Chromosomal rearrangements, specifically inversions, have been suggested to play key roles in adaptation and speciation practically since the beginning of genetics (Sturtevant 1917; reviewed by Jackson et al. 2016). Inversions were first identified by their effects on suppressing recombination (Rieseberg 2001; Faria and Navarro 2010), and in the past 30 years, their role as recombination modifiers became central to many speciation models (Trickett and Butlin 1994; Noor et al. 2001; Rieseberg 2001).

In heterozygotes for one or more inversions (i.e., heterokaryotypes), recombination can be severely reduced within the
inverted regions (Sturtevant and Beadle 1936; Crown et al. 2018). Consequently, genetic variation within inversions is expected to resist homogenization by gene flow between populations fixed for alternative arrangements relative to collinear regions of the genome (Rieseberg 2001). Chromosomal inversions may reduce gene flow sufficiently to facilitate progress toward completing speciation (Trickett and Butlin 1994; Navarro and Barton 2003; Kirkpatrick and Barton 2006; Feder et al. 2011; Guerrero et al. 2012; Wellenreuther and Bernatchez 2018).

However, even when recombination due to single crossing over is hindered in heterokaryotypes within regions spanned by inversions, gene flux, the movement of DNA sequences between alternative karyotypes (Navarro et al. 1997a), is not completely eliminated. Gene flux can occur via gene conversion and/or double crossovers (Navarro et al. 1997a,b; Stevison et al. 2011; Korunes and Noor 2016, 2019; Crown et al. 2018; Faria et al. 2019b; Fuller et al. 2019). Gene flux will tend to reduce genetic differentiation between chromosomes bearing different arrangements (Korunes and Noor 2016, 2019). Homogenization will be faster when the genetic content within alternative arrangements is neutral than when different populations are fixed for alternative arrangements and the inversion contains loci that are under divergent selection or exhibit some form of genetic incompatibility (Guerrero et al. 2012; Fuller et al. 2019). In general, a reduction of gene flux would result in a reduction of gene flow between populations in the segments spanned by inversions.

In other words, inversion polymorphisms are semipermeable barriers to gene flow. Thus, an important question becomes: Under what conditions do inversions play an effective role in adaptation, divergence, and speciation in the face of gene flow? Theoretical work about the evolutionary role of inversion polymorphisms has focused mainly on two-population models where either the alternative chromosomal arrangements themselves or their allelic content undergoes divergent selection. Kirkpatrick and Barton (2006) and Charlesworth and Barton (2018) analyzed the conditions favoring the spread of an initially rare inversion, assuming that the inversion polymorphism captures two or more adaptive loci undergoing divergent selection. A general finding of these studies is that, by suppressing recombination, an inversion protects locally favorable combinations of alleles from mixing with maladapted alleles introduced by migration (except when migration is too strong; see eq. A9 in Charlesworth and Barton 2018). In addition, Nei et al. (1967) have shown that, under a mutation-selection equilibrium model, an inversion can also invade without requiring divergent selection, as long as it captures a fitter-than-average haplotype.

Although these studies primarily tackled populations of infinite size, it is straightforward to deduce that, in populations of finite size diverging in the face of gene flow by the accumulation of new adaptive mutations, a spreading inversion that happens to capture two or more locally favorable alleles will be an effective mechanism protecting these alleles from stochastic loss (sensu Rafajlović et al. 2016, albeit in a model without inversions), thereby facilitating divergence. Furthermore, genetic patterns at neutral loci linked to an inversion polymorphism in pairs of divergent populations subject to migration have been assessed by Guerrero et al. (2012) under a coalescent framework. Their results suggest that inversion polymorphisms can store differentiation at linked neutral loci depending on the age of the inversion, on gene flux relative to the recombination rate in homokaryotypes, and on migration relative to selection.

Although a significant amount of work has been devoted to understanding the evolutionary role of inversion polymorphisms when divergent selection acts between populations, less attention has been paid to alternative models that do not involve geographically divergent selection per se and yet allow for the establishment of partial barriers to gene flow. Examples include models involving neutral and universally beneficial alleles, with or without incompatibilities (Noor et al. 2001; Navarro and Barton 2003), or modified versions of such models (cf. Feder and Nosil 2009). Such models are relevant when considering pairs of populations without any obvious ecological trade-offs. Empirical data supporting models with locally beneficial and neutral alleles (so-called conditional-neutrality models) can be found in Andersson et al. (2012) (see also references therein). Models involving genetic incompatibilities between alleles not subject to divergent selection per se have also been suggested in the literature, for instance in studies of Drosophila pseudoobscura and D. persimilis (Noor et al. 2001; Kulathinal et al. 2009). These two species exhibit higher divergence in genomic regions fixed for alternative arrangements and it is these regions that harbor loci contributing to the reproductive isolation (RI) between the two species (Noor et al. 2001; Kulathinal et al. 2009). These empirical findings may be explained (though not exclusively, see Fuller et al. 2018) by invoking genetic incompatibilities: although genetic incompatibilities established in each species could have been eliminated from collinear regions of the genome after the species’ secondary contact, incompatibilities might have been maintained within inversion polymorphisms due to suppressed recombination in heterokaryotypes (Noor et al. 2001; Ortiz-Barrientos et al. 2002; Navarro and Barton 2003). Thus, inversion polymorphisms could have played a crucial role in the persistence of these two species upon their secondary contact, allowing for additional reproductive barriers (i.e., reinforcement) to accumulate thereafter (Noor et al. 2001).

To understand the interplay between gene flux and the type of selection acting on variation within an inversion polymorphism, Feder and Nosil (2009) implemented simulations of secondary contact between two strongly differentiated populations with initially fixed alternative arrangements. After
testing five different models—some involving local adaptation, with and without trade-offs, and others involving genetic incompatibilities—the authors showed that, in the absence of divergent selection, there are conditions under which inversions may help retain species differences following secondary contact compared to collinear gene regions lacking rearrangements. However, the authors also showed that large, qualitative differences between inversions versus collinear regions will not persist for long time periods (tens of thousands of generations) even when the rate of gene flux between alternative arrangements is low (e.g., per-gamete, per-generation probability of $10^{-8}$), unless migration between populations is very weak compared to selection at loci causing RI. Given that estimates of gene conversion have been recently shown to be potentially as high as $10^{-5}$ to $2.5 \times 10^{-5}$ per base pair, per genome, per generation between rearrangements in Drosophila (Korunes and Noor 2019), this raises the question as to the efficacy of inversions in maintaining prolonged differentiation following secondary contact in cases without divergent selection.

Feder and Nosil (2009) focused on inversions containing only two or four loci and on deterministic scenarios (with populations of infinite size). Here, we present a series of simulations that extend the framework of Feder and Nosil (2009) for the cases of locally adaptive alleles and genetic incompatibilities (Models 2 and 4 in their study). Our goal is to test how the effects of more than two loci and of finite population sizes may influence the erosion of differentiation within an inversion polymorphism after secondary contact, paying particular attention to the recent empirical results implying higher rates of gene flux between chromosomal rearrangements than previously assumed (Korunes and Noor 2019).

