Sexual selection does not increase the rate of compensatory adaptation to a mutation influencing a secondary sexual trait in *Drosophila melanogaster*

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Abstract

Theoretical work predicts that sexual selection can enhance natural selection, increasing the rate of adaptation to new environments and helping purge harmful mutations. While some experiments support these predictions, remarkably little work has addressed the role of sexual selection on compensatory adaptation—populations' ability to compensate for the costs of deleterious alleles that are already present. We tested whether sexual selection, as well as the degree of standing genetic variation, affect the rate of compensatory evolution in experimental populations of *Drosophila melanogaster*. These populations were fixed for a spontaneous mutation causing mild abnormalities in the male sex comb, which is important for mating success; in some populations, the opportunity for mate choice was limited by experimentally enforced monogamy. We first showed that this mutation is deleterious, and that its expression is influenced by genetic background variation. Although populations did show some compensatory adaptation with respect to reduction in the morphological abnormalities in the sex comb, the amount of phenotypic suppression of the morphological defects did not depend on the opportunity for sexual selection. Sexual selection, therefore, may not always enhance natural selection; instead, the interaction between these two forces may depend on additional factors.

Introduction

Sexual selection has important impacts on many aspects of how organisms evolve, including on speciation rates and the degree of sexual dimorphism (e.g., Masta and Maddison 2002; Ellis and Oakley 2016). While it was once considered that sexual selection may act independently or even antagonistically to other components of natural selection (e.g. viability and fecundity), recent work has shown that these can operate concordantly. For instance, sexual selection may influence how organisms respond to selective pressures in the short term, influencing how quickly populations adapt to novel environments, in particular when the population begins at a distance from an optimum (Long *et al.* 2012). Additionally, some models predict that sexual selection should help populations filter out harmful mutations more rapidly than selection on other fitness components (viability and fecundity selection) alone (Agrawal 2001). This prediction is based partly on the observation that sexual displays are often correlated with overall condition. Any mutation that reduces an organism's nonsexual fitness is therefore also likely to affect its mating success (Rowe and Houle 1996; Chandler *et al.* 2013) or even its success in sperm competition (Clark *et al.* 2012). Sexual selection against these mutations, usually in males, should therefore enhance natural selection against them.

Empirical support for this scenario has been mixed. In some studies testing these predictions, evidence supported a role for sexual selection in purging deleterious mutations or accelerating adaptation (Radwan 2004; Sharp and Agrawal 2008; Hollis *et al.* 2009; Jarzebowska and Radwan 2010; McGuigan *et al.* 2011; Long *et al.* 2012; Almbro and Simmons 2014; Lumley *et al.* 2015; Grieshop *et al.* 2016; Jacomb *et al.* 2016). However, a handful of studies also contradict these predictions (Hollis and Houle

2011; Plesnar *et al.* 2011; Arbuthnott and Rundle 2012; Cabral and Holland 2014). The different outcomes in at least some of those cases may be due to the costs of sexual conflict, which is often confounded with sexual selection; any benefits from sexual selection's ability to purge deleterious mutations in those cases may have been canceled out by the reduction in fecundity caused by sexual conflict, such as male-induced harm (Hollis and Houle 2011; Arbuthnott and Rundle 2012; Chenoweth *et al.* 2015). Some studies also focused on large-effect mutations or exposed populations to strong mutagens (Hollis and Houle 2011; Plesnar *et al.* 2011; Arbuthnott and Rundle 2012; Cabral and Holland 2014), so they may not be representative of how selection acts in nature, where most segregating deleterious mutations are more likely to have milder effects.

Of course, deleterious mutations are not always purged by selection; they can also increase in frequency and occasionally become fixed via drift, hitchhiking or antagonistic pleiotropy, especially if their effects on fitness are only mildly deleterious (and in populations with a small effective population size). In those cases where the deleterious alleles are difficult for selection to purge, alleles at other loci that compensate epistatically for the fitness costs of these fixed deleterious alleles may be favored by selection. There is good evidence of compensatory adaptation in both microbial and multicellular organisms. For instance, alleles conferring antibiotic resistance are sometimes costly in the absence of antibiotics, but compensatory mutations can reduce those costs (Reynolds; Maisnier-Patin *et al.* 2002; Comas *et al.* 2012). In the blowfly, diazinon resistance via alleles at the *Rop-1* gene had negative pleiotropic effects, increasing fluctuating asymmetry, but these effects were ultimately

83 compensated by modifiers (McKenzie and Clarke 1988; Davies *et al.* 1996).
84 Additionally, sex chromosome dosage compensation could also be considered a form of
85 compensatory adaptation, having likely evolved in response to loss-of-function
86 mutations on Y or W chromosomes (Charlesworth 1978).

87 Although sexual selection has received a lot of attention as a possible influence
88 on the rate of purging of deleterious mutations, the role of sexual selection in
89 compensatory evolution remains largely unexplored. Nevertheless, we might similarly
90 predict that sexual selection can also accelerate compensatory adaptation, especially if
91 sexual displays are condition dependent. In one study (Pischedda and Chippindale
92 2005), the *nub*^{1} mutation, which drastically reduces the size of the wing, resulting in an
93 inhibition of males' ability to generate courtship songs, was fixed in experimental
94 populations of *Drosophila melanogaster*. As predicted, this mutation had greater fitness
95 costs in males than it did in females, but males also showed greater compensatory
96 fitness recovery over 180 generations (albeit without compensating for the effects on
97 wing morphology directly), providing some support that sexual selection may enhance
98 compensatory adaptation. However, this study was not replicated (only a single
99 lineage), since the *nub*^{1} populations were originally generated for other purposes.
100 Clearly, more study is needed on whether sexual selection can speed up compensatory
101 adaptation.

102 In this study, we address the question of whether sexual selection can impact the
103 rate of compensatory evolution using experimental evolution in *Drosophila*
104 *melanogaster*. We chose a mutation in the *sex combs distal* gene (*scd*^{1}) (Boube *et al.*
105 1997; Randsholt and Santamaria 2008), a spontaneous, partially penetrant mutation

106 affecting the development of the male sex comb, a structure that is critical for male
107 mating success (Ng and Kopp 2008) and rapidly evolving across *Drosophila* species
108 (Atallah *et al.* 2009, 2012; Kopp 2011; Malagón *et al.* 2014). In addition to further
109 mapping and characterizing the effects of this mutation across different wild type
110 genetic backgrounds, we find that despite a general compensatory response, we
111 observed no evidence that sexual selection influenced the rate of compensatory
112 evolution for the sex comb phenotypes.
113

114 **Methods**

115 *Mapping scd^1*—

116 *sex combs distal^1* (*scd^1*) is a spontaneous X-linked allele resulting in ectopic sex comb
117 bristles on the second tarsal segment of the prothoracic leg in males (Boube *et al.*
118 1997). In the base stock, it has incomplete penetrance, with only about 70% of males
119 showing the ectopic sex comb bristles, and no visible phenotype in females. In our
120 populations, males also sometimes exhibited minor defects in the primary sex comb,
121 such as a gap or partially untransformed bristles (Figure 1).

