1 **Supergene degeneration opposes polymorphism: The curious case of balanced lethals**

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Abstract

Supergenes offer some of the most spectacular examples of long-term balancing selection in nature but their origin and maintenance remain a mystery. A critical aspect of supergenes is reduced recombination between arrangements. Reduced recombination protects adaptive multi-trait phenotypes, but can also lead to degeneration through mutation accumulation. Mutation accumulation can stabilize the system through the emergence of associative overdominance (AOD), destabilize the system, or lead to new evolutionary outcomes. One such outcome is the formation of balanced lethal systems, a maladaptive system where both supergene arrangements have accumulated deleterious mutations to the extent that both homozygotes are inviable, leaving only heterozygotes to reproduce. Here, we perform a simulation study to understand the conditions under which these different outcomes occur, assuming a scenario of introgression after allopatric divergence. We found that AOD aids the invasion of a new supergene arrangement and the establishment of a polymorphism. However, this polymorphism is easily destabilized by further mutation accumulation. While degradation may strengthen AOD, thereby stabilizing the supergene polymorphism, it is often asymmetric, which is the key disrupter of the quasi-equilibrium state of the polymorphism. Furthermore, mechanisms that accelerate degeneration also tend to amplify asymmetric mutation accumulation between the supergene arrangements and vice versa. As the evolution of a balanced lethal system requires symmetric degradation of both arrangements, this leaves highly restricted conditions under which such a system could evolve. We show that small population size and low dominance coefficients are critical factors, as these reduce the efficacy of selection. The dichotomy between the persistence of a polymorphism and degradation of supergene arrangements likely underlies the rarity of balanced lethal systems in nature.

Keywords: associative overdominance, supergene, balancing selection, introgression, inversion, balanced lethal system

Background

Understanding the forces that maintain genetic variation despite the eroding forces of drift, directional selection, and recombination is one of the central problems of evolutionary biology. Supergenes, tightly linked sets of loci that underlie distinct complex phenotypes (1, 2), represent some of the most spectacular examples of long-term balanced polymorphisms in nature. Although the genetic architecture of supergenes does not always include chromosomal rearrangements, they are frequently present (3); here, we therefore refer to different variants of the supergene as arrangements. The reduced recombination between supergene arrangements allows for unique combinations of alleles to be maintained in linkage disequilibrium, thus preserving multi-trait phenotypes and allowing for adaptation to complex environments. However, the origin and maintenance of multiple supergene arrangements and their associated phenotypes within populations is still poorly understood (3-5).

Reduced recombination not only allows for multi-locus complexes that define supergenes, but also puts them at risk for degeneration. This reduction in recombination generates a pseudo-population substructure; effective population size (N_e) for the supergene region is reduced as compared to the rest of the genome. The local decreases in N_e and in effective recombination rate between supergene arrangements diminish the efficacy of purifying selection and can lead to the accumulation of deleterious alleles (3, 6, 7). Indeed, recent theoretical and empirical work has shown that supergene systems are prone to such mutation accumulation (3, 7-9). This degeneration can destabilize the system or lead to new evolutionary outcomes.

The formation of a balanced lethal system (10) has been theorized to be one such outcome (11). In a balanced lethal system, only adults possessing two distinct arrangements (i.e., heterokaryotypes) are viable. According to the rules of Mendelian inheritance, 50% of the next generation will be homozygous for either one or the other arrangement and thus inviable, meaning that half of the offspring are lost every generation. While it seems counterintuitive that such a huge genetic load could ever evolve under natural conditions, it has done so repeatedly across the tree of life, with examples in plants, insects and vertebrates (12-16). However, the degeneration of supergenes provides a potential path by which a supergene system might evolve into a balanced lethal system; a mechanism that has received very little theoretical attention (but see 17, 18). If the fitness of the homozygotes for both supergene arrangements (i.e., homokaryotypes) reaches zero, then a balanced lethal system has evolved.

Recently, Berdan and colleagues have shown that evolution of an inversion polymorphism can lead to the formation of a balanced lethal system (8). Since their focus was on the evolution of polymorphic inversions, the authors assumed that the inversion itself was inherently overdominant (*sensu stricto*), facilitating its invasion. Here, we relax this assumption, allowing only selection on linked variants. Thus in our model, associative overdominance (AOD), the heterozygote advantage experienced by a neutral variant (in this case the supergene region itself) due to selection on sites linked to the neutral locus (19-22), is the only form of balancing selection. While AOD can be caused by overdominant alleles, it can also be generated by the masking of deleterious recessive variants in multi-locus heterozygotes. Empirical and theoretical work suggests that AOD driven by deleterious recessive variants may be a strong and common force stabilizing supergene systems (6-9, 23, 24).

Here, we explore the role of AOD in maintaining a supergene polymorphism and ask when it can pave the way towards a balanced lethal system. Specifically, we examine a case where a supergene arrangement introgresses from another population. As compared to a single-population origin, the introgression scenario increases the chance of generating a balanced