We show that the effect of gene flux between alternative chromosomal rearrangements can be retarded by the finite size of populations (with up to 100,000 individuals in some cases) coming into secondary contact. This is due to a longer waiting time for actual gene flux events to be realized, and also, to some extent, due to the increased probability of the loss of favored allele combinations by random genetic drift. In particular, we find that under these conditions, inversions can retain longer-lived differentiation even with relatively high levels of gene flux ($2 \times 10^{-4}$ per gamete, per generation) when many loci of small fitness effects reside within the inversion polymorphism. Importantly, such inversion polymorphisms can protect population differentiation for tens of thousands of generations (up to 100,000, or longer in some cases) after secondary contact. This time span may provide a window of opportunity for additional differences to accumulate (further local adaptation, more incompatibilities, reinforcement, etc.), facilitating further progress toward speciation. How often these conditions are met for hybridizing populations in nature remains to be determined. Nevertheless, our results do offer a region of parameter space where inversions not containing loci causing fitness trade-offs (divergent selection) in alternate habitats may play an enhanced role in facilitating speciation over collinear regions following secondary contact.

**Model and Methods**

To assess the role of multiple loci and finite population sizes in maintaining population differentiation after secondary contact, we used individual-based computer simulations of two diploid populations with finite, constant population size (denoted by $N$ below) and discrete, nonoverlapping generations. At the start of each simulation, the two populations (referred to as Population 1 and Population 2) were fixed for different chromosomal arrangements. For convenience, we refer to the arrangement in Population 1 as standard and to the other one as inverted. We modeled 2L biallelic loci within the region polymorphic for the inversion (see below) and assumed that at the start of each simulation the two populations were fixed for alternative alleles at each locus. At generation 0, both populations consisted of adult virgin individuals coming into secondary contact. Thereafter, adult virgin individuals underwent migration, followed by soft selection, recombination, and mating (always locally in each population). We neglected mutations throughout. Migration from one population to the other occurred with a per-generation, per-individual probability of $m$, independently of the individuals’ genotypes. After migration, we applied soft fecundity selection within each population, so that the number of gametes that an adult individual contributed to the pool of offspring was binomially distributed with the number of trials equal to $2N$, and the probability equal to the fitness of the individual in the population where it ended up after migration, relative to the total fitness of all adult individuals in this population. This means that the effective migration rate, expressed in terms of the proportion of offspring produced by migrants, depends on other model specifics, and it is a dynamic property of the model.

In homokaryotypes (either for the standard or for the inverted chromosomal arrangement), consecutive loci in the simulated genomic region underwent recombination with a per-gamete, per-generation probability of $r_{2L}$ (gene conversion events were not modeled to occur in homokaryotypes). Thus, for a given gamete stemming from a homokaryotype in a given generation, the probability that there were $k = 0, 1, \ldots, 2L-1$ crossover points within the region was equal to $\binom{2L-1}{k} r_{2L}^k (1 - r_{2L})^{2L-1-k}$. We modeled the recombination rate $r_{2L}$ in two different ways, which we explain below.

First, we focused on inversions of the same size in terms of their total map distance. Throughout, we refer to this version of the model as the conserved-size model. Here, we chose the
recombination rate \( r_{2L} \) in homokaryotypes in such a way that it was smaller when the number of fitness-related loci (i.e., \( 2L \)) was larger, and that the total recombination distance (denoted by \( r \) below) spanned by the region was independent of the number of loci it contained. In particular, and in line with Feder and Nosil (2009), when \( 2L = 2 \), we set \( r_{2} = r = 0.1 \), whereas for \( 2L > 2 \), we used \( r_{2L} = 1 - (1 - r)^{1/(2L-1)} \). In addition, for heterokaryotypes we assumed that gene flux between the alternative arrangements occurred with a per-gamete, per-generation probability \( r_{\text{inv}} \), which was independent of the number of loci \( 2L \). To understand better the relationship between the parameters \( r \) and \( r_{\text{inv}} \), we note that, in the absence of mutations (as we assumed here), \( 1 - r \) is the per-generation, per-gamete probability that a gamete stemming from a homokaryotype (or from any individual in the collinear model) will be the exact copy of the region on one or the other homologous chromosome of the parental individual. Similarly, \( 1 - r_{\text{inv}} \) is the per-generation, per-gamete probability that a gamete stemming from a heterokaryotype will be the exact copy of the region on one or the other homologous chromosomal arrangement. Note that for the latter, the gamete inherits not only the alleles at a given region, but also their arrangement (either standard or inverted).

Gene flux in heterokaryotypes was implemented essentially as in Feder and Nosil (2009), except that we assumed that gene flux occurred 50% of the time by gene conversion and 50% by double crossover, whereas the corresponding ratio in Feder and Nosil (2009) was 30:70. We chose this ratio for two reasons. First, the relative proportion of the two processes remains largely unknown to date and it is likely to differ between and within species, as well as between different inversions along the genome (Korun and Noor 2019). Second, because in our simulations the ratio between the number of exchanged alleles due to double crossover and gene conversion varies depending on how many loci are exchanged in each double crossover (see below), we believe that our assumption that double crossover and gene conversion occur with the same probability avoids any bias toward one or the other process, while simplifying the model at the same time.

In our model, a double crossover in heterokaryotypes was assumed to occur within a region containing any number between \( 1 \) and \( 2L \) of consecutive loci, each combination of consecutive loci being equally likely. Note that including a double crossover with \( 2L \) consecutive loci is a legitimate choice when considering heterokaryotypes, because upon such an event, the arrangement of alleles in a given gamete will be opposite to their arrangement in the parental individual. Thus, there were \( 2L - k + 1 \) possibilities for double crossovers accounting for \( k = 1, 2, \ldots, 2L \) consecutive loci, and the total number of different possibilities was equal to \( L(2L + 1) \). This means that the average size of double crossovers (where size stands for the number of loci involved in a double crossover) was \( 2(L + 1)/3 \). In the simulations, we made a list of all possibilities for double crossover events (each possibility was determined by the corresponding start and end locus involved), and when a double crossover occurred, we chose both its size and position by sampling uniformly at random one out of all possibilities from the list. Gene conversion events were modeled so that an allele at a given locus at a given chromosome was altered to the allele at the same locus residing at the homologous chromosome. If the two alleles were identical prior to gene conversion, they remained identical also afterward. Gene conversion events occurred at a single locus, each locus being equally likely. Thus, the per-generation, per-gamete average number of converted loci in our model was \( r_{\text{inv}}/2 \) (for any number of loci in the region), whereas the per-generation, per-gamete average number of loci involved in double crossovers was \( r_{\text{inv}}(L + 1)/3 \).

One consequence of our implementation of gene flux (specifically the way we modeled double crossovers) was that the proportion of times a locus was involved in a gene flux event had a maximum for loci in the middle of the inverted region, and it decreased toward the region breakpoints (Fig. S1), in line with empirical observations (Navarro et al. 1997a; Fuller et al. 2019).