122 The identity of the gene and molecular lesion of this allele are unknown, although
123 some previous recombination mapping suggested it was near 1-30.6, and that a local
124 duplication of the 8C-9B region of the X chromosome onto the Y (*DP(1:Y)FF*), could
125 partially rescue the phenotype of *scd^1* (Santamaria and Randsholt 1995; Randsholt and
126 Santamaria 2008). We attempted to further fine map the gene through duplication
127 mapping. Virgin female flies of strain BDSC 5070 (*y^1 scd^1 ras^1 v^1 f^1*) were crossed to
128 males of strains carrying duplicated segments of the X chromosome translocated onto

129 the Y chromosome or chromosome III (Table 1; Cook *et al.* 2010; Venken *et al.* 2010),
130 and the male offspring were scored for the presence of mutant phenotypes, such as the
131 ectopic sex comb or disruptions in the primary sex comb. Assuming scd^1 is a recessive
132 loss-of-function allele, if the duplicated segment contains a functional wild-type copy of
133 the *scd* gene, then the mutant phenotype would be rescued and no male offspring from
134 these crosses would show sex comb defects. Because scd^1 is only partially penetrant,
135 we scored numerous male offspring from each cross.

136 *Influence of genetic background on penetrance and expressivity*—

137 To determine the extent of genetic variation for the penetrance and expressivity
138 of scd^1, we crossed virgin female y^1 scd^1 ras^1 v^1 f^1 flies to males of a randomly chosen
139 subset of Drosophila Genetic Reference Panel lines (Mackay *et al.* 2012). When the
140 adult F1 offspring eclosed, we fixed specimens in 70% ethanol, and then mounted male
141 prothoracic legs in 70% glycerol and scored them for the presence of ectopic sex combs
142 on the second tarsal segment, abnormalities in the primary sex comb, and primary sex
143 comb tooth number. These crosses only examine each wild-type genetic background in
144 a heterozygous state, and thus it is expected that this will underestimate the actual
145 degree of background dependence, as recessive effects of alleles in each background
146 will not be captured.

147 *Introgression of* scd^1—

148 To generate populations for experimental evolution, we introgressed the scd^1
149 mutation into FVW, a domesticated lab-maintained population founded from flies
150 collected from Fenn Valley, MI in 2010. The FVW population was maintained in

151 population cages with 10 bottles for egg-laying each generation for approximately 10
152 generations prior to beginning introgressions (Chari *et al.* 2017).

153 To begin the introgression (Supplementary Figure 1A), virgin y^1 scd^1 ras^1 v^1 f^1
154 females were crossed to FVW males. The heterozygous F1 females were then
155 backcrossed to FVW males. From the F2 offspring, we selected males showing the scd^1
156 phenotype, which were thus hemizygous for scd^1, but with phenotypically wild-type
157 eyes. We then crossed these males to virgin FVW females, to obtain female offspring
158 heterozygous for scd^1 in a partial FVW background. We then crossed these females to
159 FVW males, and selected males with ectopic sex combs, but not yellow bodies. Each
160 male from these crosses thus carries an independently derived X chromosome with
161 scd^1 in a random FVW background.

162 We obtained eight such males and crossed them to FVW females. From these
163 crosses, we obtained virgin females heterozygous for scd^1 in an FVW background. The
164 first of these virgin scd^1/scd^+ females to emerge were crossed with FVW males to obtain
165 more scd^1 males with a mostly FVW background. The later-emerging scd^1/scd^+ females
166 were kept isolated at cooler temperatures (18˚C) until the scd^1 males from the previous
167 cross emerged. We then crossed the scd^1/scd^+ females to the scd^1 males. Finally, we
168 set up sib matings among the offspring of these crosses, using only males hemizygous
169 for scd^1 (showing a sex comb phenotype) and females of unknown genotype (either
170 scd^1/scd^+ or scd^1/scd^1). Of those crosses, we kept those in which the mother was
171 inferred to be homozygous for scd^1, in which nearly all male progeny displayed scd^1
172 phenotypes. This allowed us to establish a homozygous scd^1 line with an FVW genetic

173 background, which we designated as *scd**. *scd** carried at least four independently
174 derived X chromosomes with scd^1 in an FVW background (Supplementary Figure 1A).
175 To introduce further genetic diversity (from the FVW population) into *scd**, *scd**
176 males were crossed to wild-type FVW females to obtain heterozygous scd^1 females with
177 additional genetic material from the FVW background. These females were then
178 backcrossed to FVW males. Five replicate backcrosses were set up in culture bottles,
179 each with 25-30 FVW males and 25-30 females, heterozygous for scd^1 and for alleles
180 from the FVW background. We then selected males with the scd^1 phenotype, and
181 backcrossed them to virgin *scd** females, in six replicate bottles each containing 20-25
182 males and 20-25 females, to maintain scd^1 while introducing additional genetic diversity
183 from the FVW population. This whole cycle was then repeated once to establish the
184 *scd*** base population for experimental evolution (Supplementary Figure 1B).
185 *Fitness effects of scd^1—*
186 To test whether the scd^1 allele was deleterious, we tracked changes in the
187 frequency of the scd^1 phenotype in polymorphic populations with the scd^1 allele at 0.7
188 initial frequency. We initiated four replicate populations, each consisting of 70 *scd***
189 males, 70 *scd*** females, 30 FVW males, and 30 FVW females. Populations were
190 placed in population cages with four culture bottles for mating and oviposition for five
191 days, after which the flies were discarded and the bottles transferred to fresh cages at
192 24°C. After adult flies began emerging, they were allowed to mate for three to four days.
193 The old bottles were then removed, and fresh bottles were placed in the cage for egg
194 laying. After two days of egg laying, the flies were discarded and the bottles moved to
195 fresh cages. This cycle was repeated for a total of nine generations.

196 For each of the first five generations, and at generation nine, we scored male sex
197 comb phenotypes. 50 males were picked randomly, and the first prothoracic legs from
198 each male were mounted on glass slides in 70% glycerol/PBS to check for the presence
199 of an ectopic second sex comb and other abnormalities. While this does not give an
200 exact measurement of the frequency of the *scd*^{1} allele because of this allele's
201 incomplete penetrance (though penetrance is almost complete in the FVW background;
202 see below), it should provide a reasonable proxy. Even though reductions in the
203 frequency of the mutant phenotype could also be driven by selection for suppressor
204 alleles, this should still give an indication of whether or not the *scd*^{1} phenotype is
205 deleterious.