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102   polymorphism, as there will be pre-existing AOD due to private deleterious recessive mutations,
103   resulting from divergence between populations. Empirical data indicate that introgression might
104   be a common mechanism through which supergene polymorphisms originate (25, 26). Starting
105   from introgression of a supergene arrangement we use simulations to understand the range of
106   conditions under which a supergene can persist and potentially evolve into a balanced lethal
107   system.
108
109   **Model**
110
111   We explored how admixture between populations, fixed for alternative arrangements of a
112   supergene, could generate a supergene polymorphism via AOD. Following admixture, AOD is
113   generated by the masking of recessive private deleterious mutations within the supergene region.
114   We hypothesized that the extent of AOD and its evolutionary consequences should be based on
115   four major factors:
116
117   (1) the local mutational load of the populations,
118
119   (2) the dominance coefficient of the deleterious mutations,
120
121   (3) the rate of gene conversion (GC; the only recombination occurring between supergene
122   arrangements in our model), and
123
124   (4) the population size.
125
126   We simulated two isolated diploid populations (P_1 and P_2, N=2,500 individuals per population)
127   using SLiM v2.6 (27). The genome consisted of 2 chromosomes of 10 Mb (genome length L =
128   20 Mb). We used parameters based on estimates from *Drosophila melanogaster*. Mutations occurred
129   at a rate of μ=4.5 x 10^{-9} per bp per generation (28). Recombination rate was set to r = 4.80 x 10^{-8}
130   per base pair per meiosis (29); recombination occurred via a combination of crossing over, at a
131   rate of ρ = 3.0 x 10^{-8} per base pair per meiosis (29, 30), and gene conversion at a rate of γ = 1.8
132   x 10^{-8} per base pair per meiosis (31) (γ is the rate of initiation of a gene conversion event). Gene
133   conversion track length was modeled as a Poisson distribution, with the parameter λ set to 500
134   bp (31).
135
136   To generate differences in mutational load of the populations, we used burn-ins of two different
137   durations (T_{BI}=200,000 or T_{BI}=500,000 generations). Since the populations were isolated, the
138   burn-in length corresponds to divergence time. Fitness was multiplicative, and all mutations were
139   assumed to be deleterious. The magnitude of their fitness effects ($|s|$) was drawn from a
140   Gamma (Γ) distribution (α = 0.5, β = 10), with fixed dominance coefficient h. Separate burn-ins
141   were run for two different dominance coefficients: h=0 and h=0.1. Given that not all simulations
142   used the same burn-in, stochasticity between burn-ins could affect possible metrics of interest
143   when comparing across different values of h or burn-in length (see *Significant variation with*
144   *evolutionary consequences exists between burn-ins* below). To take into account this source of variation,
145   we generated 10 different burn-ins per parameter combination (2 population sizes and 2
146   dominance coefficients, for a total of 40 burn-ins).
147
148   We modelled the supergene as a chromosomal inversion. The inversion occurred at a fixed
149   position on chromosome 1 and encompassed 50% of its length (i.e., 25% of the genome). The
150   inverted arrangement was assumed to be fixed in P_2, while the P_1 population carried the standard
151   arrangement. Recombination in the inverted region in the heterokaryotype was restricted to gene

152   conversion only. The second chromosome served as a control region since indirect effects of the
153   inversion could extend beyond the inverted region to the rest of chromosome 1.
154
155   The polymorphic period for each replicate began with the migration of a single randomly chosen
156   individual from P_2 to P_1 in generation 1. This migration step established the supergene system,
157   with the inverted arrangement from P_2 and the standard arrangement from P_1 forming the two
158   arrangements of the supergene. After this migration event, we only followed evolution of P_1.
159   Simulations ended when the immigrating inverted arrangement was fixed or lost, or after 200,000
160   generations if it remained polymorphic. For each of the 10 burn-ins per parameter combination,
161   we ran 10,000 replicates for a total of 100,000 replicates per parameter set (over 16 parameter
162   sets in total).
163
164   We examined the emergence of AOD and its evolutionary consequences for the inversion
165   polymorphism, using a full block design and varying two post-burn-in parameters (GC and
166   population size) for each of the four combinations of the burn-in parameters (see Table 1). The
167   absence of GC was modelled as having all recombination events occur as crossing overs, so that
168   the total rate of recombination, r, remained constant. To explore the effect of a smaller
169   population size, we assumed that a bottleneck occurred before the migration event, with only
170   100 individuals among the 2,500 in P_1 contributing to generation 1 of the AOD period. The
171   polymorphic period differed from the burn-in period in that beneficial mutations could also
172   occur at a 1,000x lower rate than their deleterious counterparts (4.5 x 10^{-12} per bp per generation).
173   All beneficial mutations were co-dominant (h=0.5) and their fitness effects ($|s|$) were drawn
174   from an exponential distribution with parameter $\kappa = 0.001$.
175
176   To further explore the relationship between population size and the emergence of balanced
177   lethals, we considered two additional population sizes (N=250 and N=500; resulting from a
178   bottleneck of the N=2,500 population, as described above) and tested these with the two
179   different burn-in lengths. Both GC and h were kept at 0, as these factors were found to be
180   critical for the emergence of balanced lethals using our initial parameter combinations (see
181   *Balanced lethals can only evolve in a highly restricted parameter space* below). As before, we performed
182   10,000 replicate runs of each of these parameter sets, for each of the 10 burn-ins, for a total of
183   100,000 replicates.
184
185   To reduce computational time we did not simulate neutral mutations. We simulated allelic
186   content only in 200 kb of the 20Mb genome, divided into 5 kb segments, uniformly distributed
187   along the genome. Recombination can occur anywhere in the genome, but deleterious and
188   beneficial mutations only occur in regions where we simulated allelic content. Finally, we scaled
189   our parameters by a factor of 10, keeping $L\mu$ and Lr constant, so that evolution occurred at an
190   accelerated rate (a common practice; see (32) for an example).
191
192   Since we assumed a constant population size, only relative fitness values were considered. For
193   the rest of the paper, we refer to fitness relative to the mean fitness of the population.
194

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Parameter	Value
Mutation rate (μ)	4.5 x 10^{-9} per bp per generation
Genome length (L)	20 Mb
Recombination (r)	4.80 x 10^{-8} per bp per meiosis
Gene conversion initilization (γ)	0 or 1.8 x 10^{-8} per base pair per meiosis
Gene conversion tract length (λ)	500 bp
Burn-in length (T_{BI})	200,000 or 500,000 generations
Dominance coefficient (h)	0 or 0.1
Population size post-burn-in (N)	100 or 2,500 (250 and 500 for selected combinations of other parameters)

Table 1 - Parameters for the simulations

Results and Discussion

Significant variation with evolutionary consequences exists among burn-ins

The mutational load of the populations, following the burn-in, is affected by dominance and burn-in length. As expected, the number of fixed mutations between different populations increases with burn-in length, but the number of segregating mutations remains stable (figure S1). Mutations accumulate in a linear fashion, with the 500,000 generations burn-in having ~2.5x more fixed mutations than the 200,000 generation burn-in. Increasing the dominance coefficient (h) strongly decreases the number of segregating mutations (figure S1B). Additionally, increasing the dominance coefficient slightly decreases the average number of fixed mutations, however the distributions overlap (figure S1A). Thus, by simply picking randomly a single burn in for each value of h, there is a non-negligible chance that the average trend observed would be reversed, highlighting the necessity of using multiple burn-ins. These effects are mirrored for mutational load (figure S2). The mutational load of the supergene region, especially in P_1 (the focal population), is strongly correlated with the strength of AOD at the beginning of the simulation (figure 1A, figure S3). We calculate AOD following Ohta (19), using $s' = \min(s'_1, s'_2)$, where:

$s'_1 = 1 - \overline{W}_{AA}/\overline{W}_{AB}$
$s'_2 = 1 - \overline{W}_{BB}/\overline{W}_{AB}$

Where A is the inverted arrangement (originating in P_2) and B is the standard arrangement (originating in P_1). Positive values of s' indicate that the heterokaryotype is more fit than either homokaryotype (i.e., the presence of AOD) and negative values indicate the reverse. Variation in mutational load and in the strength of AOD is found across parameter combinations, as well as among burn-ins within parameter combinations (figure 1A).