In a second version of the model, we assumed that the size of the inversion increased with the number of fitness-related loci within it. We refer to this version of the model as the increasing-size model. Here, we set the recombination rate \( r_{2L} \) between each pair of consecutive loci in homokaryotypes to be independent of the number of fitness-related loci within the inversion, that is, \( r_{2L} = r = 0.1 \). Thus, the region harboring these loci can be an entire chromosome, or a significant proportion of a large chromosome. Specifically, for \( L \geq 3 \), there will be pairs of distant loci that recombine freely, despite being on the same chromosome (e.g., the first and the last locus in the model with eight loci). More generally, the number of loci and \( r_{2L} \) determined the average per-generation, per-gamete number of crossover points in the region, and this was approximately equal to 1.9 for the largest number of loci we used (i.e., 20; see the formula above for the probability of \( k \) crossover points within the region). In this version of the model, the total rate of gene flux in heterokaryotypes (denoted by \( r_{\text{inv},2L} \) below) was assumed to depend linearly on the number of fitness-related loci, that is, \( r_{\text{inv},2L} = r_{\text{inv}} \cdot L \), where \( r_{\text{inv}} \) had the same value as in the conserved-size model explained above. This is the simplest scaling assuring that the per-generation, per-gamete, per-locus probability of gene conversion (i.e., \( r_{\text{inv}} \)) was independent of the number of loci in the region. We chose to use gene conversion rate as a base against which to scale gene flux in this version of the model because, unlike double crossovers, any gene conversion event was assumed to account for a single locus. Note that this means that when the inversion contained exactly two fitness-related loci (i.e., \( 2L = 2 \)), the conserved-size model and the increasing-size model were
equivalent, whereas this was not true for \(2L > 2\). This setting allowed us to assess the impact of increasing the number of fitness-related loci within the inversion beyond two while either keeping the total map distance of the inversion constant or increasing it (roughly linearly) with the number of fitness-related loci within the inversion.

We further assumed that mating was random within each local population, that is, the pool of \(2N\) gametes obtained after selection and recombination was randomly divided into \(N\) pairs, thus producing \(N\) offspring in each population (this corresponds to sampling without replacement). After reproduction, all adult individuals were replaced by their offspring, which were then treated as the next generation of adults.

As mentioned above, we studied two out of the five models analyzed by Feder and Nosil (2009), specifically their Model 2 that involves alleles that are locally favored in one habitat and neutral in the other, and Model 4 that involves universally beneficial alleles with negative fitness epistasis (i.e., incompatibilities). In both models and in line with Feder and Nosil (2009), we considered two types of loci, denoted here by \(A_i\) and \(B_i\) for \(i = 1, 2, \ldots, L\) (the difference between the two types of loci is explained further below). Initially, all loci were biallelic: the two alleles at locus \(A_i\) (\(B_i\)) are denoted by \(A_i\) and \(a_i\) (i.e., \(B_i\) and \(b_i\) for locus \(B_i\)). Furthermore, the alleles denoted by uppercase letters at each locus were initially fixed in Population 1, whereas the alternative alleles (denoted by lowercase letters) were initially fixed in Population 2.

Selection was implemented in the two models as follows. In Model 2, the alleles denoted by uppercase letters at loci \(A_i\), \(i = 1, 2, \ldots, L\) were advantageous over the alternative alleles at these loci in the first population, whereas both allelic types at these loci were selectively neutral in the second population. The opposite was true for loci \(B_i\), \(i = 1, 2, \ldots, L\): although at these loci both allelic types were selectively neutral in the first population, the alleles denoted by lowercase letters were advantageous over the alternative alleles in the second population. Throughout, we use the term “fitness-related loci” instead of “loci under selection,” to account for the fact that, in this model, alleles at a given locus were under selection only in one population, but neutral in the other. We considered two different models with respect to how loci \(A_i\) and \(B_i\) were ordered in the genomic region simulated. First, we considered the case where loci of the type \(A_i\) were next to each other on the first half of the region (taking places 1, 2, \ldots, \(L\)) and loci of the type \(B_i\) were on the second half of the region (thus, taking places \(L + 1, L + 2, \ldots, 2L\)). We refer to this version of the model throughout as the “half-half array.”

Second, we considered the case where loci of the two types were arranged consecutively (i.e., interdigitated), such that the order of loci was \(A_1, B_1, A_2, B_2, \ldots, A_L, B_L\). We refer to this version of the model as the “consecutive array.” These two versions of the model (relative to how the two types of loci are arranged in the inversion) present two extreme cases chosen here for convenience. Of course, real systems will present a continuum of possibilities between these two extremes.

We denoted selection coefficients per locus by \(s_{2L}\). For individuals that were homozygotes for the advantageous allele, heterozygotes, or homozygotes for the disadvantageous allele (relative to the alternative allele), a fitness-related locus (see above) made a fitness contribution equal to \(1 + s_{2L}\), \(1 + s_{2L}/2\), and 1, respectively, with fitness being multiplicative across loci. Note that we conservatively scaled selection to avoid modeling the trivial case of extremely strong selection. To do so, we chose the selection coefficient per locus to depend on the number of loci to achieve the same maximal fitness when varying the number of loci within the inversion polymorphism. Denoting the maximal fitness by \(1 + s\), and recalling that only \(L\) loci were under selection in each population (whereas \(L\) loci were neutral), it follows that \(s_{2L} = (1 + s)^L - 1\), with \(s_2 = s\), as expected.

In Model 4 with universally beneficial alleles and negative epistasis, alleles \(A_i\) at loci \(A_i\), were favored in both populations, whereas at loci \(B_i\), the universally beneficial alleles were those denoted by lowercase letters (i.e., \(b_i\)). As in the previous model, loci contributed to fitness in a multiplicative manner with the selection coefficient per locus \((s_{2L})\) depending on the number of loci \(2L\) within the inversion polymorphism. Genetic incompatibilities were modeled as occurring between universally favored alleles \(A_i\) and \(b_i\) at pairs of loci \(A_i\) and \(B_i\) (note the same index \(i\)). We accounted for incompatibilities slightly differently from Feder and Nosil (2009). Namely, for each individual we counted the number of incompatibilities at each pair of loci \(A_i\) and \(B_i\) as follows: (1) If an individual is a homozygote for allele \(A_i\) and a heterozygote at locus \(B_i\), we accounted for two incompatibilities at this pair of loci; (2) for a homozygote for allele \(A_i\), and a homozygote for allele \(b_i\), we accounted for four incompatibilities for this pair of loci; (3) for an individual that is a heterozygote at both loci \(A_i\) and \(B_i\), we accounted one incompatibility; and (4) for an individual that is a heterozygote at locus \(A_i\), but a homozygote for allele \(b_i\) at locus \(B_i\), we accounted for two incompatibilities at this pair of loci. All other allele combinations at these two pairs of loci gave zero incompatibilities. For each individual, we thus computed the number of incompatibilities at each pair of loci \(A_i\) and \(B_i\), \((i = 1, 2, \ldots, L)\), and summed them up to obtain the total number of incompatibilities (denoted by \(n_{inc}\)) that the individual carried. To each individual that carried at least one incompatibility (i.e., \(n_{inc} \geq 1\)), we assigned a fitness of \((1 - e^{-P_{2L}})^{n_{inc}}\), with \(e^{-P_{2L}}\) denoting the strength of the negative epistasis per incompatibility. Note that, because we assumed that incompatibilities may arise between pairs of loci, the maximum number of incompatibilities in the model with \(2L\) loci is equal to \(4L\), and this is obtained when an individual is a homozygote for alleles
Table 1. Parameters, their symbols, explanations, and values used in computer simulations. We also note that we performed simulations with \( N = 100, 200, 300, 500, 700, 900, \) but only for a limited set of parameters for Model 4.