206 *Experimental evolution—*

207 To test whether sexual selection influences the rate of compensatory adaptation,
208 we set up two treatments. In the low sexual selection (LSS) treatment, we removed
209 sexual selection by enforcing monogamous mating. Each generation, we set up 100
210 vials, each containing one male and one virgin female. After a three-day interaction
211 period, we anesthetized the flies using CO_2, discarded males, and placed the females in
212 a population cage with four bottles containing culture media for egg laying. After four
213 days, the bottles were removed and incubated at 24˚C. When adult flies began eclosing,
214 we selected virgins for the next generation. Thus, while this treatment did preclude mate
215 choice, it still allowed for fecundity and viability selection (Arbuthnott and Rundle 2012).

216 In the high sexual selection (HSS) treatment, we followed a similar protocol
217 except allowed the opportunity for sexual selection. Each generation, 100 males and
218 100 virgin females were allowed to interact in a population cage, along with an open

219 culture bottle for food and moisture. After the three-day interaction period, we placed the
220 cage in a refrigerator to knock the flies out, and then we sorted males and females.
221 Males were discarded, and females were placed in fresh cages with four culture bottles
222 for a four-day egg-laying period. After egg laying, females were discarded, and the
223 bottles were placed in an environmental chamber at 24˚C until adults began emerging,
224 at which point we collected virgins for the next generation.

225 Additionally, we also set up a treatment with low levels of genetic variation (LV)
226 to test whether compensatory adaptation is limited when segregating genetic variation is
227 diminished; in other words, testing whether the mutational target size of the
228 compensatory response was large enough that *de novo* mutations could contribute in
229 the time frame of the experimental evolution regime. In this treatment, each population
230 was established from the offspring of a single-pair mating between a randomly chosen
231 virgin *scd*** female and a randomly chosen *scd*** male. These populations were kept
232 under the same regime as the HSS treatment.

233 Finally, we set up a wild-type control (WTC) treatment using wild-type FVW flies.
234 WTC populations were also maintained under the same regime as the HSS treatment.
235 These provide a control for lab domestication and unknown aspects of the experimental
236 protocol.

237 All populations were initiated using randomly selected *scd*** flies (see above),
238 except for the LV treatments as described. We set up three replicate populations of
239 each treatment except for WTC, in which we performed two replicates. Experimental
240 evolution was conducted for a total of 24 generations. We assayed male sex comb

241 phenotypes as described earlier at generations 1, 7, 13, 19, and 24, using 30 randomly
242 selected males from each population at each time point.
243 To test how male sex comb traits changed over the course of experimental
244 evolution, we fit generalized linear mixed models using the glmmTMB (Hadfield 2010)
245 and lme4 (Bates *et al.* 2015) packages in R. For sex comb tooth number for both
246 primary and ectopic/secondary combs, we assumed a Poisson distribution and used a
247 log link function. The model included generation, treatment, and their interaction as
248 fixed effects; we also included individual fly, and in some cases replicate population
249 nested within treatment, as random effects (some models failed to converge when
250 replicate nested within treatment was included as a random effect). Additionally we also
251 tested for lineage specific zero-inflation in the data, but found no evidence for this, so
252 excluded this to reduce the number of fit parameters. To test whether the frequency of
253 defects in the primary sex comb changed over time, we fit a mixed logistic model (sex
254 comb defects present/absent), again with generation, treatment, and their interaction as
255 fixed effects, and replicate nested within treatment, as well as individual fly, as random
256 effects.
257
258 **Results**
259 *Mapping scd^1*—
260 Though we could not map scd^1 to a specific gene, we were able to further narrow
261 down its location to an ~85-kb (cytological region 8F8-9A1) region on the X
262 chromosome through duplication mapping (Table 1, Figure 2). This region contains only
263 two complete annotated protein-coding genes, *btd* and *Sp1* (both of which influence

264 aspects of leg development and morphogenesis), and three annotated long non-coding
265 RNAs, CR42657, CR44016, and CR53498. Interestingly, the two parent strains carrying
266 the duplications that rescued *scd*^{1} had smaller than average sex combs in the absence
267 of the *scd*^{1} mutation (Table 1), similar to a past study involving this mutation (Randsholt
268 and Santamaria 2008), suggesting that the *scd* gene product is a suppressor of sex
269 comb development.

270 *Influence of genetic background on penetrance and expressivity—*

271 When females of the original *scd*^{1} stock strain were crossed to males of various
272 wild-type strains to generate males that were hemizygous for *scd*^{1} and heterozygous for
273 different genetic backgrounds, the penetrance and expressivity of *scd*^{1} varied widely
274 (Figure 3) demonstrating segregating variation for them. The progenitor *scd*^{1} strain from
275 the Bloomington stock center had the lowest penetrance (frequency of flies exhibiting an
276 ectopic sex comb on the second tarsal segment) and among the lowest expressivity
277 (number of teeth in the ectopic sex comb). Some of the other genetic backgrounds,
278 even in a heterozygous state, resulted in nearly complete penetrance for *scd*^{1}, including
279 the outbred population (FVW) for experimental evolution (described below).
280 Interestingly, the FVW outbred population only showed intermediate levels of
281 expressivity of the mutant phenotype, consistent with segregating variation in this
282 population. Overall this result suggests genetic background has a strong impact on the
283 phenotypic expression on *scd*^{1}. It also suggests that the partial penetrance initially
284 observed in the stock may reflect the accumulation of suppressor/compensatory
285 mutations in the base stock center strain itself.

286 *Fitness effects of scd*^{1}*—*

287 The frequency of male flies exhibiting the *scd*^{1} phenotype decreased, on
288 average, across five generations of experimental evolution in populations polymorphic
289 for *scd*^{1} (Figure 4). With a starting allele frequency of 0.7 the frequency decreased to an
290 average frequency of 0.4 (across the multiple replicates) in males. These results are
291 consistent with the mutation having moderate deleterious effects.

292 *Experimental evolution—*

293 In the populations carrying the *scd*^{1} allele, defects such as gaps in the sex comb
294 were observed occasionally, and at significantly higher frequencies in the High Sexual
295 Selection (HSS) populations, and marginally significant frequencies in the Low Sexual
296 Selection (LSS) populations, than in the wild-type populations. The frequency of these
297 gaps appeared to decrease in the HSS populations across the 24 generations of the
298 experiment, although the interaction between generation and treatment was not
299 significant (Figure 5B; Table 2). In addition, the ectopic sex combs induced by the *scd*^{1}
300 mutation became smaller on average in both the HSS and LSS treatments (Figure 5C;
301 Table 3), losing on average ~0.5 teeth across the 24 generations of experimental
302 evolution. Again, we saw no evidence for differences in rate of compensation between
303 the EE treatments with and without sexual selection. For the Low genetic Variation (LV)
304 treatment, no significant change over time was observed, as expected (generation
305 effect = 0.0017, SD = 0.0044, p = 0.70). No significant changes were observed in the
306 primary sex comb tooth number across 24 generations in any of the experimental
307 treatments (Figure 5A; Table 4).

