Fitness-dependent recombination can be evolutionarily advantageous in diploids under mutation–selection balance

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ABSTRACT

Recombination's omnipresence in nature is a most intriguing problem in evolutionary biology. The question of why recombination exhibits certain general features is no less interesting than that of why it exists at all. One such feature is its dependence on genotype fitness. The evolution of fitness-dependent recombination in diploids remains theoretically unexplained, despite empirical evidence for this phenomenon, mostly from diploids. Here, using numerical analysis of modifier models with infinite population size, we show that fitness-dependent recombination can be evolutionarily advantageous in diploids under selection against deleterious mutations. With unlinked modifier locus, this advantage originates entirely from benefits of recombination-rate plasticity within the selected system (indirect effect). With a linked modifier locus, it is accompanied by benefits of variation in modifier linkage to the selected system induced by fitness dependence of recombination rates between the selected loci (direct effect). Together with our earlier results on cyclical selection and Red Queen dynamics, these findings suggest that the evolutionary advantage of fitness-dependent recombination based on an indirect effect can be universal in diploids. Remarkably, fitness-dependent recombination is often favored in situations where constant non-zero recombination is rejected, thereby relaxing the constraints on the evolution of non-zero recombination in classical models with non-plastic recombination.

Keywords: recombination, fitness dependence, diploids, modifier's direct and indirect effects, purifying selection, synergistic epistasis

INTRODUCTION

Meiotic recombination is a process of reshuffling the parental genetic material, which takes place when a sexual organism produces its gametes. For about a century, recombination's omnipresence in nature has been a most intriguing question, given the evolutionary ambiguity of this process (Weismann 1889; Fisher 1930; Maynard Smith 1971; Bell 1982). Indeed, the new allelic combinations generated in this process serve as the raw material to meet selection demands. On the other hand, it can also break down the existing combinations, including even the most successful ones. However, today, the question of why recombination exhibits certain general features has become no less interesting than the initial question of why recombination exists at all (Lenormand et al. 2016). One such feature is recombination's sensitivity to external and/or internal conditions affecting the proportion of recombinants in the progeny. Harold Plough was the first to show that recombination rates (RRs) can rise when the organism is exposed to an ecological stressor (Plough 1917). Empirical studies have provided accumulating evidence for the ecological plasticity of recombination (for recent reviews, see Bomblies et al. 2015; Modliszewski and Copenhaver 2017; Stapley et al. 2017). Earlier, it was noticed that recombination's ecological plasticity is genotype-specific (Elliott 1955; Wilson 1959; Nakamura 1966), although the pattern of such specificity remained obscure. In 1986, Zhuchenko et al. (1986) demonstrated that stressor-induced changes in RRs can be modulated by genotype fitness in a negative-feedback manner, so that less stress-tolerant genotypes show more pronounced increases in RR. Moreover, genotype fitness can affect RRs even when no ecological stressors are imposed, i.e., when variation in fitness among individuals results from their differential genetic background (e.g., deleterious mutations) rather than from their differential stress tolerance (Tucić et al. 1981). In general, one can think of 'fitness-dependent' (Zhuchenko et al. 1986) or 'fitness-associated' (Agrawal et al. 2005) recombination; herein, we use the former term, abbreviated as FD recombination. Empirical evidence for this phenomenon is still very limited, for both stressor-induced (Kilias et al. 1979; Zhuchenko et al. 1986; Korol et al. 1994; Khlebova 2010; Zhong and Priest 2011; Jackson et al. 2015; Hunter et al. 2016) and mutation-induced (Tucić et al. 1981; Tedman-Aucoin and Agrawal 2012) changes in RR. Importantly, this evidence comes from diploids—mainly from fruit flies (Kilias et al. 1979; Tucić et al. 1981; Korol et al. 1994; Zhong and Priest 2011; Tedman-Aucoin and Agrawal 2012; Jackson et al. 2015; Hunter et al. 2016), but also from plants, such as tomato (Zhuchenko et al. 1986) and wheat (Khlebova 2010).