| Symbol | Explanation | Values |
|--------|-------------|--------|
| \( N \) | Number of diploid individuals in each population | 1000, 5000, 10000, 20000, 100000 |
| \( m \) | Per-individual, per-generation migration probability (migration rate) | 0.01, 0.1 |
| \( r \) | Per-gamete, per-generation recombination probability between loci in the two locus models; with more loci (2\( L \)), the per-gamete, per-generation recombination probability between a pair of consecutive loci is set to \( r_{2L} = 1 - (1 - r)^{1/(2L - 1)} \) (in the conserved-size version of the models), or to \( r \) (in the increasing-size version of the models). | 0.1 |
| \( r_{\text{inv}} \) | Per-gamete, per-generation recombination probability (gene flux) between the alternative chromosomal arrangements in the conserved-size version of the models; in the increasing-size version of the models, the total rate of gene flux is set to \( r_{\text{inv}} \) \( L \). | 0, 2 \( \times 10^{-4} \) |
| \( s \) | Total selection strength | 0.1 |
| \( 2L \) | Number of loci within an inversion polymorphism | 2, 4, 6, 8, 10, 20 |
| \( ep \) | Strength of the negative epistasis | 0.1, 0.5, 0.95 |

\( A_i \) and for alleles \( b_i \) at all loci \( A_i \) and \( B_i \). To assure meaningful comparison between the models with different number of fitness-related loci within the inversion, we set \( ep_{2L} = 1 - (1 - ep)^{1/L} \), so that when \( 2L = 2 \), we have \( ep_2 = ep \). Note that our implementation of incompatibilities differs from that in Feder and Nosil (2009), where it was assumed that an individual carrying any combination of incompatible alleles had a fitness equal to \( 1 - ep \) (independently of how many incompatibilities it had and of their linkage phase on chromosomes, as long as there was at least one). Our model is similar to that proposed by Turelli and Orr (2000), except that here we assumed multiplicative fitness and we did not specify a “breakdown score of incompatibilities” beyond which the fitness of an individual would be 0. An alternative way to pose the model would be to account also for the multiplicative contributions of fitness effects at pairs of loci where incompatibilities could arise but have not done so (similarly to Blanckaert and Bank 2018). Such extensions could be dealt with in future work.

Similar to the model with locally favored and neutral alleles, we also analyzed two different orderings of the loci \( A_i \) and \( B_i \), that is, we considered the half-half array ordering, and the consecutive array ordering (see above).

The parameter values we tested corresponded largely to those tested by Feder and Nosil (2009) but here we focused on a much larger rate of gene flux (by four orders of magnitude) than that used by them, and even larger in the increasing-size version of the models with more than two loci (see above). In addition, and unlike Feder and Nosil (2009), here we examined the effects of finite population size using populations of size \( N = 1000 \) (but, for some parameter values, we also performed simulations with \( N = 5000, N = 10, 000, N = 20, 000, \) and \( N = 100, 000 \)). Finally, we assessed the effect of increasing the number of loci within the inversion (\( 2L = 2, 4, 6, 8, 10, \) or 20), while keeping the total selection strength constant (details explained above). For the full list of parameter values we used, see Table 1, as well as Tables S1 and S2.

In addition, for each model and each parameter set, we ran comparative simulations without any inversion polymorphism (i.e., considering only collinear regions). In these simulations, we set the recombination rate between pairs of loci to be equal to the corresponding recombination rate in homokaryotypes in the model with inversion polymorphism. In comparison, Feder and Nosil (2009) assumed a recombination rate of 0.5 in their simulations of collinear regions.

In each simulation, we tracked the evolution of the allelic content of the inversion polymorphism for 100,000 generations after the populations come into secondary contact. The number of independent realizations we performed per parameter set was 200 (for \( N = 1000 \) and \( N = 5000 \)), 40 (for \( N = 10,000 \) and \( N = 20,000 \)), or 20 (for \( N = 100,000 \)). During each simulation, we recorded (in steps of \( \Delta t = 50 \) generations) the allelic frequencies at each locus within the inversion polymorphism, the frequencies of alternative chromosomal arrangements in each population, as well as \( F_{ST} \) and \( D_{ST} \) at each locus. To facilitate comparisons between the simulation results corresponding to different parameter sets, we calculated the average time of sustained differentiation weighted by the differentiation (\( T_w \); hereafter referred to as the average weighted time of differentiation):

\[
T_w = \frac{1}{2L} \left( \sum_i \left( \sum_k \Delta t \Delta p_k (A_i) + \sum_k \Delta t \Delta p_k (B_i) \right) \right). 
\]
Here, the factor $1/(2L)$ serves to average over all fitness-related loci, and index $k$ accounts for all sampling time points when the recorded allele frequency difference $\Delta p_i(A_i)$ (or $\Delta p_i(B_i)$) at a given locus $A_i$ (or $B_i$) was nonzero (here, $\Delta p_i(A_i)$ represents the frequency difference of allele $A_i$ between Population 1 and Population 2; and similarly for $\Delta p_i(B_i)$). The last such sampling point for locus $A_i$ ($B_i$) is denoted by $\tau_{A_i}$ ($\tau_{B_i}$). Note that in the absence of mutations, which we assumed, once the allele frequency difference between the two populations becomes zero, it stays zero infinitely. However, we did not observe fixation in many of the cases we simulated because we ran simulations only up to a maximum of 100,000 generations after secondary contact. Therefore, the upper bound for $T_w$ in our simulations was 100,000 generations, even if the actual average weighted time of differentiation would, in fact, be longer. Finally, $\Delta \tau$ in equation (1) stands for the time span between consecutive sampling points in our simulations ($\Delta \tau = 50$). Essentially, the term in the bracket in equation (1) integrates the allele frequency difference between the two populations over time and over all loci within the region (but in a discretized form).

We note that in the model without gene flux, due to our scaling of the strength of selection and of negative epistasis, the number of loci within the inversion does not play a role in the temporal dynamics of between-population differentiation. Consequently, all results for the cases without gene flux were obtained by averaging over the simulation results corresponding to different numbers of loci (except for $D_{xy}$ and $F_{ST}$, where we show the corresponding statistics for each locus obtained in a single randomly chosen stochastic realization of the model).