308 **Discussion**

309 Some, but not all, previous work has found that sexual selection may facilitate
310 populations in purging deleterious mutations (Radwan 2004; Hollis *et al.* 2009;
311 Jarzebowska and Radwan 2010) or accelerating rate of adaptation (Jacomb *et al.* 2016;
312 Parrett and Knell 2018). Few studies, however, have addressed whether sexual
313 selection may facilitate compensatory adaptation, where populations evolve traits to
314 compensate for the fitness costs of deleterious mutations. While compensatory
315 adaptation itself is well documented in other systems (Reynolds; Maisnier-Patin *et al.*
316 2002; Estes *et al.* 2011; Chandler *et al.* 2012; Comas *et al.* 2012; Chari *et al.* 2017), in
317 this experiment, we found no effect of the sexual selection regime on the rate of
318 compensatory adaptation (with respect to the sex comb) to a mutation that causes
319 morphological abnormalities in male sex combs in experimental *Drosophila* populations.
320 This is perhaps surprising, because our experiments show that the scd^1 mutation is
321 deleterious (Figure 4), and given the importance of the *Drosophila* sex comb for male
322 mating success (Ng and Kopp 2008), we expected that the fitness costs of this mutation
323 would involve male sexual fitness. Thus, we predicted that the costs of this mutation
324 would be higher in the HSS treatment, in which there was a high opportunity for female
325 mate choice, than in the LSS treatment, with reduced opportunity for sexual selection. It
326 is possible that this mutation has effects on other aspects of fitness in males or females
327 (viability, fecundity), but unfortunately our experiments did not directly measure specific
328 fitness components. Even so, theoretical work predicts that sexual selection should act
329 in concert with natural selection because of condition dependence (Whitlock and
330 Agrawal 2009); that is, mutations that reduce nonsexual fitness should also reduce
331 mating success, since sexual displays are often indicators of overall condition. While

332 some work has supported this prediction, our findings add to a growing body of work
333 suggesting that this is not always the case (Hollis and Houle 2011; Plesnar *et al.* 2011;
334 Arbuthnott and Rundle 2012, 2014; Cabral and Holland 2014; Power and Holman 2015;
335 Chenoweth *et al.* 2015). It is possible that the relatively minor degree of compensation
336 observed here made it difficult to detect differences between treatments.

337 Even though sexual selection did not impact the rate of compensatory evolution,
338 we did observe evidence of weak compensatory adaptation for the scd^1 mutation in both
339 the HSS and LSS treatments with respect to phenotypic suppression of the mutant
340 phenotypic effects. On average, the ectopic sex combs lost about half a tooth (starting
341 with a mean of ~ 3.5 teeth) over the course of 24 generations in these populations; in
342 other words, the expressivity of the mutation declined slightly. One possible explanation
343 for the similar response in both the high and low sexual selection treatments is simply
344 that the compensatory response (in terms of phenotypic suppression) was sufficiently
345 weak that any subtle difference between these treatments would be difficult to detect
346 given our design.

347 As expected, we did not observe any significant trend in the LV treatment, in
348 which populations experienced genetic bottlenecks prior to beginning the experiment
349 (LV populations were treated the same way as HSS populations). Combined with the
350 observation that genetic background has strong influences on the penetrance and
351 expressivity of this mutation (Figure 3), this suggests that compensatory adaptation
352 relies heavily on the presence of standing genetic variation, rather than new mutations.
353 An interesting side note is that the initial strain (obtained from the Drosophila stock
354 center) carrying scd^1 has among the lowest penetrance/expressivity for this mutation of

355 all the genetic backgrounds that we tested. This may suggest that the stock strain has
356 already undergone compensatory adaptation, and that alleles suppressing the
357 phenotypic expression of *scd*^{1} had become fixed throughout the maintenance of this
358 stock (which were subsequently removed when we outcrossed the mutation), though
359 unfortunately we do not have any data on how long the *scd*^{1} stock strain has been
360 maintained.

361 While we were unable to map *scd*^{1} to a specific gene, we were able to localize it
362 to an ~85 kb region containing only a few candidates. The only protein-coding candidate
363 genes in this region, *Sp1* and *btd*, both have known roles in leg development (Estella
364 and Mann 2010), but are not specifically known to influence sex comb development.
365 There are also three long non-coding RNAs in this region (CR42657, CR44016, and
366 CR43498). Interestingly, all three of these RNAs show evidence of male-specific
367 expression in modENCODE RNA-seq data available on FlyBase (Graveley *et al.* 2011;
368 Brown *et al.* 2014). However, CR44016 shows expression only at very low levels and
369 only in adult males, not pupae or larvae, suggesting it is unlikely to be involved in the
370 development of sex combs. CR42657 and CR43498 both show expression in pupae
371 and/or larvae, as well as adult males (but not adult females); however, these RNAs
372 seem to be expressed in the testis and accessory gland and not other tissues (though
373 expression in legs specifically was not assessed in the modENCODE dataset). This
374 suggests that these male-specific reproductive tissues may be driving these sex-specific
375 expression patterns, not a role in sex comb development. Further work is necessary to
376 identify the molecular nature of *scd*^{1}.

There are a number of important limitations to point out about our study. First, much of the focus was on compensation by suppression of the phenotypic effects of the *scd*^{1} mutation on the sex combs directly. While we observed similar levels of phenotypic compensation with both our high and low sexual selection treatments (LSS and HSS), it is possible that compensatory evolution differed with respect to the fitness components (viability, fecundity, and sexual/mating components), which were not evaluated. Thus we limit our interpretation to the effects on morphological compensation/suppression, recognizing that we cannot rule out differential patterns of compensatory response for fitness *per se*. Indeed, this pattern has been observed previously (Pischedda and Chippindale 2005; Chari *et al.* 2017). Additionally, this experiment was performed over a relatively short time period (25 generations); if we continued the experiment over a longer period, subtle differences in the rate of morphological compensation may have become apparent.

In summary, we found evidence of moderate compensatory adaptation to a deleterious mutation. However, while compensatory adaptation did depend on the presence of standing genetic variation, it did not depend on the opportunity for sexual selection, in spite of the affected phenotype's known role in mating. This adds to a growing body of studies suggesting that sexual selection does not always enhance natural selection. Future work should tease apart when and why sexual and natural selection act in concert and when they are likely to operate differently (Martínez-Ruiz and Knell 2017).

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405 **Figure Legends**

406 **Figure 1.** (A) Wild-type *Drosophila* leg showing a normal male sex comb (black arrow).
407 (B) scd^1 leg showing the primary sex comb (black arrow) as well as a smaller ectopic
408 sex comb on the second tarsal segment (white arrow).