Within the adaptationist program, phenotypic traits (at least those that are heritable, affect reproductive success, and originate from earlier existing traits) are believed to evolve as adaptations (Williams 2008). Since FD recombination meets all of these criteria, it seems natural to consider it as

an evolvable phenotype, or at least to test for this possibility. Analysis of natural populations infers that variation in RRs may indeed be adaptive (Ritz et al. 2017). Theoretical models developed to date have clearly demonstrated the evolutionary advantage of FD recombination in haploids (Gessler and Xu 2000; Hadany and Beker 2003a,b; Agrawal et al. 2005; Wexler and Rokhlenko 2007). However, extending these results to diploids required making additional specific assumptions, such as cis/trans effects (Agrawal et al. 2005). In contrast, our recent study showed that FD recombination can also be evolutionarily advantageous in diploids under cyclical selection (Rybnikov et al. 2017b). The same result was obtained for another type of condition dependence, i.e., ecological plasticity of recombination, again for diploids exposed to cyclical selection (Zhuchenko et al. 1985; Rybnikov et al. 2017b).

To address the discrepancy between the results obtained in the aforementioned diploid models, it was suggested that more complex selection regimes, such as cyclical selection, favor FD recombination more than less complex ones, such as directional selection or mutation–selection balance (Agrawal et al. 2005). Alternatively, as we discussed in our recent paper (Rybnikov et al. 2017b), what may really affect the evolutionary advantage/disadvantage of FD recombination in the considered models is the presence or absence of variation in fitness among genotypes. Importantly, one should consider only those genotypes in which recombination may have fitness consequences, i.e., genotypes heterozygous for at least two selected loci; in all other genotypes, RRs are ‘immaterial’, in terms of Otto and Barton (1997). Herein, as in our previous study (Rybnikov et al. 2017b), we refer to the former genotypes as ‘recombination-responsive’. To further explore this assumption, here we examine the evolution of FD recombination in diploids under mutation–selection balance, which is a relatively simple selection regime compared to cyclical selection. The wild-type genotype is assumed to have the highest fitness, while mutations at any locus are deleterious. If mutations at different loci affect fitness in a purely multiplicative way, recombination is known to be neutral. However, if the presence of a mutant allele(s) simultaneously at several loci decreases fitness more markedly (synergistic epistasis), then recombination can appear evolutionarily advantageous, since it facilitates purging mutant alleles from the population (Feldman et al. 1980; Kondrashov 1984; Charlesworth 1990; Gabriel et al. 1993; Barton 1995; Lynch et al. 1995; Otto and Barton 1997; Otto and Feldman 1997).

Under this scenario, we estimate the optimal constant RR in the selected system, and then test whether FD recombination can be evolutionarily more advantageous. We compare different recombination strategies using the modifier approach (Kimura 1956; Nei 1967), i.e., based on the dynamics of selectively neutral recombination-modifying alleles in an infinite panmictic population

subject to diploid selection against deleterious mutations. It should be noted that comparisons between 'asexual reproduction' and 'sexual reproduction without recombination' on the one hand, and between 'sexual reproduction without recombination' and 'sexual reproduction with recombination' on the other, show that the effect of sex *per se* (i.e., of segregation) is much stronger than that of recombination (Charlesworth 1990). Yet, the latter is no less important, bearing in mind that segregation is typically accompanied by recombination. Here, we study the evolution of recombination assuming obligate sexual reproduction with total panmixia, which seems realistic for many higher diploid eukaryotes.

Importantly, as in our previous studies (Rybnikov et al. 2017b,a), we exploit selected systems with *three* loci, which is the minimal number for our purposes. Indeed, recombination may have fitness consequences in genotypes with at least two heterozygous loci (*AaBb*). However, if we need such recombination-responsive genotypes to vary in fitness, at least one more locus is required (unless couple- and repulsion-phase double heterozygotes differ in fitness due to cis/trans effects, which we do not assume here).