## Results

In Model 2 involving neutral and locally beneficial alleles, our simulations showed that when the fitness-related loci were captured by an inversion polymorphism (either with or without gene flux), the persistence time of differentiation weighted by the differentiation after secondary contact ($T_w$; see Model and Methods) was longer than in the absence of inversion polymorphism: the difference between the two ranged from thousands to tens of thousands of generations in most cases (Fig. 1A, and see Figs. S2–S12; but note that the actual persistence time of differentiation after secondary contact was larger than $T_w$, due to the weighting we made when defining $T_w$). This was true both when gene flux between the alternative arrangements was absent, as well as when it was relatively high (in particular, four orders of magnitude higher than that assumed in Feder and Nosil 2009). Notably, when gene flux was present, the weighted persistence time of differentiation increased rapidly with increasing the number of fitness-related loci within the inversion. This was not the case in the model without inversions (although in this case a slight increase of $T_w$ with increasing the number of loci occurred in the consecutive-array, conserved-size variation of the model; Figs. 1C and 1D). Our results, thus, showed that the advantage of inversions in maintaining population differentiation after secondary contact increased with increasing the number of fitness-related loci within the inversion, with $T_w$ sometimes (e.g., Figs. 1B, S7, S16F, S24F, S28B, and S29F) reaching a plateau after six, or more loci were involved. This was true for all parameter values and variations of the model we considered, but the advantage was typically stronger for lower than for higher migration rates and in the conserved-size than in the increasing-size variation of the model (compare Figs. 1 and S7). Our simulations showed that there were also some differences between the model variations with respect to the ordering (half-half array vs. consecutive array) of the two types of loci ($A_i$ that carried locally beneficial alleles in Population 1, and $B_i$ that carried locally beneficial alleles in Population 2), especially in the conserved-size model. For example, in the case with 20 loci, differentiation was maintained during longer time periods following secondary contact in the consecutive-array than in the half-half array version of the model with inversions and gene flux (approximately by a factor of 3; compare upper and lower panels in Fig. 1). However, regardless of the ordering of loci, the model results confirmed the advantage of inversions (with gene flux) over collinear regions in maintaining population differentiation.

Stronger and more persistent population differentiation after secondary contact in the presence of inversion was also observed when comparing $F_{ST}$ and $D_{xy}$ patterns arising in individual realizations of the models with and without inversions (Figs. S3–S6 and S9–S12). However, we found that $F_{ST}$ ($D_{xy}$) values were relatively low when the migration rate was high (i.e., of the order of the total selection strength) even in the presence of inversion polymorphism (panels D–F in Figs. S3–S6 and S9–S12). Note that stochastic fluctuations between individual realizations were high (not shown).

In contrast to the model involving neutral and locally beneficial alleles, we found that differences in simulations involving genetic incompatibilities with and without inversions depended more strongly on the parameter values. When migration was weak in comparison to selection, and negative epistasis was weak, inversions maintained population differentiation after secondary contact for longer than collinear regions, despite gene flux, and this difference was greater when the number of loci was larger (Figs. 2A, S16A, S20A, and S24A). In the conserved-size version of the model, this effect was much stronger in the half-half array than in the consecutive-array case (compare Fig. 2A with Fig. S16A). In contrast, in the increasing-size version of the model, this effect was similar for the half-half array and the consecutive array scenarios (compare Fig. S20A with Fig. S24A).
Figure 1. Simulation results for the conserved-size model involving locally favored and neutral alleles (Model 2). The figure shows the weighted time of differentiation ($T_w$) averaged over 200 independent realizations of the model, and over all loci within the region, as a function of the number of loci. Note the logarithmic scale on the vertical axis on this and all subsequent figures. Gene flux in heterokaryotypes for the models with inversions is $r_{Inv} = 2 \cdot 10^{-4}$ (red) or $r_{Inv} = 0$ (black horizontal lines). Results for the model without inversions are shown in blue. The vertical lines around the symbols and the gray regions (for $r_{Inv} = 0$) depict the range between the minimum and maximum values of $T_w$ obtained in individual simulations. The panels differ by the migration rate ($m$) and by the ordering of the loci $A_i$ and $B_i$, as indicated in the figure. Remaining parameters: selection strength $s = 0.1$, number of individuals in each population $N = 1000$, and recombination rate in homokaryotypes $r = 0.1$. This figure shows that, despite gene flux, inversions maintain population differentiation after secondary contact for notably longer time periods than collinear regions, and this effect becomes stronger as the number of fitness-related loci increases.

Note, however, that in this case in the model with the inversion, differentiation was maintained throughout the whole time span simulated for all numbers of loci we used. This precluded us from assessing precisely how the weighted time of differentiation depended on the number of loci in the model with inversions but we expect it would increase with increasing the number of loci similar to our other simulations. Notably, and accounting for this limitation of our results, we found that the difference in persistence time of population differentiation in the presence versus in the absence of inversion polymorphism was higher in the increasing-size than in the conserved-size version of the model. This was because in the increasing-size model, the total recombination rate along the collinear region (model without any inversion polymorphism) increased with increasing numbers of loci within the region, and this significantly reduced the persistence time of population differentiation after secondary contact in the absence of inversions (down to 100 generations on average; Figs. S20A and S24A; blue circles). Note that in the increasing-size model, the total rate of gene flux also increased (linearly) with the number of loci. However, in this case, gene flux occurred only in heterokaryotypes and the frequency of heterokaryotypes after secondary contact was typically low for the parameters considered (Figs. S13A, S17A, S21A, and S25A). Thus, for populations of modest to small sizes, this meant that the effective gene flux rate was still relatively low despite being higher in the conserved-size model.

For weak migration and intermediate negative epistasis, we found that strong population differentiation was maintained long after secondary contact (at least for 100,000 generations) independently of the number of loci in the model with inversion polymorphism and gene flux (red circles in Figs. 2B, S16B, S20B, and S24D). The same was true without inversion...
polymorphism in the case of the conserved-size version of the model (blue circles in Figs. 2B and S16B). However, in the increasing-size version of the model without inversion polymorphism, population differentiation was lost more quickly when more fitness-related loci were involved (blue circles in Figs. S20B and S24B). As a result, the weighted time of differentiation in this case was at least 500 times larger when loci were in the inversion than in the collinear region (compare red and blue circles in Figs. S20B and S24B; note that we say at least, because we ran simulations only up to 100,000 generations, and, for the parameters considered here, population differentiation was not lost during this time span in the model involving inversions with or without gene flux).

Conversely, for weak migration and very strong epistasis, population differentiation was maintained for at least 100,000 generations both in the model with and without inversions (Figs. 2C, S16C, S20C, and S24C).

For strong migration and weak negative epistasis, population differentiation was lost quickly after secondary contact both with and without inversions (Figs. 2D, S16D, S20D, and S24D), whereas for intermediate strength of negative epistasis, we found slightly longer persistence times of population differentiation in the case with inversions (with or without gene flux) than in the case without inversions, but this effect was mild (Figs. 2E, S16E, S20E, and S24E). Because in these cases differentiation was lost quickly after secondary contact, even when gene flux was fully suppressed, it is likely that loss of the haplotype that was initially
fixed in one or the other population occurred largely by chance due to random genetic drift.