The strength of AOD at the beginning of the simulation partially explains the probability of invasion (figure 1B). We consider that the inverted arrangement has successfully invaded if it is still present in P_1 after N generations. As AOD increases, so does the probability of invasion. This is in line with theoretical predictions that AOD should aid supergene invasion (23).
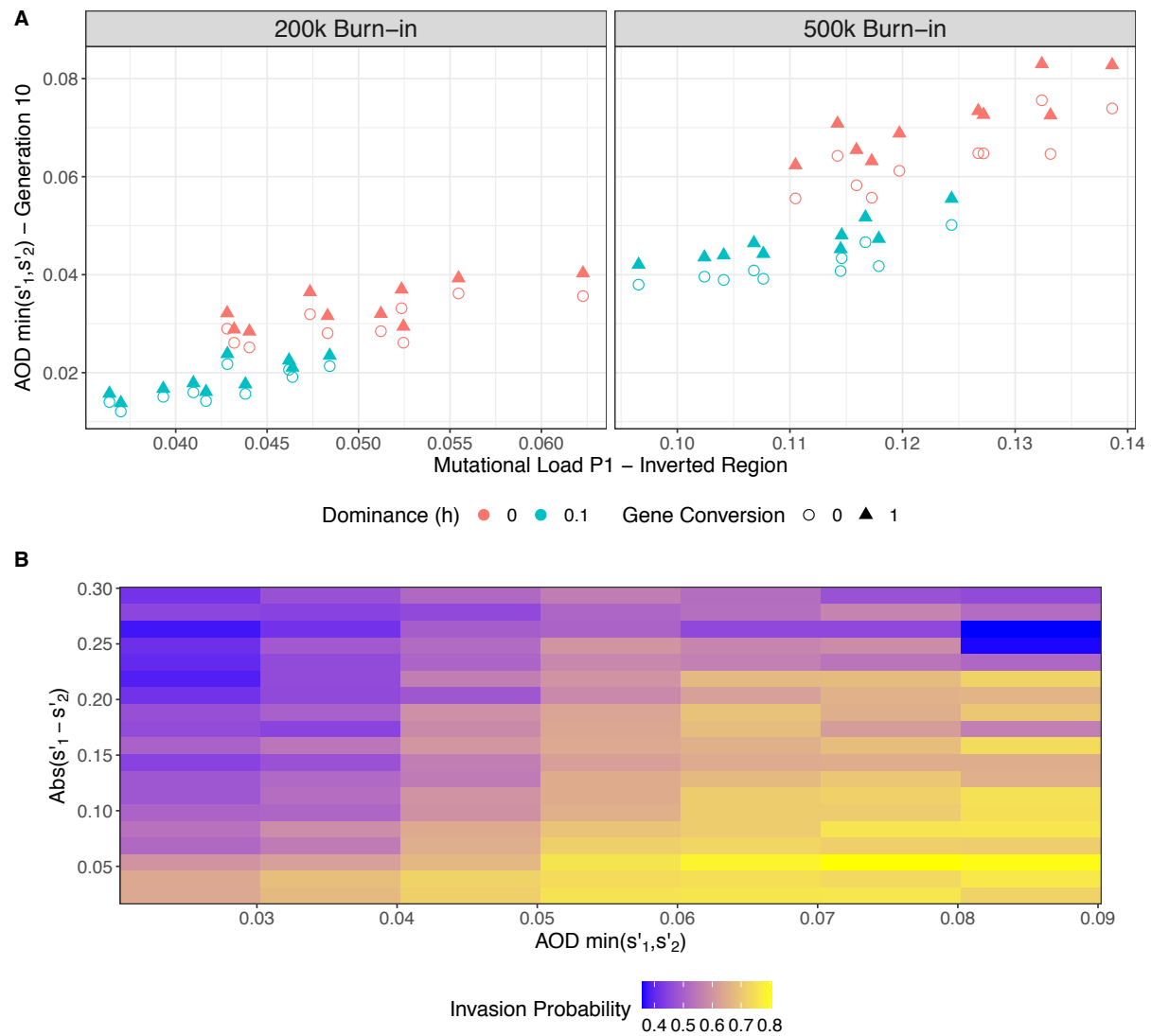
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Figure 1. Associative overdominance (AOD) generated from mutational load can facilitate invasion of a supergene. A.) AOD correlates with mutational load. Shown is the average AOD at generation 10, across replicates, for each burn-in (200k or 500k generations), compared to the mutational load of the P1 population estimated from the burn-in. Colors indicate the dominance coefficient (0-red, 0.1-blue) and shape indicates the level of gene conversion (circle - absence, triangle - presence). The data shown is for N=2,500. B.) Both symmetry and strength of AOD contribute to the probability of invasion. Each tile represents a specific parameter space (+/-0.005 change in AOD and +/-0.0075 change in absolute (s'_1-s'_2)). Coloring represents the probility that a simulation from that parameter space resulted in invasion. The number of obervations per tile differs, but all are greater than 20. The data shown is for N=100, GC=0, and h=0.