METHODS

Life cycle

We consider an infinite population of obligate sexual diploids with total panmixia. The life cycle includes random mating, selection at the diploid level, and meiosis resulting in gametes of the next generation. The generations do not overlap. Let x_{ij} be a diploid genotype made up of haplotypes i and j. Its frequency p^s after selection (as an adult) can be calculated based on its frequency p before selection (as a zygote) and its absolute fitness W:

$$p^s(x_{ij}) = \frac{p(x_{ij}) \cdot W(x_{ij})}{\sum_{ij} p(x_{ij}) \cdot W(x_{ij})} \quad (1)$$

Then, let g_k be gamete of haplotype k. Its frequency p in the gamete pool can be calculated based on frequencies of adults and probabilities of recombination events:

$$p(g_k) = \sum_{ij} p^s(x_{ij}) \cdot P^r_{ij \to k} \quad (2)$$

where $P^r_{ij \to k}$ is the probability of obtaining gamete g_k from genotype x_{ij} via recombination (Barton 1995).

Frequency p^m of a given gamete after mutation can be calculated based on probabilities of mutation events:

$$p^m(g_k) = \sum_l p(g_l) \cdot P^m_{l \to k} \tag{3}$$

where $P^m_{l \to k}$ is the probability of obtaining gamete g_k from gamete g_l via mutations.

Finally, frequencies of zygotes in the next generation can be calculated based on frequencies of the corresponding gametes, given random mating:

$$p_{t+}(x_{ij}) = p^m_t(g_i) \cdot p^m_t(g_j) \tag{4}$$

Genetic system

Each genotype has $n = 3$ selected loci (*A*, *B* and *C*), and a selectively neutral modifier locus (*M*) affecting RRs between the other loci. The loci are arranged as *M–A–B–C*. Also, in an additional experiment, we examined simpler systems, with $n = 2$ selected loci.

Each selected locus is represented by two possible alleles: wild type (*A*, *B* or *C*) and mutant (*a*, *b* or *c*). The locus-associated component of fitness (W_l) depends on the number of mutant alleles at this locus:

$$W_l = \begin{cases} 1, & \text{for } AA, BB, \text{ or } CC \\ 1 - hs, & \text{for } Aa, Bb, \text{ or } Cc \\ 1 - s, & \text{for } aa, bb, \text{ or } cc \end{cases} \tag{5}$$

Here, *s* and *h* represent the deleterious effect of mutations in the homozygous state and the dominance of mutations, respectively.

The whole-genotype fitness (*W*) deviates from a purely multiplicative one, due to interlocus epistasis (*e*):

$$W = \prod_l W_l \cdot (1 + e) \tag{6}$$

The effect of epistasis is defined by the number of mutant loci (a locus is regarded as 'mutant' if it bears at least one mutant allele):

$$e(n_M) = \begin{cases} 0, & \text{for } n_M \le 1 \\ e_2, & \text{for } n_M = 2 \\ e_3, & \text{for } n_M = 3 \end{cases} \tag{7}$$

Here, e_2 and e_3 represent two-locus epistasis and three-locus epistasis, respectively.

For simplicity, deleterious effect and dominance were assumed to be equal for all three selected loci. Likewise, two-locus epistasis was assumed to be equal for all three pairs of selected loci. It should be noted that here, epistasis is modeled as a multiplier to the purely multiplicative fitness, rather than as an addend, as used in other studies. Thus, in our model, epistasis actually varies across genotypes, although it is formally described by only two parameters.