Finally, when both migration and negative epistasis were strong, the persistence time of differentiation after secondary contact was, in most cases, by several orders of magnitude longer in the case with inversions (despite gene flux) than without inversions (Figs. 2F, S16F, S20F, and S24F). This effect was stronger when the number of fitness-related loci was larger, except in the consecutive-array version of the model: in this case, in the conserved-size model without inversions, the persistence time of differentiation increased with increasing the number of loci (blue circles in Fig. S16F), reaching the maximum persistence time (within the time span we simulated) for 10 loci. In the consecutive-array, increasing-size version of the model without inversions, the persistence time of differentiation also increased with increasing numbers of loci, but this increase was slower than in the conserved-size version of the model. For 20 loci, the persistence time of differentiation was shorter by at least two orders of magnitude in the case without inversions than in the case with inversions and gene flux (Fig. S24F). Note that the observed increase of persistence time of differentiation with increasing numbers of loci in the model without inversions in the case of high migration was opposite to what happened in the case of low migration (compare, e.g., blue circles in Fig. S20A with blue circles in Fig. S20F). This was because in the case of high migration, the timescale of recombination between consecutive loci was either less than the migration timescale (in the conserved-size model), or the two timescales were similar (in the increasing-size model); and with increasing numbers of loci, the number of recombination events needed to purge incompatibilities increased. As a consequence, the persistence time of differentiation after secondary contact in the model without inversions increased with increasing numbers of loci in the high-migration case, whereas the opposite was true in the weak-migration case of the increasing-size model (where the timescale of recombination between consecutive loci was less than the migration timescale).

We also note that, in the model with inversions and gene flux, the persistence time of differentiation was, in many cases, much longer in the consecutive-array than in the half-half array model (compare, e.g., red circles corresponding to four loci in Fig. 2F and in Fig. S16F, as well as Fig. S20F and in Fig. S24F). In the remaining cases, differentiation was maintained throughout the time span simulated in both versions of the model, and longer simulations would be needed to confirm this effect.

The patterns described for the model involving universally beneficial alleles with genetic incompatibilities were also reflected in $F_{ST}$ (and $D_{xy}$) patterns from individual realizations of the model (Figs. S14, S15, S18, S19, S22, S23, S26, and S27). As in the model with locally beneficial and neutral alleles, stochastic fluctuations between individual realizations were high (not shown).

The findings outlined above were retained when considering populations of larger size ($N = 5000$; Figs. S28 and S29), but the advantage of an inversion polymorphism in maintaining population differentiation after secondary contact was slightly less than when $N = 1000$ (compare Figs. 1A and 1B with Figs. S28A and S28B, and Fig. 2 with Fig. S29).

To further assess the effect of population size on the advantage of inversions over collinear regions in maintaining population differentiation after secondary contact, we performed simulations with population sizes of up to 100,000 individuals for a subset of parameter values, focusing on cases with 20 loci (Figs. 3 and 4, and see also Figs. S30–S33). We found that the advantage of inversions over collinear regions was either fully retained when we increased the population size (Figs. S31, 4A, S33A, S33C, and S33D; but note that in these cases, differentiation was maintained during the time span simulated in the model with inversions for all values of the local population size, and this precluded us to assess the actual effect of the population size), or was less but still noticeable (Figs. 3 and 4B, see also Figs. S30 and S32). Interestingly, by increasing the population size beyond the critical population size $N_c = 10,000$ in cases shown in Figures 3A, 3B, and 3D, or beyond $N_c = 20000$ in the case shown in Figure 3C (but note that, in this case, $T_w$ for $N = 20,000$ is still slightly larger than that for $N = 100,000$; see also Fig. S30), the average weighted time of differentiation in the model with the inversion and gene flux ceased to depend on the population size, suggesting that a deterministic limit was attained. In the case shown in Figure 4B (see also Fig. S32), $T_w$ dropped sharply between the two largest population sizes we used (20,000 and 100,000), suggesting that $N_c > 20,000$ in this case. Finally, in the consecutive-array, conserved-size version of the model involving universally beneficial alleles with genetic incompatibilities, population differentiation was strong and maintained throughout the entire simulated time period after secondary contact (100,000 generations) either with or without inversions (Figs. 4C and 4D).

**Discussion**

Earlier studies suggested that chromosomal inversions are semipermeable barriers to gene flow, especially due to genetic exchange between the alternative arrangements by gene flux (Guererro et al. 2012; Koruntes and Noor 2016, 2019). Theoretical work by Feder and Nosil (2009) suggested that in the absence of strong local adaptation, population differentiation may tend to be eroded by gene flux and that this can occur quickly after secondary contact even with low gene flux, unless strong selection and weak migration are at work. These results were difficult to reconcile.
Figure 3. Simulation results for the conserved-size model involving locally favored and neutral alleles arranged at 20 loci (Model 2). The figure shows the weighted time of differentiation ($T_w$) averaged over independent realizations of the model, and over all loci within the region, as a function of the population size. Note the logarithmic scale on the vertical and horizontal axis. Gene flux in heterokaryotypes for the models with inversions is $r_{inv} = 2 \times 10^{-4}$ (red). Results for the model without inversions are shown in blue. The vertical lines around the symbols depict the range between the minimum and maximum values of $T_w$ obtained in individual simulations. The panels differ by the migration rate ($m$) and by the ordering of the loci $A_i$ and $B_i$, as indicated in the figure. Remaining parameters: selection strength $s = 0.1$, recombination rate in homokaryotypes $r = 0.1$, and number of independent realizations of the model is 200 (for $N = 1000$ and $N = 5000$), 40 (for $N = 10,000$ and $N = 20,000$), or 20 (for $N = 100,000$). This figure shows that the advantage of inversions (with gene flux) over collinear regions in maintaining population differentiation after secondary contact decreases with increasing the population size $N$. However, even for population sizes of $N = 100,000$, inversions (despite gene flux) maintain population differentiation after secondary contact for notably longer time periods than collinear regions. For depiction of the results on a linear scale, see Figure S30.

with the repeated empirical observation of stronger population differentiation within regions polymorphic for inversions across different organisms (e.g., Kulathinal et al. 2009; Jones et al. 2012; Korunes and Noor 2019, and references therein). However, Feder and Nosil (2009) based their results on models involving infinitely large populations and focused mainly on situations in which just a few loci reside within the inversion.

Here, we extended the analysis of Feder and Nosil (2009) for two models: one involving locally favored and neutral alleles (Model 2 in that study, also known as the conditional-neutrality model), and the other involving universally beneficial alleles with negative epistasis (Model 4 in that study). We found that, in situations with finite population sizes and/or many (as opposed to few) loci, inversion polymorphism can, relative to collinear regions, delay the erosion of genetic differentiation between populations for tens of thousands of generations after secondary contact. Our results suggest that the relative roles of finite population size and numbers of loci within the inversion in prolonging the time of differentiation after secondary contact depend on model specifics (e.g., Model 2 compared to Model 4) and on model parameters. Our findings differ from Feder and Nosil (2009) for three reasons. First, finite population sizes introduce a waiting time for gene flux events to occur. This waiting time depends on the population size and the migration-selection quasi-equilibrium that is reached in each case but it is, in general, longer in smaller than in larger populations. Second, even when gene flux does occur, any genotype it gives rise to must escape loss by drift. Thus, given that the timescale of stochastic loss decreases with decreasing effective population size, it follows that favored genotypes are more rapidly lost by genetic drift in smaller than in larger populations.
Figure 4. Same as in Figure 3 but for the conserved-size version of the model involving universally beneficial alleles with genetic incompatibilities (Model 4). Note that blue and red circles overlap in panels C and D. This figure shows that the advantage of inversions (with gene flux) over collinear regions in maintaining population differentiation after secondary contact is either roughly independent of the population size, $N$ (panel A; but note that differentiation is maintained throughout the whole time span simulated in the model with inversions for all values of $N$), or it decreases with increasing $N$, but it is notable even for population sizes of $N = 100,000$ (panel B), or that differentiation is maintained throughout the whole time span simulated both in the model with and without inversions (panels C and D). For depiction of the results for panel 4B on a linear scale, see Figure S32.