AOD is evolutionarily unstable because it evolves

AOD is determined by the mutational loads of the two supergene arrangements. Its strength therefore varies as the supergene arrangements evolve. Nei *et al.* found that AOD could aid the invasion as well as the fixation of a new inversion (33). Yet, in many circumstances such an advantage conferred by AOD might only be transient as the inversion will accumulate recessive deleterious mutations over time which might in turn prevent the fixation of the inversion homokaryotype (34). In our model, where the inversion is introduced by migration, invasion probability is also dependent on the symmetry of the mutational load of the two supergene arrangements in addition to the strength of AOD (figure 1B). This is consistent with results from

253   single-locus overdominance models which show that higher levels of heterozygosity can be
254   maintained under symmetrical overdominance, because drift is less likely to cause the loss of a
255   polymorphism maintained at intermediate frequency (35, 36). A critical difference between AOD
256   and single-locus overdominance is that AOD can easily evolve (34), while the overdominance
257   coefficient is generally a fixed property of the mutation.
258
259   AOD is determined by large regions of the genome that are prone to mutation accumulation due
260   to reduced recombination (8, 9). The rate of mutation accumulation in either of the supergene
261   arrangements is tied to both effective recombination rate and the relative strengths of drift and
262   purifying selection (correlated with the number of copies of the arrangement). A large difference
263   between the fitness of the supergene arrangements implies a large frequency difference between
264   the major (more frequent) and minor (less frequent) supergene arrangements. This in turn
265   translates into purifying selection being far less effective in the minor arrangement, which leads
266   to an increase in mutation accumulation (8). This has two consequences: first the relative fitness
267   of the major arrangement will increase even more, further increasing its frequency, and therefore
268   the efficacy of purifying selection, in a feedback loop. Second, if mutations are only partly
269   recessive, mutation accumulation in the minor type will also decrease the fitness of the
270   heterokaryotype and thus reduce AOD. These processes drive the frequency of the minor
271   arrangement down, increasing its chance of being lost by drift. To illustrate this point, consider a
272   single-locus model with overdominance and assume that one homozygote is inviable (see
273   Appendix). In order for the frequency of the corresponding allele to remain above 10%,
274
275    $9*(\overline{W}_{MAJOR}-\overline{W}_{AB}) + \overline{W}_{AB} > 0$.
276
277   Using our notation, this can be simplified (regardless of dominance) to:
278
279    $s' > 1/9$.
280
281   The critical factors for the maintenance of the supergene polymorphism are the fitness
282   differential between the major arrangement and heterokaryotype and the mean fitness of the
283   heterokaryotype. An increase in dominance coefficient will reduce both terms; as predicted, we
284   observe a negative relationship between dominance coefficient and invasion probability (figure
285   S4). Together, our results point towards the symmetry of the mutational load of the two
286   supergene arrangements (along with the magnitude of AOD) as being a key factor determining
287   the fate of the supergene polymorphism.
288
289   AOD readily evolves in our model: it generally decreases in the beginning of the runs and then
290   increases again over time (figure 2). This remains true even when focusing only on simulations
291   where the population remains polymorphic to the end. This indicates that this increase is not due
292   to a sieving effect (i.e., cases with weak AOD are removed at early time points leaving only cases
293   with strong AOD at later time points). In addition, the initial decrease in AOD occurs even in
294   the absence of gene conversion and with fully recessive deleterious mutations, excluding the
295   possibility that the mutational load of the immigrating arrangement decreases via gene flux and
296   selection. Thus, this initial decrease is likely due to the erosion of linkage disequilibrium between
297   the supergene arrangement from P_2 and the rest of the P_2 genome. Initially, the entire
298   chromosome 1 (50% of the genome) contributes to overdominance, but over time
299   recombination will reduce this to the supergene alone. Indeed, this drop in AOD corresponds to
300   a drop in heterokaryotype fitness and a slight increase in the fitness of the inversion
301   homokaryotype (figure S5). After the initial decrease, AOD increases in all runs that remain
302   polymorphic over 200,000 generations, due to mutation accumulation. This increase is stronger

303 in the absence of GC, with fully recessive deleterious mutations, and in smaller populations
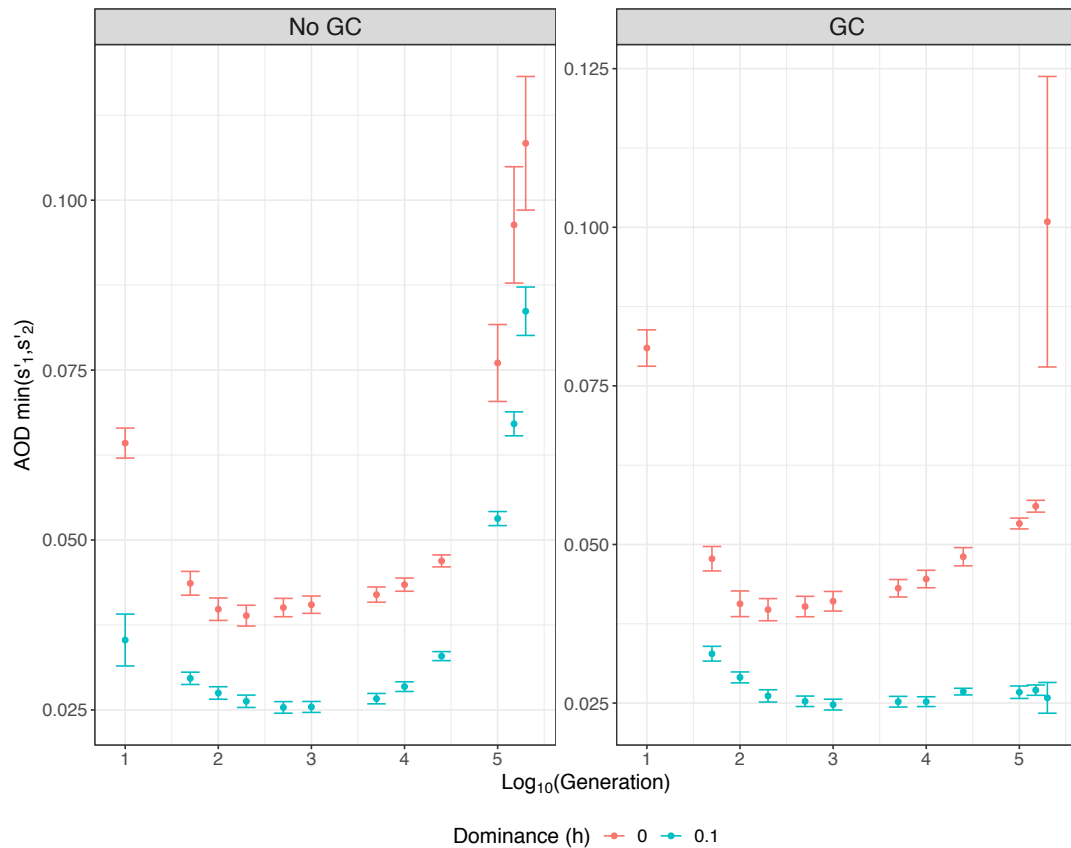304 (figure S6).
305

306

307 Figure 2. Associative Overdominance (AOD) evolves in a non-monotonous manner over time. Shown are changes
308 in AOD over time, only for simulations where the polymorphism lasted 200,000 generations (N=2,500,
309 Burn-in=500k generations). AOD for each generation is calculated as an average per burn-in, per
310 parameter set. Error bars represent standard error between burn-ins. Colors indicate the dominance
311 coefficient (0-red, 0.1-blue) and facets indicate the level of gene conversion (GC; presence or absence)).