Recombination strategies

Alleles at the modifier locus define RRs between the selected loci (r_{AB} and r_{BC}). For simplicity, RRs within the selected system are assumed to be equal ($r_{AB} = r_{BC} = r_S$). The modifier locus is assumed to be either unlinked ($r_{MA} = 0.5$) or linked ($r_{MA} = 0.05$) to the selected system. No crossover interference is assumed. The relations between alleles at the modifier locus are assumed to be purely co-dominant. Alleles at the modifier locus confer different recombination strategies. We consider two types of strategies: (*i*) constant recombination, implying equal RRs for all genotypes (C-strategy), and (*ii*) FD recombination, implying varying RRs among genotypes according to their fitness (FD-strategy). Under the FD-strategy, RRs within the selected system (r_S) depend negatively on genotype absolute fitness (W). Specifically, the genotype with the highest fitness ($W_{\max}$) has the lowest recombination rate ($r_{\min}$) and vice versa. For genotypes with intermediate fitness values, RRs are obtained by linear interpolation:

$$r_S(W) = \begin{cases} r_{\min}, & \text{for} \quad W = W_{\max} \\ r_{\min} + (r_{\max} - r_{\min}) \cdot \dfrac{W_{\max} - W}{W_{\max} - W_{\min}}, & \text{for} \quad W_{\min} < W < W_{\max} \\ r_{\max}, & \text{for} \quad W = W_{\min} \end{cases} \tag{8}$$

In our modeling, we assume that a precondition for the evolutionary advantage of FD recombination is variation in fitness among *recombination-responsive genotypes* (double and triple heterozygotes). In this respect, when estimating the lowest and highest fitness values ($W_{\min}$ and $W_{\max}$), we took into account only such genotypes. However, in models with two selected loci, which were examined in an additional experiment, there exists only one recombination-responsive genotype (double heterozygote), and such normalization would result in division by zero (equation 8). In this case, we estimated the lowest ($W_{\min}$) and highest ($W_{\max}$) fitness values among *all genotypes*.

Criteria for comparison of recombination strategies

First and foremost, we compared alternative recombination strategies in terms of *individual selection*, based on allele dynamics at the modifier locus (Kimura 1956; Nei 1967). Specifically, strategy S_1 was regarded as evolutionarily more advantageous than strategy S_2 if the modifier allele for S_1 succeeded in the two following tests. First, it had to invade populations in which the modifier allele for S_2 prevailed. Second, it had to resist, when it itself prevailed, backward invasion by the modifier allele for S_2. In both tests, 'prevailing' meant an allele frequency of 0.95. In both tests, modifier alleles were allowed to compete for 10,000 generations. Before the competition started, the selected system was allowed to evolve with a monomorphic modifier locus until it reached mutation–

selection balance. The latter was diagnosed when allele frequencies at each selected locus changed by less than 10^{-12} per generation.

The optimal constant RR within the selected system (r_S^*) was estimated as follows. The strategy conferring minimal RR ($r_S = 0$) was compared with the strategy conferring one step higher RR ($r_S = \delta r$). If the latter appeared to be evolutionarily more advantageous (see above), it was compared to the strategy conferring one more step higher RR ($r_S = 2\delta r$), and so on. These pair-wise comparisons were conducted until a strategy with higher RR failed to overcome its opponent (i.e., either failed to invade, or succeeded to invade but failed to resist the backward invasion). Once this happened, the lower of the two competing RRs was regarded as the inferior estimate for the optimal constant RR ($\underline{r_S^*}$). Analogously, we sought the superior estimate for the optimal constant RR ($\overline{r_S^*}$). We started with a strategy conferring maximal RR ($r_S = 0.5$), and moved downward ($r_S = 0.5–\delta r$, $r_S = 0.5–2\delta r$, etc.). Then, we repeated the procedure between the obtained inferior and superior estimates using the one order lower step, to obtain new, more precise estimates. In total, we used three iterations, with steps equal to 0.01, 0.001 and 0.0001. The final inferior and superior estimates differed by no more than 0.0001; the average between these two values was used as the optimal constant RR (r_S^*).

Aside from allele dynamics at the modifier locus, we also used additional criteria to compare recombination strategies, appealing to *group selection*; these included population mean fitness, population genetic variation, and population adaptation time. Population mean fitness was calculated as fitness of all genotypes weighted by their frequencies. Population genetic variation (v) was calculated as mean standard deviation of allele frequencies within the selected system:

$$v = \frac{1}{n}\sum_i \sqrt{p_i(1-p_i)} \qquad (9)$$

where p_i is allele frequency at the i-th selected locus, and n is the number of selected loci. Population adaptation time was considered to be the number of generations needed for the selected system to reach mutation–selection balance.