populations, thereby potentially delaying the elimination of population divergence. However, we note that this effect may be counteracted to some degree by a shorter fixation time of a genotype in smaller than in larger populations. Third, distributing selection across many loci in inversions, as opposed to being concentrated on a couple of genes, increases genetic interference, requiring a larger number of favorable gene flux events toward forming favorable genotypes, thereby decreasing the effectiveness of selection (on a haplotype level). This results in populations residing in a quasi-steady migration-selection equilibrium for a long period of time after secondary contact even for gene flux values as high as $2 \times 10^{-4}$ per gamete, per generation.

We also note that genetic drift can cause stochastic loss of differentiation without requiring gene flux or recombination, by driving to fixation in both populations the haplotype that was initially fixed in either local population. Unlike the effect of drift explained above, this effect would cause differentiation to be lost more quickly in smaller than in larger populations, the opposite of what we found in models with inversions for the parameters we used to assess the effect of the population size. However, this effect of genetic drift likely was a factor in the cases we analyzed with $N = 1000$ for Model 4 with strong migration and weak or intermediate negative epistasis. In these two cases, differentiation was lost quickly after secondary contact in the model with the inversion, even in the absence of gene flux. For the remaining cases, however, the effect of genetic drift in causing the loss of inversion polymorphism in smaller populations (down to $N = 1000$) appeared negligible. To further assess the importance of this effect, we performed additional simulations with even smaller population sizes (i.e., $100 \leq N < 1000$) for Model 4, and for a limited set of parameters (Fig. S32B). These results showed that the average weighted time of differentiation continued to increase when decreasing $N$ down to $N = 300$, but with further reduction of the population size, the average weighted time of differentiation started decreasing (with relatively large stochastic fluctuations in all cases examined). Thus, at a very low value of $N$ (that, in
general, will depend on model parameters), the effect of genetic drift in situations with chromosomal inversions changes from causing loss of differentiation more quickly in the smallest populations (which is how drift is commonly perceived), to instead contributing to maintaining differentiation for a longer time in smaller than in larger populations.

The rate of gene flux tested here, that is, \( r_{inv} = 2 \times 10^{-4} \), is close to the upper bounds of the corresponding rates inferred from empirical data (between \( 10^{-4} \) and \( 10^{-6} \) proportions of converted sites per genome, per generation across taxa; Korunes and Noor 2019). Recent data from the *D. pseudoobscura-D. persimilis* system imply that double crossover rates, although lower than for homokaryotypes or collinear regions, are nonnegligible in heterokaryotypes (\( 10^{-4} \) per site, per genome, per generation) (Stevison et al. 2011). Gene conversion rates between \( 10^{-5} \) and \( 2.5 \times 10^{-5} \) per site, per genome, per generation, involving tracts of DNA sequences between 200 and 400 bp long, have also been observed for these species (Korunes and Noor 2019).

A direct way to estimate gene conversion rates is simply based on the number of converted (diagnostic) SNPs relative to the total number of SNPs in a given region (Korunes and Noor 2019). Although double crossovers occur mainly in the central parts of large inversions due to interference (Navarro et al. 1997a; Fuller et al. 2019), gene conversion rates seem to be similar across the entire inverted regions (without interference), including near breakpoints (Korunes and Noor 2016; Crown et al. 2018).

Several cautionary considerations should be noted, however, when comparing the rate of gene flux, and especially of gene conversion, used in our model to the empirical estimates. First, like related models (e.g., Kirkpatrick and Barton 2006; Feder and Nosil 2009; Guerrero et al. 2012; Charlesworth and Barton 2018), our model treated loci and not sites or SNPs *per se* as the units of gene flux. Second, the loci in our model were assumed to be related to individuals’ fitness and the model did not explicitly account for fully neutrally evolving loci. Thus, the gene conversion rate used in our model (i.e., \( 10^{-4} \) per gamete, per generation in the *conserved-size* model) corresponded to the per-gamete, per-generation probability that a *fitness-related locus* was converted. In other words, the gene conversion rate in our model is the probability that the SNPs belonging to a fitness-related locus are among the per-generation, per-gamete converted SNPs. The proportion of fitness-related SNPs is likely to vary within and between species, and along the genomes, and it is unknown in general (as was also pointed out by Korunes and Noor 2019).

Although our model did not resolve the genomic region of interest on the per-site level, a comparison of our model results with respect to varying the number of fitness-related loci within the region in both the *conserved-size* and *increasing-size* model versions provides insight into a range of possibilities. Notably, with smaller rates of gene flux in our model, the parameter space where inversions are more effective in maintaining differentiation than collinear regions would be larger than what we report here.

Despite assuming high rates of gene flux in the model, our results show that inversions can delay population fusion after secondary contact for a longer period of time when they contain a larger number of loci under selection or involved in generating hybrid incompatibilities. However, the positional effects of the different mechanisms of gene flux, considering also interference between crossover events, need to be modeled in more detail. The previous deterministic analysis by Feder and Nosil (2009) suggested that spreading selection across greater number of loci in inversions could delay homogenization due to the longer time it takes for genes with lower per-allele selection strength to spread between populations. Our results advance understanding of the process by showing that finite population size and interference among linked loci can amplify the effects of multiple versus a few loci in curtailing the homogenization of rearranged regions of the genome following secondary contact.

We also showed that the ordering of fitness-related loci along the inverted region (*half-half array* as opposed to *consecutive-array* ordering) can have a strong effect on the model outcome (as was also pointed out by Blankaert and Bank 2018, although in a model without inversions). In our model with an inversion and gene flux between the alternative arrangements, differentiation time was typically much longer in the *consecutive-array* model than in the *half-half array* model, but more so in Model 4 than in Model 2. This is because in Model 4, incompatibilities needed to be resolved at specific pairs of loci due to the explicitly assumed interaction (i.e., negative epistasis) between alleles at pairs of loci, whereas in Model 2, there were no explicit between-alleles interactions (except for implicit, indirect selection). We expect that model outcomes with a random ordering of loci would be between the two extreme orderings we examined here, but this remains to be tested.