409 **Figure 2.** Duplication mapping of scd^1. Purple bars represent duplications that rescued
410 the scd^1 mutant phenotype; pink bars represent duplications that failed to rescue scd^1.
411 (A) Entire region of the X chromosome in which duplications were tested. (B) Close-up
412 of the putative scd^1-containing region (red box). Because the duplication carried by
413 strain 30333 is sufficient to rescue the scd^1 phenotype, we hypothesize that the *scd*
414 gene must lie entirely within this region; at the same time, the neighboring duplications
415 (30522 and 30334) did not rescue the scd^1 phenotype, so *scd* must lie at least partially
416 outside of these regions.

417 **Figure 3.** (A) Penetrance and (B) expressivity of the scd^1 mutation varies among
418 different wild type genetic backgrounds. In these experiments, stock scd^1 females were
419 crossed to males of different wild-type strains, and phenotypes were scored in F1 males
420 (which were hemizygous for scd^1 and heterozygous for a different wild genetic
421 background). Penetrance was measured as the proportion of males showing an ectopic
422 sex comb on the second tarsal segment of the prothoracic leg, while expressivity was
423 measured as the number of sex comb teeth on the ectopic sex comb. Error bars
424 indicate 95% confidence intervals.

425 **Figure 4.** The scd^1 mutation is deleterious. Average frequency of scd^1 over time, across
426 4 replicate populations, each initialized with 70% scd^1 males. Error bars indicate 95%
427 confidence intervals.

428 **Figure 5.** Plots of model fits for sex comb traits in experimental evolution populations.
429 (A) Number of teeth in the primary sex comb across 24 generations of experimental
430 evolution in all experimental treatments. (B) Proportion of male flies with defects in the
431 primary sex comb in all four treatments. (C) Number of teeth in ectopic/secondary sex
432 combs in males in the HSS and LSS treatments.
433
434