Statistical analysis

Statistical analysis was performed in Statistica V 10.0.

RESULTS

We examined 24,000 combinations of four selection parameters: deleterious effect of mutations (s), dominance of mutations (h), two-locus epistasis (e_2), and three-locus epistasis (e_3). The deleterious effect was scanned from 0.05 (slightly deleterious mutation) to 1 (lethal mutation), with a step of 0.05. Dominance was scanned from 0.25 (partially recessive mutation) through 0.5 (co-dominant mutation) to 0.75 (partially dominant mutation). The two- and three-locus epistases were scanned from -0.05 to -1 with a step of 0.05; positive values were not considered, since they are expected to be incompatible with selection for non-zero RRs (Charlesworth 1990). Mutations were assumed to be unidirectional, with a rate of 10^{-4} per selected locus.

For each case, we first estimated the optimal constant RR within the selected system. This was estimated separately for unlinked and linked modifier loci, since modifier locus linkage is known to affect competition between different modifier alleles (for reviews, see Korol et al. 1994; Otto 2009). The proportion of cases with non-zero optimal constant RR tended to increase with higher dominance of deleterious mutations; this held for both unlinked and linked modifier loci. Cases with intermediate optimal constant RR were observed only with linked modifier loci, where their proportion was higher under lower dominance of deleterious mutations (Fig. S1). Once the optimal constant RR within the selected system was estimated for each case, we traced the competition between two strategies: (*i*) implying that all genotypes have the optimal constant RR (OC-strategy), and (*ii*) implying that different genotypes have different RRs, varying around the optimal constant RR according to their fitness (FD-strategy). We tested whether the modifier allele for the FD-strategy can displace that for the OC-strategy.

Zero optimal constant RR within the selected system

The first question is whether FD recombination can be favored in situations where any constant non-zero recombination is rejected. To address this, we compared OC-strategy with *zero* optimal constant RR, on the one hand, and FD-strategy with RRs varying from 0 to δr, on the other (hereafter, we refer to such FD-strategy as 'recombination-increasing FD-strategy', or '+FD-strategy'). The first round of simulations was conducted with amplitude $\delta r = 0.01$. The +FD-strategy appeared to be favored in a remarkably high proportion (~26%) of cases with an unlinked modifier locus, and even more so (~47%) with a linked modifier locus. The models presented herein are numerical, which does not allow strict inferences about conditions that favor/disfavor FD recombination. Nevertheless, it is possible to discriminate parameter combinations leading to different outcomes using numerical classification, e.g., logit analysis. Interestingly, one of the most informative parameters appeared to be monotonicity of absolute fitness across recombination-responsive genotypes, like

W(*AaBbCC*) > *W*(*AaBbCc*) > *W*(*AaBbcc*). Typically, when monotonicity did not hold, +FD-strategy was rejected; when it did, other parameters also mattered. Among the latter, the most influential were two- and three-locus epistases and absolute fitness of the best double heterozygote, e.g., *W*(*AaBbCC*). Specifically, with an unlinked modifier locus, +FD-strategy was the most favored under close values of two- and three-locus epistases (Fig. 1A) and under intermediate absolute fitness of the best double heterozygote (Fig. 1B). Effect of selection intensity (deleterious effect of mutations) seemed to be weaker, manifested as flatter landscapes (Fig. 1C, D). With a linked modifier locus, +FD-strategy was favored in wider areas of the parameter space. In particular, three-locus epistasis stopped being so restrictive, and only its extremely high values resulted in rejection of +FD-strategy.

