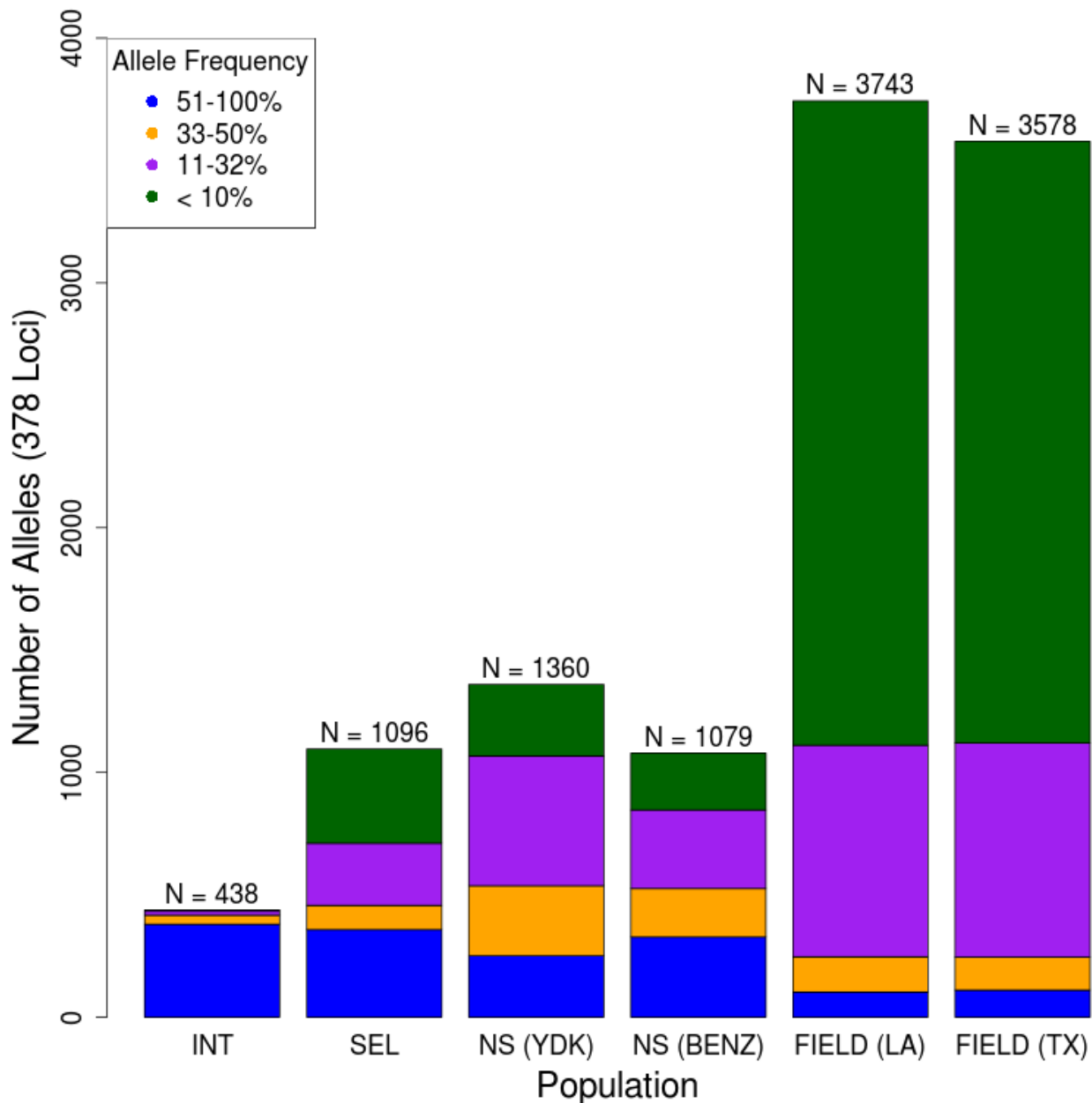
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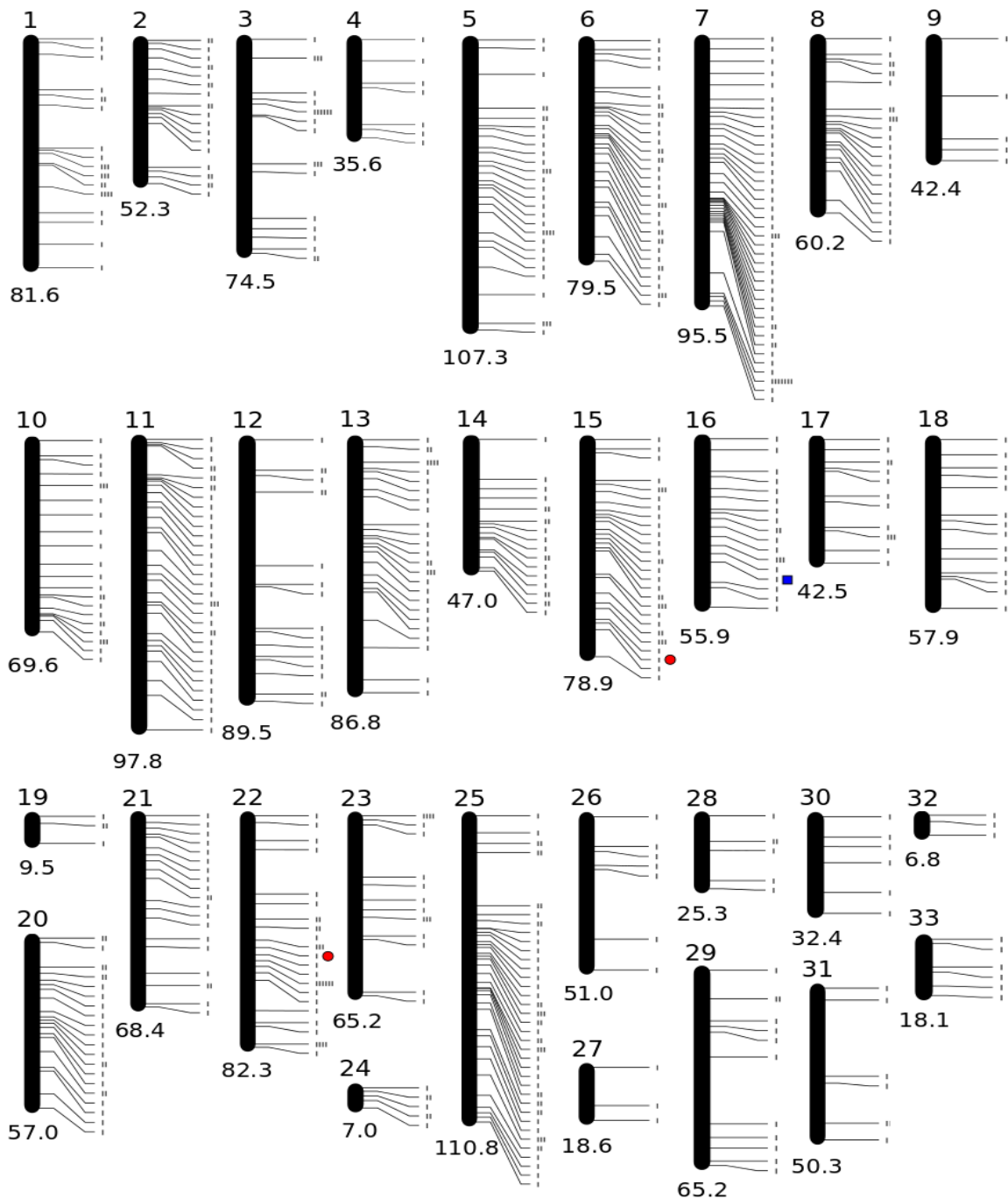
825 **Figures**  
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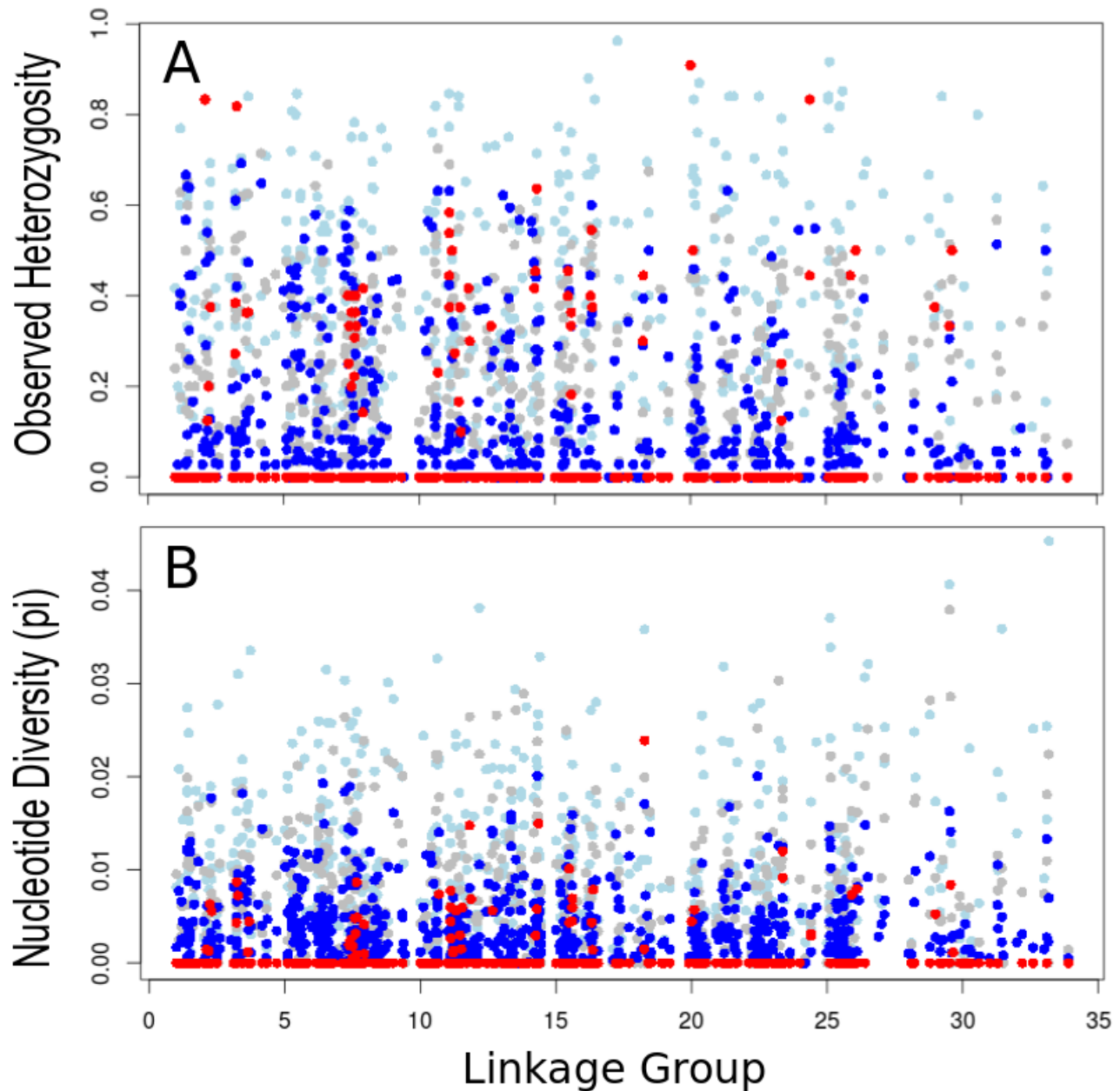
828 **Figure 1** – Mean numbers of unique alleles detected among 378 loci depends upon the number of  
829 alleles sampled per population. For all but the inbred line (in green), as sample size increases, so does  
830 the number of unique alleles detected per locus. Due to low survivorship in the inbred line, no mean  
831 was computed for an allelic sample size per locus of 24.  
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835 **Figure 2** – Total numbers of unique alleles detected in sib-mated (INT), Bt-selected (SEL), non-  
836 selected (NS), and field-collected (FIELD) populations based upon random sampling of 18 haplotypes  
837 per population per locus. Alleles were binned and color-coded according to the frequencies at which  
838 they were present out of 18 total haplotypes. Numbers above each bar represent the total number of  
839 unique alleles found per population out of 378 loci.  
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843 **Figure 3** – *Heliothis virescens* linkage map with a total length of 1919.5 cM. Centimorgan lengths are  
844 below each linkage group. Each tick mark represents an individual marker that mapped to a particular  
845 position in the linkage group. Red circles next to linkage groups 15 and 22 represent the positions of  
846 the *HevCaLP*, and the *ABCC2*, respectively. The blue square represents the position of the delta-11-  
847 desaturase on linkage group 16.