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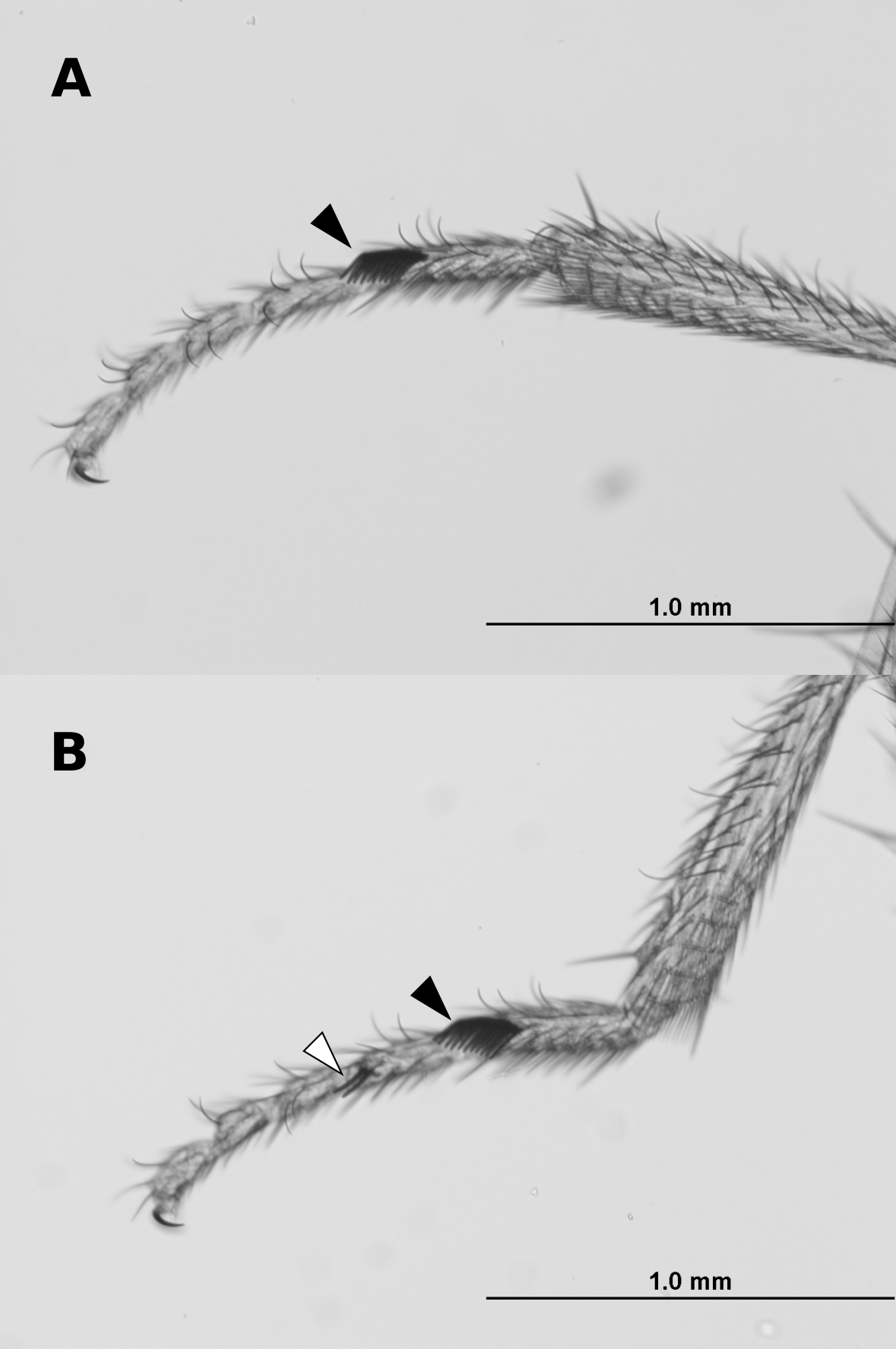
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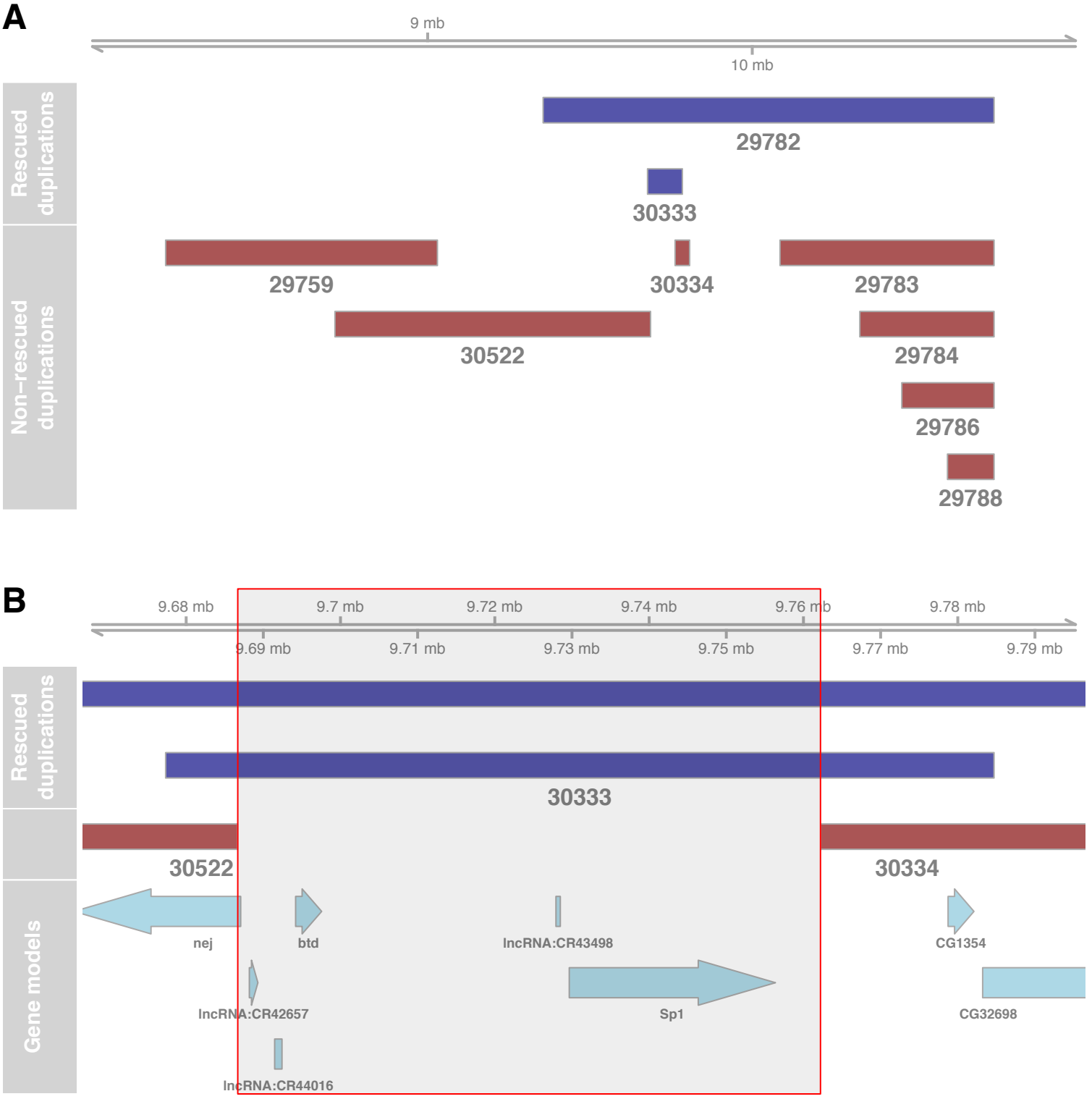
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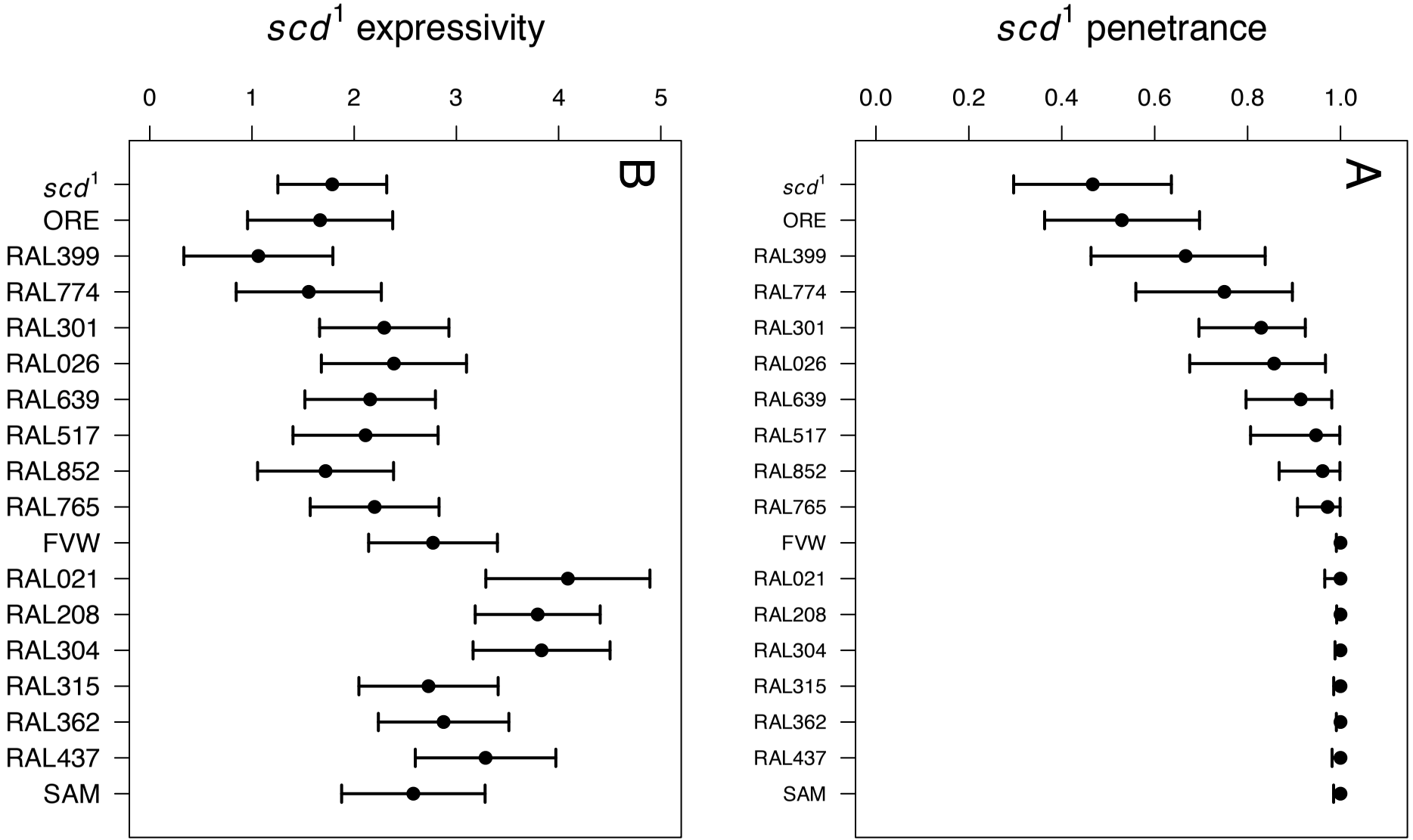
scd1 expressivity