312

313 *Maintenance of supergene polymorphism can be accomplished in three different ways*

314

315 To understand the fate of the supergene and its possible long-term maintenance, we focus on
316 cases where the polymorphism is maintained over the full simulation period (i.e., 200,000
317 generations). We examine fitness across karyotypes at the final generation, considering
318 individuals with fitness values < 0.01 to be inviable and individuals with fitness values > 0.01 to
319 be viable. We identify three qualitatively different outcomes:

320

321 (1) both homokaryotypes remain viable ($\overline{W}_{BB} > 0.01$ & $\overline{W}_{AA} > 0.01$),

322

323 (2) one homokaryotype becomes inviable, while the other remains viable ($\overline{W}_{BB} < 0.01$ & $\overline{W}_{AA} >$
324 0.01 or $\overline{W}_{AA} < 0.01$ & $\overline{W}_{BB} > 0.01$), and

325

326 (3) both homokaryotypes become inviable and only heterokaryotoypes contribute to subsequent
327 generations (i.e., a balanced lethal system; $\overline{W}_{BB} < 0.01$ & $\overline{W}_{AA} < 0.01$).

328

9

329 Outcome 1 only occurs in the large populations (N=2,500) and is negatively correlated with GC
330 and positively correlated with h and divergence time before introgression (figure S7). Outcome 2
331 occurs almost exclusively in large populations (N=2,500; 99.99% of cases), without GC (99.92%
332 of cases), and is negatively correlated with h and positively correlated with divergence time
333 (figure S8A). Finally, outcome 3 (a balanced lethal system) only occurs in the small populations
334 (N=100) and is negatively correlated with h and positively correlated with divergence time (figure
335 S8B). Overall, either partially recessive mutations or GC, or both, reduce the chance of the
336 supergene remaining polymorphic for up to 200,000 generations (i.e. at least 80 N generations).
337
338 We further explore how fitnesses change over time when the polymorphism persists. When both
339 homokaryotypes remain viable (outcome 1), there is an initial increase in fitness of all three
340 karyotypes (figure 3). In our simulations there are three ways for a homokaryotype to increase in
341 fitness: (i) purging of deleterious mutations via gene flux, (ii) reducing linkage disequilibrium with
342 the rest of the genome, therefore lessening the mutational load generated by private mutations,
343 and (iii) accumulation of beneficial mutations. Recombination can occur within arrangements via
344 crossing over and GC or between arrangements via GC only. The introgressed inverted
345 arrangement cannot purge its initial mutational load by recombination within arrangements, since
346 all inverted arrangements share the same load. We observe increases in fitness, both in the
347 presence and absence of GC. This indicates either accumulation of beneficial mutations or the
348 lessening of linked mutational load generated by private mutations (figure 3). After the initial
349 increase, fitness of the homokaryotypes plateaus, before decreasing again. This decrease is due to
350 the accumulation of deleterious mutations, which do not affect the fitness of the heterokaryotype
351 when h=0. It is important to remember that we only run our simulations for 200,000 generations.
352 Given that karyotype fitnesses are still evolving, outcome 1 is likely a transient state, as s' is quite
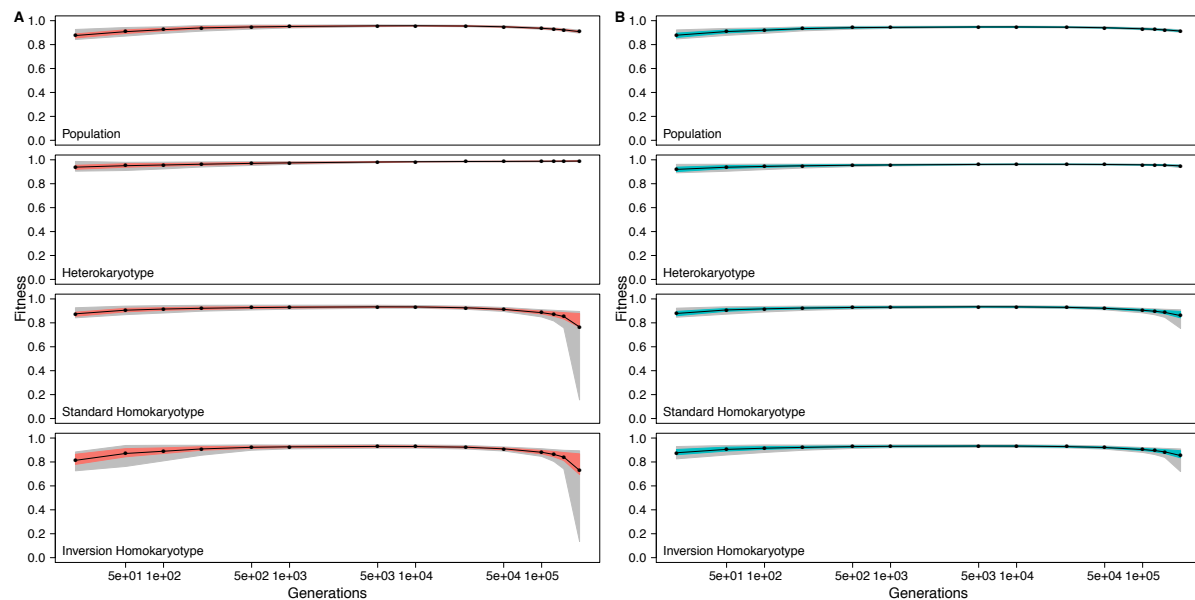353 small and asymmetric mutation accumulation could result in the loss of the polymorphism.
354

Figure 3. Evolution of fitness when all karyotypes remain viable for different values of h (A. h=0, B. h=0.1). Fitness of the population, relative fitness of the heterokaryotype, relative fitness of the standard homokaryotype, and relative fitness of the inversion homokaryotype are shown. Time is always shown on a log scale, actual time points are: 10, 50, 100, 200, 500, 1000, 5000, 10000, 25000, 50000, 100000, 125000, 150000, and 200000 generations. Only data from N=2,500 runs that lasted 200,000 generations are shown.