We caution, however, that the difference between many versus a few loci we report here is valid when (1) gene flux scaled by the effective population size is large enough (but not too large) to create a sufficient number of chromosomes possessing all favorable alleles to ensure homogenization; (2) per-allele selection strength scaled by population size is low enough so that rearrangements containing a small number of alleles under selection (following gene flux) will predominantly be lost; (3) effective population sizes are small (but not too small) to intermediate, with strong dependence on the details of the model and on parameter values; in some cases, the advantage of an inversion harboring many loci over collinear regions is retained even for populations with 100,000 individuals; and (4) favorable alleles in one population are neutral in the other population, or universally favorable alleles across populations cause incompatibilities in hybrids. We note that when fitness trade-offs exist such that alleles...
beneficial in one habitat are detrimental in the other, inversions might still retard the genetic homogenization of finite populations but the effects of concentrating selection on a few versus many loci may differ. However, we leave the exploration of this model for future work. The extent to which the above conditions hold in nature will therefore have a significant bearing on the degree to which multiple loci prolong inversion differentiation following secondary contact. Finally, when additional genes are added and increase the strength of selection (per region), the parameter space within which rearrangements will have a relevant role in population differentiation will vary drastically and remains to be explored in more detail.

One limitation of our study shared with earlier work is that we considered a two-population system because such a simple scenario is convenient for modeling. However, if testable quantitative predictions applicable to empirical systems are to be obtained, it will be necessary to use spatially explicit models involving many local populations (Flaxman et al. 2012) with a spatial and/or temporal selection gradient (e.g., mimicking hybrid zones). Such models would provide a significant step toward understanding observations in many hybrid zones where genetic regions harboring inversion polymorphisms have been detected (e.g., Rieseberg et al. 1999; Ayala et al. 2012; Westram et al. 2018; Faria et al. 2019a,b). In addition, it would be appealing to model inversions that harbor alleles under divergent selection together with alleles that are universally beneficial (or deleterious) and/or alleles involved in incompatibilities, comparing cases with diffuse versus concentrated genetic architectures.

### Conclusions

Our results support the hypothesis that inversions may be a strong mechanism contributing to maintenance of population differentiation, and potentially speciation, when divergence has a polygenic basis (Feder et al. 2005; Kirkpatrick and Barton 2006). Crucially, by maintaining population differences after secondary contact, inversions can act as a partial barrier to gene flow between populations, thus allowing for a longer period of time compared to collinear gene regions during which populations may accumulate additional differences, including incompatibilities. This may facilitate the evolution of additional RI and “progress” in speciation through other mechanisms, such as reinforcement (Noor et al. 2001; Butlin and Smadja 2018). Note that we do not claim that inversion polymorphisms are impermeable barriers to gene flow. Rather, unless new inversions arise or divergence by new mutations occurs, populations will eventually homogenize in the face of gene flow and in the absence of divergent selection. However, for many parameter sets we tested, homogenization can be delayed for tens of thousands of generations after secondary contact and this delay can allow for additional barriers to accumulate.

Besides suggesting that chromosomal rearrangements have a role in maintaining population differences after secondary contact, previous theoretical work showed that they can also facilitate adaptive population differentiation in cases of primary contact, resulting in the emergence of clustered genomic architectures (Yeaman 2013). Moreover, reduced recombination rates within heterokaryotypes containing multiple weakly diverged loci will be an effective mechanism protecting such loci from stochastic loss (Feder et al. 2014; see also Rafajlović et al. 2016 for the effect of recombination on stochastic loss in a model without inversions), thereby facilitating divergence with gene flow. Overall, all these results, together with those presented here, suggest an important role of inversions in population divergence and speciation, even in the face of gene flux.
Drosophila pseudoobscura
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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. List of simulated versions of Model 2, and the corresponding combinations of parameter values used in the simulations.

Table S2. List of simulated versions of Model 4, and the corresponding combinations of parameter values used in the simulations.

Figure S1. Proportion of times that a locus within the region simulated is involved in a gene flux event, conditional on the gene flux occurring, when the number of loci within the region is 2 (A), 4 (B), 6 (C), 8 (D), 10 (E), or 20 (F).

Figure S2. Simulation results for the conserved-size model involving locally favored and neutral alleles (Model 2).

Figure S3. Simulation results for the conserved-size, half-half array model with 20 loci involving locally favored and neutral alleles (Model 2).

Figure S4. Same as in Figure S3, but for the corresponding $F_{ST}$ patterns.

Figure S5. Same as in Figure S3, but for the consecutive-array, conserved-size model involving locally favored and neutral alleles (Model 2).

Figure S6. Same as in Figure S5, but for the corresponding $F_{ST}$ patterns.

Simulation results for the increasing-size model involving locally favored and neutral alleles (i.e., Model 2 in which the total recombination distance within the region considered, as well as the total rate of gene flux, increase with increasing numbers of loci).

Figure S7. Same as in Figure S2 but for the increasing-size version of the model with locally favored and neutral alleles (Model 2).

Figure S8. Same as in Figure S3 but for the increasing-size version of the half-half array model with locally favored and neutral alleles (Model 2).

Figure S9. Same as in Figure S8, but for the corresponding $F_{ST}$ patterns.

Figure S10. Same as in Figure S8, but for the corresponding $F_{ST}$ patterns.

Figure S11. Same as in Figure S9, but for the consecutive-array version of the increasing-size model with locally favored and neutral alleles (Model 2).

Figure S12. Same as in Figure S11, but for the corresponding $F_{ST}$ patterns.

Figure S13. Simulation results for the half-half array, conserved-size model involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S14. Simulation results for single randomly chosen realizations of the conserved-size, half-half array model with 20 loci involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S15. Same as in Figure S14, but for the corresponding $F_{ST}$ patterns.

Figure S16. Simulation results for the consecutive-array version of the conserved-size model involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S17. Same as in Figure S13, but for the consecutive-array version of the conserved-size model involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S18. Same as in Figure S14, but for the consecutive-array version of the conserved-size model involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S19. Same as in Figure S18, but for the corresponding $F_{ST}$ patterns.

Figure S20. Same as in Figure S16, but for the increasing-size version of the half-half array model involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S21. Same as in Figure S13, but for the increasing-size version of the half-half array model involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S22. Same as in Figure S14, but for the increasing-size version of the half-half array model involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S23. Same as in Figure S22, but for the corresponding $F_{ST}$ patterns.

Figure S24. Same as in Figure S16, but for the increasing-size version of the consecutive-array model involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S25. Same as in Figure S13, but for the increasing-size version of the consecutive-array model involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S26. Same as in Figure S14, but for the increasing-size version of the consecutive-array model involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S27. Same as in Figure S26, but for the corresponding $F_{ST}$ patterns.

Figure S28. The effect of population size: simulation results for the conserved-size half-half array model involving locally favored and neutral alleles (Model 2), and with local population size set to $N = 5000$.

Figure S29. The effect of population size: simulation results for the conserved-size, half-half array model involving universally beneficial alleles with genetic incompatibilities (Model 4), and with local population size set to $N = 5000$.

Figure S30. Simulation results for the conserved-size model involving locally favored and neutral alleles (Model 2) arranged at 20 loci.

Figure S31. Simulation results for the increasing-size version of the model with locally favored and neutral alleles (Model 2) arranged at 20 loci.

Figure S32. Same as in Figure S30 but for the half-half array, conserved-size version of the model involving universally beneficial alleles with genetic incompatibilities (Model 4).

Figure S33. Same as in Figure S31 but for the increasing-size version of the model involving universally beneficial alleles with genetic incompatibilities (Model 4).