849 **Figure 4** – Observed heterozygosity (A) and nucleotide diversity (B) estimates per mapped marker site.  
850 For comparative purposes, estimates for the Louisiana field population were included (light blue).  
851 Grey and blue circles represent estimates in non-selected (YDK), and Bt-selected (YHD2) populations,  
852 respectively. Red circles represent estimates in the inbred line following 10 generations of sib-mating.

853 **Tables**

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855 **Table 1** - Population history, sample sizes (N) and ddRAD-seq read summary data. Filtered N refers to  
856 the population sample size following removal of individuals with low read counts.

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Population	Population History	Total N	Filtered N	Mean Number Reads per Individual
Inbred Line	10 generations of sib-mating	13	13	334689
YHD2	Bt-selected	43	41	346264
BENZ	Unselected	42	40	318636
YDK	Unselected	46	44	335728
LA2012	Field-collected	30	27	398336
TX2012	Field-collected	30	28	306164

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875 **Table 2** – Genome-wide nucleotide diversity values per 350 bp locus across populations. All values were calculated using a conservatively  
 876 chosen set of 378 loci for which at least 10 individuals were genotyped within each population. Genome-wide values represent population-  
 877 level  $\pi$  and  $S_K$  averaged across all loci. The abbreviations Bt-sel and NS stand for Bt-selected and non-selected, respectively.  
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Population	Mean Observed Heterozygosity	Genome-wide $\pi$ (2.5, 97.5% CIs)	Max $\pi$	Genome-wide $S_K$ (2.5, 97.5% CIs)	Max $S_K$
Inbred line	0.06	0.0006 (0.0004, 0.0008)	0.013	0.15 (0.10, 0.19)	2.95
Bt-Sel (YHD2)	0.15	0.0040 (0.0035, 0.0043)	0.020	1.67 (1.54, 1.81)	6.53
NS (YDK)	0.26	0.0067 (0.0061, 0.0073)	0.028	1.66 (1.53, 1.80)	6.20
NS (BENZ)	0.22	0.0051 (0.0046, 0.0056)	0.026	1.35 (1.24, 1.47)	5.46
Field (LA)	0.46	0.0094 (0.0087, 0.0100)	0.034	5.35 (5.03, 5.68)	15.55
Field (TX)	0.40	0.0092 (0.0085, 0.0099)	0.033	5.02 (4.71, 5.35)	15.63