362 In the half-lethal outcome (outcome 2), one arrangement degrades by mutation accumulation,
363 while the other does not (figure S9). Initially, fitnesses of all karyotypes increase, due to the

10

364 accumulation of beneficial mutations and the reduction of linkage disequilibrium between the
365 supergene and the other P_2 mutations situated outside of the supergene. After reaching a plateau,
366 the fitness of one of the homokaryotypes drops steeply to "lethal" (fitness < 0.01), due to the
367 accumulation of deleterious mutations, while the other remains relatively fit (figure S9). The fitter
368 arrangement also accumulates mutations over time, but at a much slower rate (figure S9).
369 Outcome 2 is likely to be less transient than outcome 1 (where both homokaryotypes remain
370 viable), because there is the possibility for *s'* to increase as the fitter homokaryotype slowly
371 degrades. This indicates that AOD is able to maintain a long-term polymorphism when at least
372 one of the homokaryotypes is inviable. Indeed, many supergene systems have a single lethal
373 homokaroytype, including e.g. the ruff (37, 38) and the fire ant (39, 40). We discuss outcome 3
374 below (see section *Balanced lethals can only evolve in a highly restricted parameter space*).

375

376 *The degradation of supergene arrangements and the maintenance of the polymorphism via AOD are at odds*

377

378 The parameter space within which the supergene polymorphism might potentially be maintained
379 over long time-scales by AOD alone is small. Events occurring with a frequency below 10^{-4} were
380 ignored. Outcomes 1 and 2 only occur in 5 and 2 parameter combinations, respectively, out of
381 16. This suggests, that within our model, AOD alone is only capable of maintaining a long-term
382 balanced polymorphism in exceptional circumstances. We hypothesize that this is due to the fact
383 that degeneration of the supergene arrangements, the process that increases AOD, typically also
384 leads to homokaryotype asymmetry.

385

386 To test this idea, we examine how our four investigated parameters (GC, dominance coefficient,
387 divergence time, and population size) affect critical properties (Table 2). For a polymorphism to
388 be maintained, the inverted arrangement must invade, but not fix. This creates a challenge; the
389 underlying cause of the necessary fitness advantage of the heterokaryotype, should not also
390 generate a fitness advantage for the inverted homokaryotype. For instance, increased divergence
391 between populations increases both invasion and fixation probability. Stronger initial AOD
392 means that the immigrating arrangement quickly spreads in the population, thus spending less
393 time at low frequency, where mutation accumulation is more likely. Once this happens, and if the
394 two arrangements are approximately equivalent in load, it is unpredictable which one is fixed or
395 lost.

Parameter	Invasion	Fixation	Mutation Accumulation major	Mutation Accumulation Minor	Efficacy of purifying selection	Homokaryotype Symmetry	Magnitude of AOD
Gene Conversion	+	=	-	-	+	+	=
Dominance coefficient	-	+	-/=	-	+	+	-
Divergence Time	+	+	+	+	-	=	+
Population Size	-	-	-	-	+	-	-

396 Table 2. Shown are the relationship between the parameter and process (yellow positive, blue negative, grey no
399 relationship). Note that dominance coefficient refers to the coefficient for deleterious mutations only.

400

401 If invasion succeeds, the processes that will decide the fate of the polymorphism are the rates of
402 mutation accumulation in the major and minor arrangements. Only mutation accumulation in
403 the major arrangement will increase *s'* and symmetry. Mutation accumulation in the minor
404 arrangement will not affect *s'* but will decrease symmetry. Due to the feedback loop between the
405 effective population size of each supergene arrangement and mutation accumulation (discussed
406 above in *AOD is evolutionarily unstable because it evolves*), mutation accumulation is (much) faster in
407 the minor arrangement. The presence of the feedback loop thus makes maintenance of the
408 polymorphism by AOD alone unlikely.

409

410   Several factors impact mutation accumulation in the major and minor arrangements and the
411   resulting fate of the polymorphism (figure 4). First, GC, by allowing recombination between the
412   two arrangements, can reduce asymmetry, promoting the polymorphism. Surprisingly, we do not
413   find a detectable reduction of the magnitude of AOD associated with GC. This might be due to
414   the low population-wide GC rate ($N\gamma$) relative to the rate of mutation accumulation. Second,
415   incompletely recessive deleterious mutations are partially expressed in the heterokaryotype. This
416   results in lower AOD, making the polymorphism more likely to be lost. However, the partial
417   expression in the heterokaryotype also allows purifying selection to act on the mutations,
418   especially those in the minor arrangement. This slows the rate of mutation accumulation and the
419   build-up of the asymmetry (figure 4). Third, population size has an unexpected role here. Small
420   populations mean that maintenance of the polymorphism becomes unlikely, as drift becomes
421   stronger, with the exception of one case: the balanced lethal system, which we discuss below. On
422   the other hand, larger population size means that selection can act upon smaller fitness
423   differences. With stronger purifying selection, a tiny initial asymmetry in mutation load can
424   trigger a 'snowball effect', leading to the loss of one of the two arrangements, even if they start
425   off in perfect symmetry. Finally, the divergence time between the two populations may be the
426   only factor that helps to maintain the polymorphism following invasion. A stronger initial
427   mutational load in both arrangements means a stronger initial AOD plus lower asymmetry
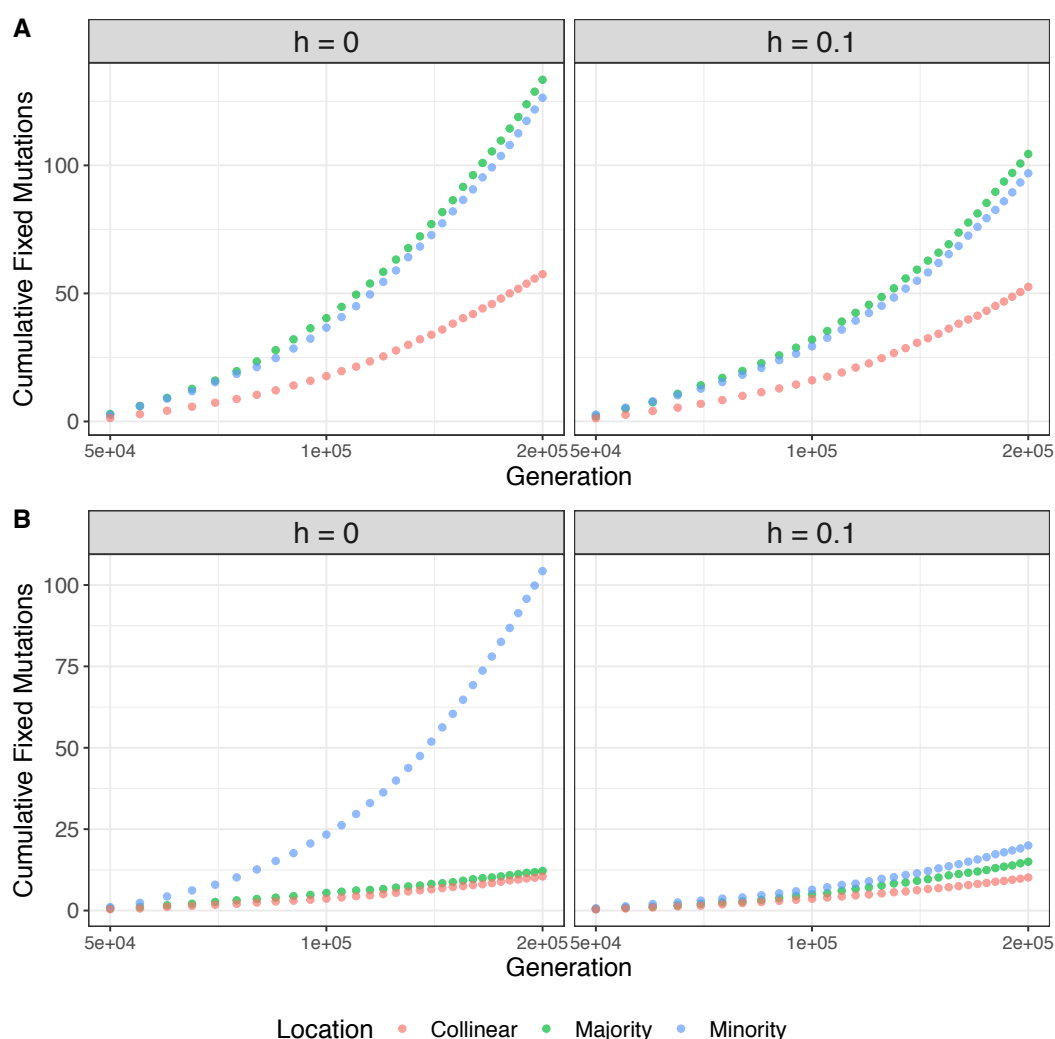428   between arrangements because of the larger sample of mutations.