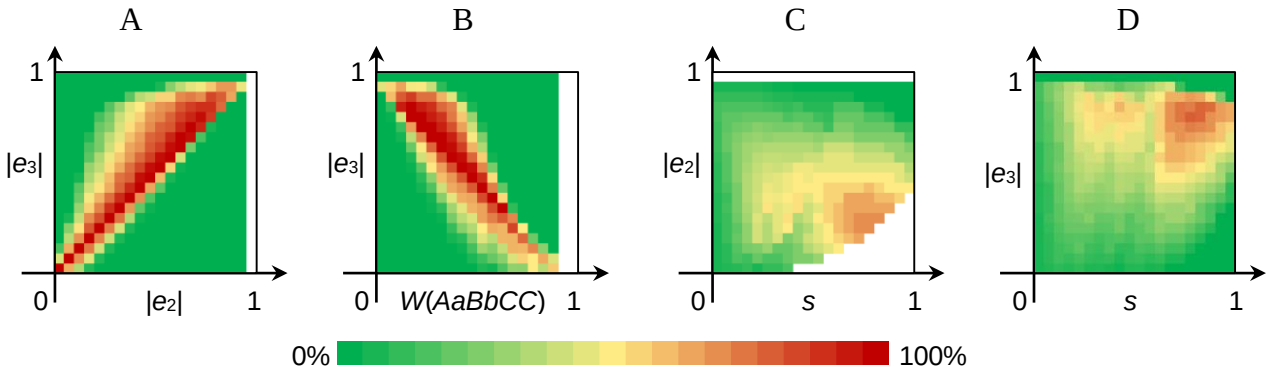

Figure 1. Effect of the main parameters on the proportion of cases in which +FD-strategy was favored over the corresponding OC-strategy with *zero* optimal constant RR.

(A) Two-locus epistasis (absolute values)/three-locus epistasis (absolute values). (B) Absolute fitness of the best double heterozygote/three-locus epistasis (absolute values). (C) Selection intensity/two-locus epistasis (absolute values). (D) Selection intensity/three-locus epistasis (absolute values). Here and in Fig. 2, the proportions represent the ratios between the number of cases in which +FD-strategy was favored and the total number of cases with the given combination of parameter values. White areas indicate that the corresponding combinations of parameter values were absent. 'Favored' +FD-strategy means that the modifier allele for the +FD-strategy displaced that for the corresponding OC-strategy (those rare cases in which the two modifier alleles coexisted were classified as 'non-favored').

Due to the importance of the above results, we paralleled the $\delta r = 0.01$ simulations with those of $\delta r = 0.05$, 0.1 and 0.5. We found that the proportion of cases in which +FD-strategy is favored tends to grow with the amplitude, up to ~30% of cases with unlinked modifier locus, and up to ~57% of cases with linked modifier locus. Yet, in several cases, large-amplitude +FD-strategies appeared to

be less successful (compared to the corresponding OC-strategy) than small-amplitude +FD-strategies. This gave rise to the natural question of whether there exists an *optimal* amplitude of RR plasticity. To address this, we let +FD-strategies with different amplitudes compete with one another. We found that indeed, in such cases, a modifier allele for a certain intermediate amplitude of RR plasticity displaced those for both smaller and larger amplitudes (Fig. S1).

Intermediate optimal constant RR within the selected system

As already noted, cases with *intermediate* optimal constant RR were observed only with a linked modifier locus. For each such case, we compared the OC-strategy with three different FD-strategies: (*i*) with RRs varying *above* the optimal constant RR, i.e., in the range of r_S^* to $r_S^* + \Delta r$ ('recombination-increasing FD-strategy', or '+FD-strategy'); (*ii*) with RRs varying *below* the optimal constant RR, i.e., from $r_S^* - \Delta r$ to r_S^* ('recombination-decreasing FD-strategy', or '–FD-strategy'); and (*iii*) with RRs varying *around* the optimal constant RR, i.e., from $r_S^* - \Delta r$ to $r_S^* + \Delta r$ ('fringe FD-strategy', or '±FD-strategy'). Again, we examined various amplitudes (δr), from 0.01 to 0.1; within this range, the results were strongly robust. The +FD-strategy was favored in a predominant proportion of cases (~92%). The evolutionary advantage of the ±FD-strategy was much less pronounced, albeit still sound (~43%), whereas –FD-strategy was always rejected. As in cases with zero optimal constant RR, the most informative parameters were the two- and three-locus epistases and absolute fitness of the best double heterozygote. Typically, the ±FD-strategy was favored when the three-locus epistasis was stronger that the two-locus one; higher fitness of the best double heterozygote tended to reduce the evolutionary advantage of FD recombination. For the +FD-strategy, these constraints almost disappeared: it was rejected only under marginal combinations of epistases, and only under very high fitness of the best double heterozygote (Fig. 3).