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880 **Table 3** – Pairwise estimates of genetic divergence across 378 ddRAD-seq loci calculated according to Weir and Cockerham's  $F_{ST}$ . Estimates  
 881 of  $F_{ST}$  are above the diagonal, while corresponding bootstrapped confidence intervals (2.5%, 97.5%) are presented below.  
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	<b>Inbred Line</b>	<b>Bt-Sel (YHD2)</b>	<b>NS (YDK)</b>	<b>NS (BENZ)</b>	<b>Field (LA)</b>	<b>Field (TX)</b>
<b>Inbred Line</b>	-	0.6378	0.3272	0.6209	0.3873	0.3899
<b>Bt-Sel (YHD2)</b>	(0.5479, 0.7342)	-	0.2818	0.4854	0.3137	0.3175
<b>NS (YDK)</b>	(0.3096, 0.3465)	(0.1823, 0.3796)	-	0.3863	0.1656	0.1664
<b>NS (BENZ)</b>	(0.5705, 0.6645)	(0.3643, 0.5922)	(0.3345, 0.4288)	-	0.2552	0.2565
<b>Field (LA)</b>	(0.3663, 0.4078)	(0.2380, 0.3930)	(0.1521, 0.1850)	(0.2134, 0.2931)	-	0.0004
<b>Field (TX)</b>	(0.3681, 0.4106)	(0.2403, 0.3971)	(0.1529, 0.1861)	(0.2148, 0.2951)	(-0.0115, 0.0156)	-

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884 **Table 4** – Linkage group (LG) correspondence with *B. mori* chromosome (Chr). Linkage groups with an asterisk contained one or more  
885 markers that aligned uniquely to an unmapped *B. mori* sequence. Where linkage groups contained markers that aligned to more than one *B.*  
886 *mori* chromosome, italicized marker names correspond to the italicized *B. mori* chromosome.  
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LG	<i>B.mori</i> Chr	Number Markers Aligned to <i>B.mori</i> Chr	Total markers in LG	LG Length (cM)	Average Marker Spacing (cM)	Names of Markers Aligned to <i>B.mori</i> Chr
1	25	3	25	81.6	3.3	19754, 29329, 20282
2	8	6	23	52.3	2.3	22394, 27499, 21475, 17667, 17852, 25500
3*	17	3	24	74.5	3.1	376, 21931, 25112
4	-		7	35.6	5.1	
5*	4	5	36	107.3	3.0	2556, 29595, 22095, 8695,15160
6	5	6	40	79.5	2.0	18575, 4268, 21041, 23679, 66, 5723
7	22	9	49	95.5	1.9	13710, 2328, 1443, 3654, 13880, 22897, 3123, 2430, 19858
8*	11	9	25	60.2	1.7	22584, 939, 3060, 18820, 23156, 17932, 202, 22290, 21173
9	23	2	7	42.4	6.1	4275, 1286
10	3	5	26	69.6	2.7	5015, 5329, 4265, 4545, 23262
11	9	8	37	97.8	2.6	19938, 6113, 16969, 19392, 1572, 89, 22588,19343
12	-		18	89.5	5.0	
13	13	6	31	86.8	2.8	2074, 15962, 26388, 1193, 16684,18825
14	19	1	21	47.0	2.2	14242
15	6	7	35	78.9	2.3	25943, 4579, 25083, 1752, 3385, 2784, 18178
16	23	4	20	55.9	2.8	2485, 16350, 806, 20599
17	7	2	14	42.5	3.0	29343, 343
18	26	1	14	57.9	4.1	1113
19	11, 22	2	4	9.5	2.4	3536, <i>16185</i>
20*	10	6	25	57.0	2.3	17820, 2978, 18970, 12277, 16316, 12984

890 **Table 4 continued.**

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<b>LG</b>	<b><i>B.mori</i> Chr</b>	<b>Number Markers Aligned to <i>B.mori</i> Chr</b>	<b>Total markers in LG</b>	<b>LG Length (cM)</b>	<b>Average Marker Spacing (cM)</b>	<b>Names of Markers Aligned to <i>B.mori</i> Chr</b>
21	21	3	21	68.4	3.3	26113, 20542, 7624
22	15	2	33	82.3	2.5	21411, 5773
23	14	1	17	65.2	3.8	4156
24			7	7.0	1.0	
25	12,1	4	53	110.8	2.0	20280, 9211,12382, 23604
26			7	51.0	7.3	
27	2	1	3	18.6	6.2	5781
28			6	25.3	4.2	
29	16	1	12	65.2	5.4	23481
30			6	32.4	5.4	
31			7	50.3	7.2	
32			3	6.8	2.3	
33	14, 28	2	6	18.1	3.0	6601, 20184

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