429

430

431   Figure 4. Mutation accumulation varies across parameter space. Shown is cumulative accumulation of mutations in
432   (A) small populations (N=100) and (B) large populations (N=2,500) in the major arrangement (green), the

433   minor arrangement (blue), and the unlinked collinear region (red). Facets indicate the dominance
434   coefficient of deleterious mutations. For all data there is no gene conversion and only runs that lasted
435   200,000 generations are shown.

436

437   Up to this point we have only considered balancing selection caused by AOD driven by
438   deleterious recessive mutations. However, other forms of balancing selection may help to
439   stabilize the polymorphism. Negative frequency-dependent, spatially and/or temporally varying
440   selection are well-known forms of balancing selection that are found in supergene systems (1, 3,
441   5, 41). One particular mechanism, disassortative mating, is predicted to evolve in systems with
442   strong heterozygote advantage (e.g., AOD 42). Indeed, there is evidence of disassortative mating
443   in many classic supergene systems that also show signs of mutation accumulation (25, 26, 43-45).

444
445

446   *Balanced lethals can only evolve in a highly restricted parameter space*

447

448   The evolution of a supergene system into a balanced lethal system only happens under a
449   stringent set of conditions. We only observe the evolution of balanced lethals in small
450   populations. The probability of a balanced lethal outcome decreases with population size and is
451   not observed for N>=500. (figure 5). This is because the feedback loop between arrangement
452   frequency and mutation accumulation is disrupted by drift in small populations. Specifically,
453   purifying selection is going to be less effective in general, and in the major arrangement in
454   particular. Additionally, arrangement frequencies will fluctuate more between generations due to
455   drift. Together, these hamper the snowballing of an initial asymmetry by selection, occasionally
456   allowing for both supergene arrangements to degrade simultaneously (figure 5). However, in a
457   small population, loss of the polymorphism is the most common outcome, meaning that
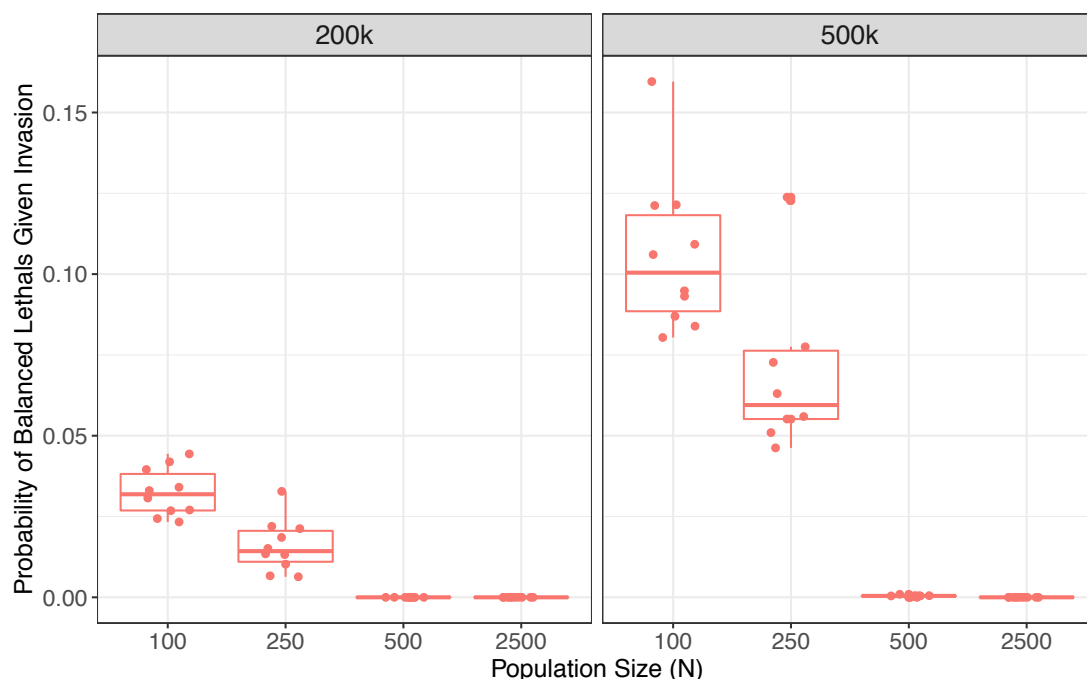458   balanced lethal systems remain rare.