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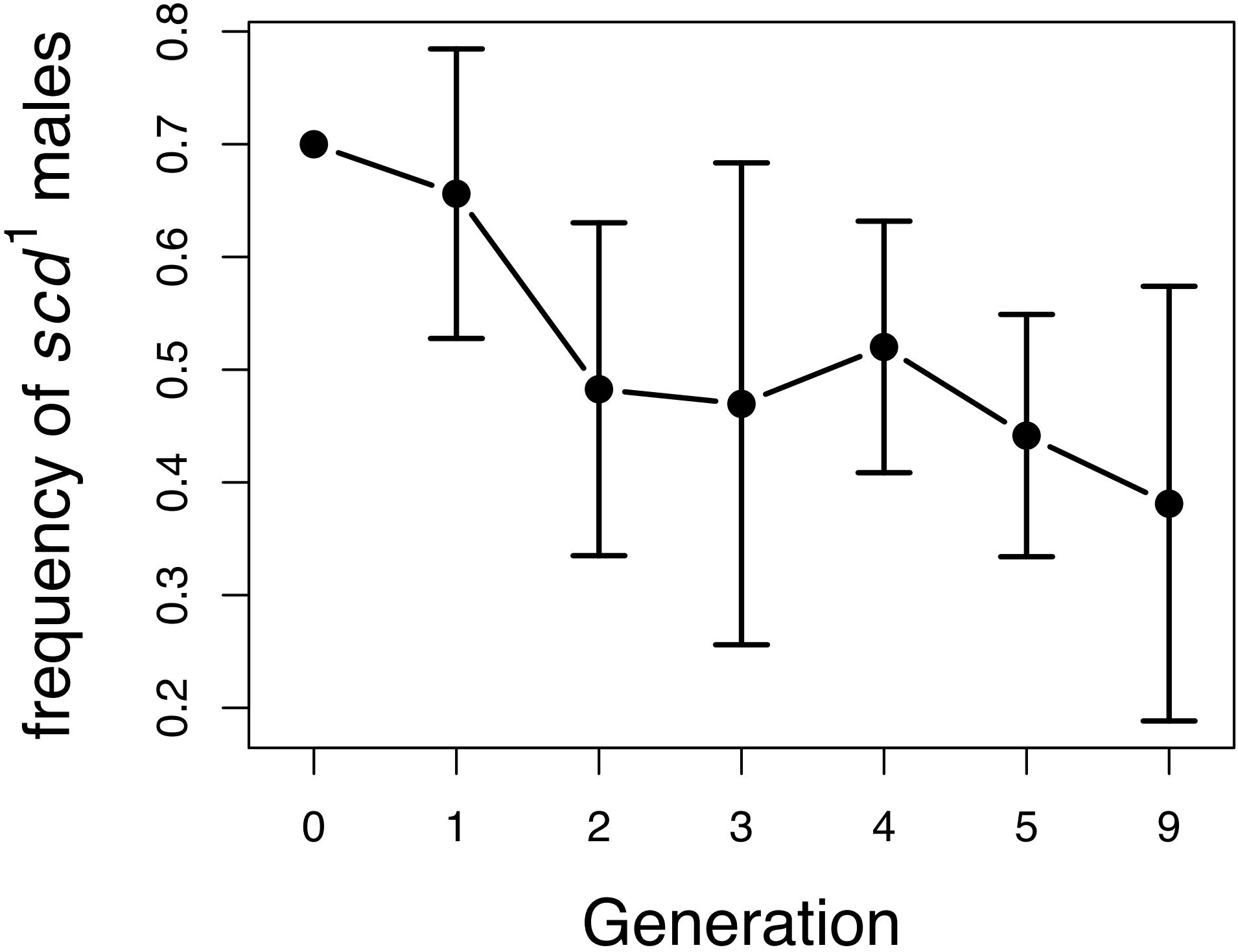
frequency of scd1 males
0.2
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0.4
0.5
0.6
0.7
0.8
0
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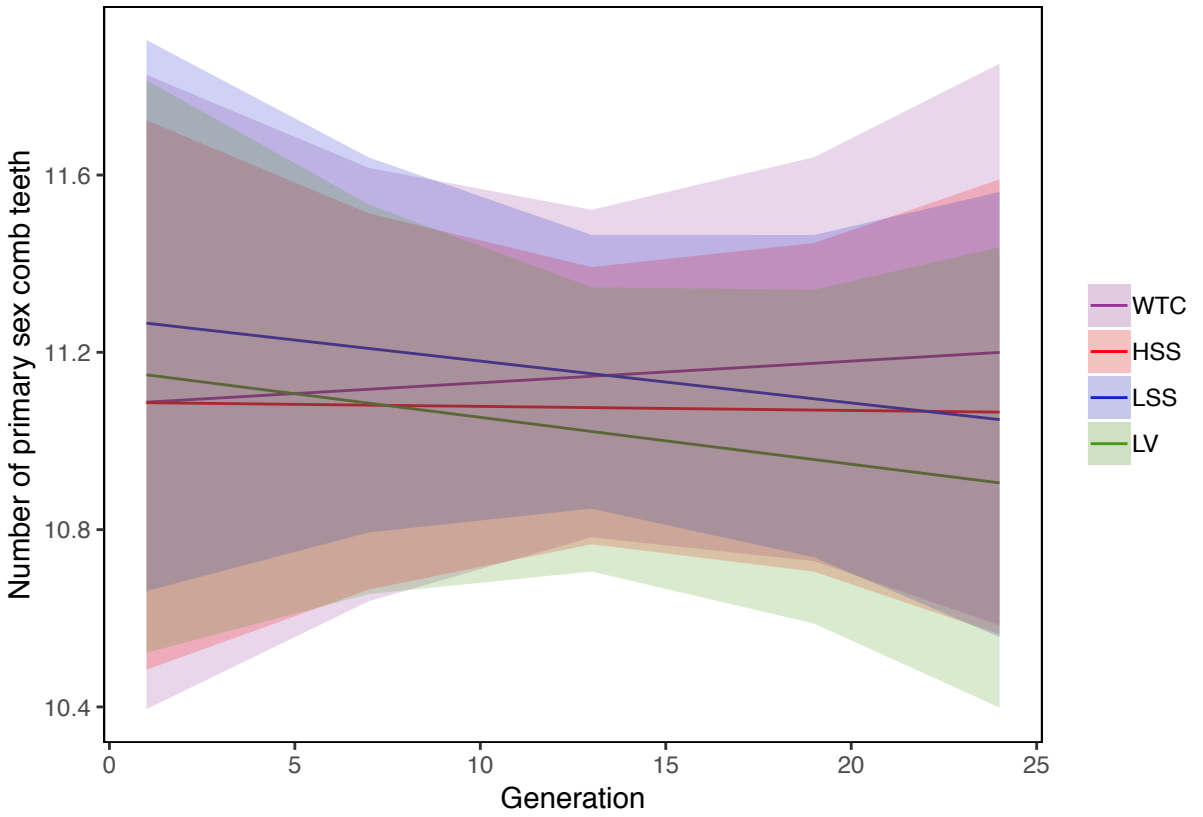
2
3
4
5
9
Generation