459

461   Figure 5. Balanced lethals evolve under restricted conditions. Probability of balanced lethals given invasion is shown
462   as a function of the population size in the absence of gene conversion and for fully recessive mutations
463   ($h=0$). Facets indicate burn-in length (200k or 500k generations). Each point is a burn-in.

464

465 Another crucial factor is the dominance coefficient of the deleterious mutations. While we
466 observe the evolution of balanced lethals when mutations are not fully recessive, the likelihood
467 of observing this outcome is reduced. This is because partially recessive mutations are expressed
468 in the heterokaryotype, reducing AOD and destabilizing the polymorphism. In contrast, the
469 presence of GC does not seem to affect the outcome in line with the fact that it has little impact
470 on AOD overall.

471

472 **Conclusions**

473

474 While AOD can lead to the establishment of a supergene system, it is unlikely to maintain
475 polymorphism over long time periods when acting alone. Supergenes degenerate over time as the
476 decrease in effective population size and recombination rate leads to mutation accumulation.
477 Initial asymmetries often mean that one of the two supergene arrangements degenerates more
478 rapidly, which makes polymorphism sensitive to loss through drift.

479

480 We observe only two potentially persistent polymorphic states in our model, a half-lethal system
481 and a fully balanced lethal system, both of which occur only in small parts of parameter space.
482 Importantly, both states are found in natural supergene systems (3, 11). As AOD alone is
483 unlikely to maintain the supergene over long time scales, other forms of balancing selection, or
484 disassortative mating, might be key to maintaining supergene polymorphisms. This matches
485 empirical evidence from multiple supergene systems, where complex multi-dimensional selection
486 is involved in the maintenance of polymorphism (3, 4, 43, 46, 47).

487

488 In our simulations, balanced lethals only occur in small populations under restrictive conditions.
489 This finding is in good qualitative agreement with the fact that, despite a broad representation
490 across the tree of life, balanced lethal systems appear to be quite rare (10). We anticipate that
491 advances in genomics will provide insights into the frequency with which supergenes degrade
492 and evolve into balanced lethal systems.

493

494

495 **Methods**
496 The simulation code and analysis code is available at gitlab.com/slottelab/BL_sims

497

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500

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509

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615

Supplemental Figure Legends

617

618 Figure S1. Differences in fixed and segregating mutations between burn-ins. A.) Number of
619   fixed mutations between P1 and P2, B.) Number of segregating mutations in P2. Colors
620   indicate the dominance coefficient (0-red, 0.1-blue).

621

622 Figure S2. Differences in mutational load between burn-ins. A.) Mutational load of P1 B.)
623   Mutational load of P2. Colors indicate the dominance coefficient (0-red, 0.1-blue).

624

625 Figure S3. Mutational load of P2 correlates with associative overdominance (AOD). Facets
626   indicate burn-in length, shape indicates rate of gene conversion (absent or present) and
627   color indicates the dominance coefficient (0-red, 0.1-blue). The data shown is for
628      $N=2,500$.

629

630 Figure S4. Dominance decreases invasion probability. Probability of invasion is shown for all
631   parameter sets. Facets indicate N (100 or 2,500), and gene conversion (GC; absent or
632   present). Colors indicate the dominance coefficient (0-red, 0.1-blue). Each point is a
633   different burn-in.

634

635 Figure S5. Changes in fitness within the first 1,000 generations after invasion. Shown are
636   normalized fitnesses for A.) the inversion homokaryotype, B.) the standard
637   homokaryotype, C.) the heterokaryotyope, and D.) the mean fitness of the entire
638   population. Normalized fitness for each generation is calculated as an average per burn-
639   in per parameter set. Error bars represent standard error between burn-ins. Facets
640   indicate gene conversion (GC; absent or present) and colors indicate the dominance
641   coefficient (0-red, 0.1-blue). $N=2,500$ and burn-in length = 500,000 for all graphs.

642

643 Figure S6. The evolution of associative overdominance (AOD) under different parameters.
644   Shown is the evolution of AOD over time for simulations where the polymorphism
645   lasted 200,000 generations A.) $N=100$, and B.) $N=2,500$. AOD for each generation was
646   calculated as an average per burn-in per parameter set. Error bars represent standard
647   error between burn-ins. Colors indicate the dominance coefficient (0-red, 0.1-blue) and
648   facets indicate the level of gene conversion (absent or present). Burn-in length=500,000
649   for all graphs. Note the change in y-axis scale.

650

651 Figure S7. Polymorphism of the inversion with fit homokaryotypes given invasion. Probability
652   that the supergene is polymorphic at the end of the simulation and both homokarotypes
653   are fit is shown for all parameter sets. acets indicate N (100 or 2,500), and gene
654   conversion (GC; absent or present). Colors indicate the dominance coefficient (h; 0-red,
655   0.1-blue). Each point represents a different burn-in.

656

657   Figure S8. A. Probability of a half lethal system given invasion. Probability that the supergene is
658   polymorphic at the end of the simulation and one homokarotypes is lethal is shown for
659   all parameter sets. B. Balanced lethal outcome given invasion. Probability that the
660   supergene has eveoved into a balanced lethal system after invasion. For both plots facets
661   indicate N (100 or 2,500), and gene conversion (GC; absent or present). Colors indicate
662   the dominance coefficient (h; 0-red, 0.1-blue). Each point represents a different burn-in.

663

664   Figure S9. Evolution of karyotype fitnesses in a half lethal system. Fitness of the population,
665   relative fitness of the heterokaryotype, relative fitness of the inversion homokaryotype,
666   and relative fitness of the standard homokaryotype are shown for cases where (A) The
667   inversion homokaryotype becomes lethal, (B) The standard homokarotype becomes
668   lethal. Time is always shown on a log scale, actual time points are: 10, 50, 100, 200, 500,
669   1000, 5000, 10000, 25000, 50000, 100000, 125000, 150000, and 200000 generations. For
670   all data shown N=2,500, gene conversion is absent and all mutations are fully recessive
671   (h=0).