A


Number of primary sex comb teeth
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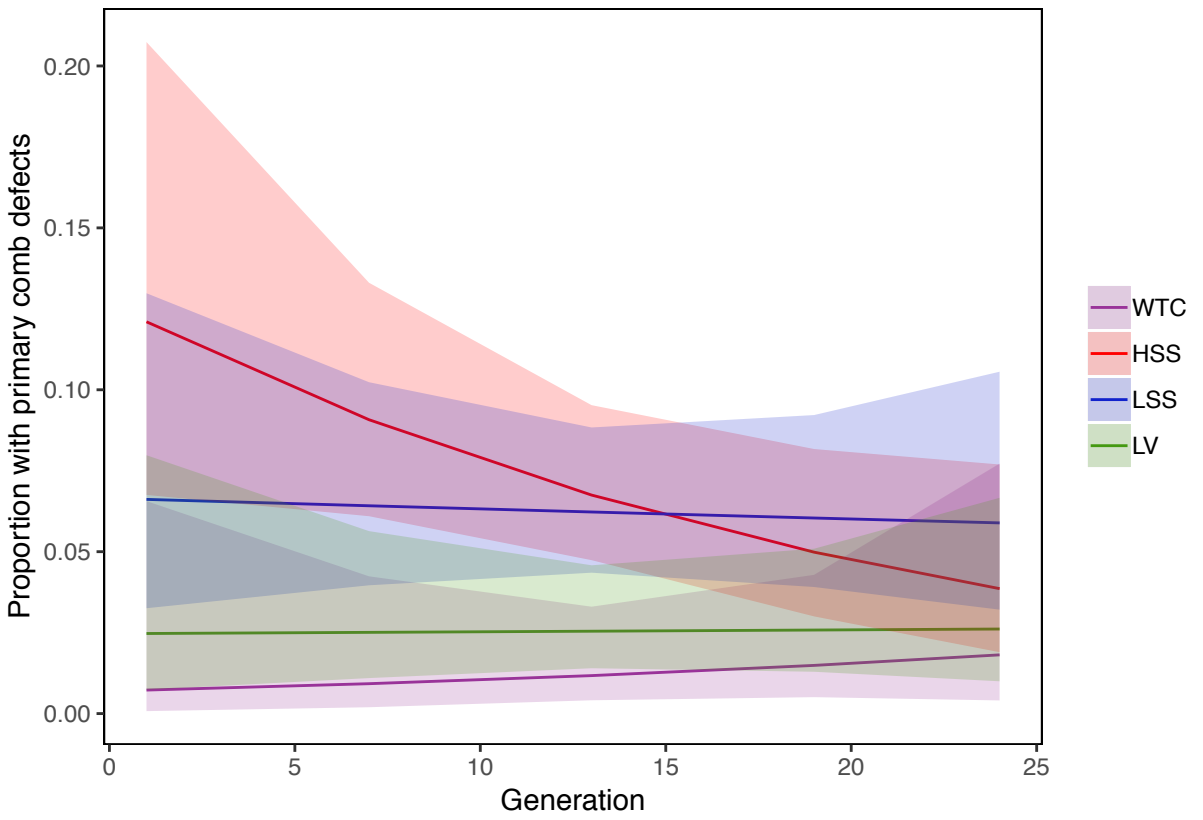
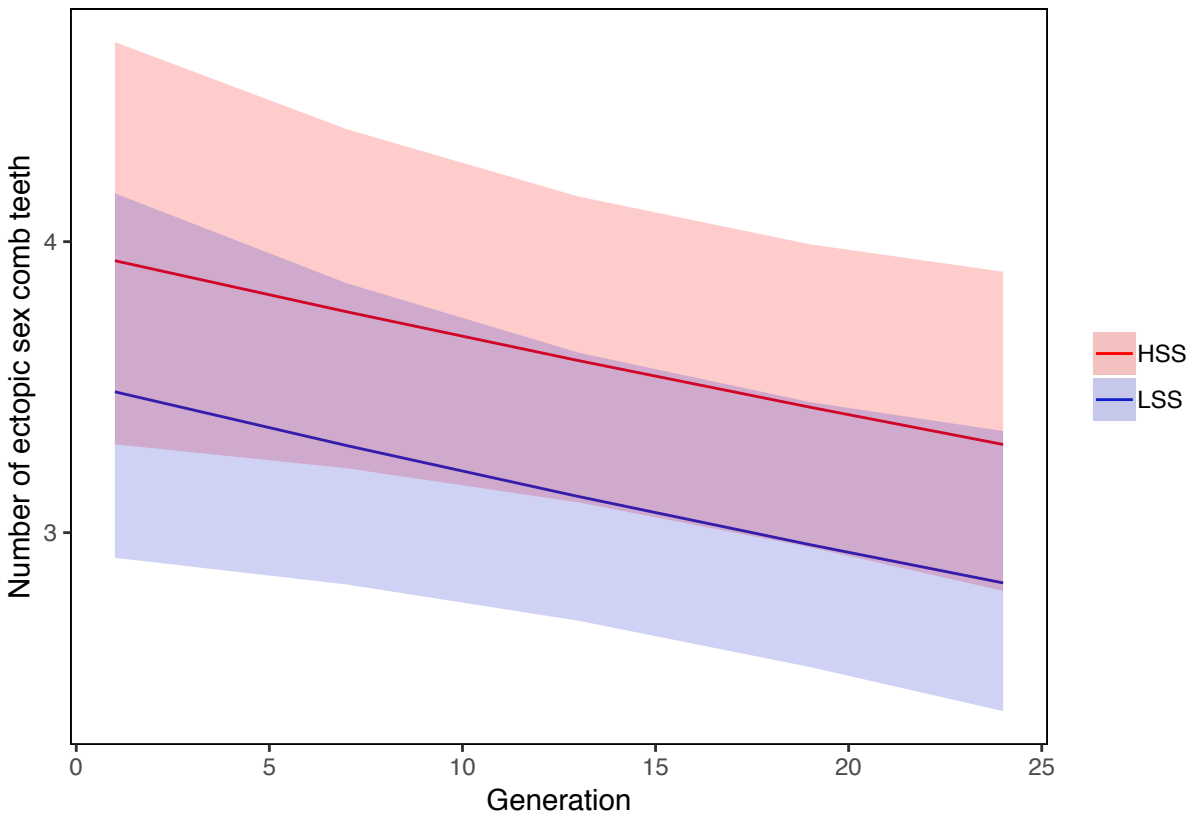
11.2
10.8
10.4
0
5
10
15
20
25
Generation
WTC
HSS
LSS
LV

B


Proportion with primary comb defects
0.20
0.15
0.10
0.05
0.00
0
5
10
15
20
25
Generation
WTC
HSS
LSS
LV

C


Number of ectopic sex comb teeth
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0
5
10
15
20
25
Generation
HSS
LSS