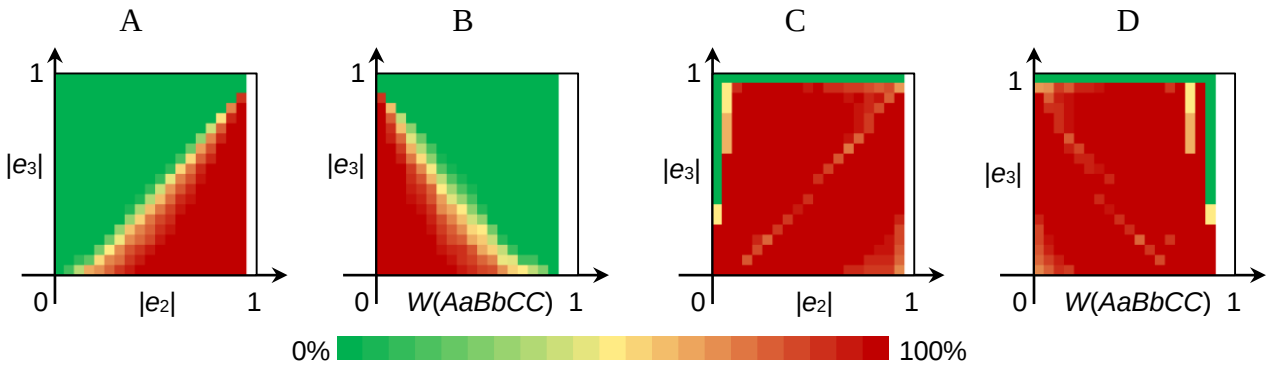

Figure 2. Effect of the main parameters on the proportion of cases in which FD-strategy was favored over the corresponding OC-strategy with *intermediate* optimal constant RR.

(A) Two-locus epistasis (absolute values)/three-locus epistasis (absolute values), ±FD-strategy. (B) Absolute fitness of the best double heterozygote/three-locus epistasis (absolute values), ±FD-strategy. (C) Two-locus epistasis (absolute values)/three-locus epistasis (absolute values), +FD-strategy. (D) Absolute fitness of the best double heterozygote/three-locus epistasis (absolute values), +FD-strategy.

Relative roles of direct and indirect effects of the modifier

In all of the above described simulations, each pair of competing modifier alleles differed only in their effect on RR between the selected loci (r_{AB} and r_{BC}), but not on RR between the modifier locus and the adjacent selected locus (i.e. r_{MA}). Following the common terminology (Agrawal et al. 2005), the competing modifier alleles differed in their indirect effect, but not in their direct effect. Under FD recombination, r_{AB} (as well as r_{BC}) varied among genotypes. As a 'by-product', RR between the modifier locus and the intermediate selected locus (i.e., r_{MB}) also varied among genotypes, since r_{MB} depends simultaneously on r_{MA} and r_{AB}. In other words, although a direct effect on r_{MA} was absent, a direct effect on r_{MB} did emerge under FD recombination. Thus, the evolution of FD recombination in this situation was driven simultaneously by two effects: (*i*) indirect effect on r_{AB} and r_{MB} and (*ii*) direct effect on r_{MB}. To discriminate between these two effects in situations with a linked modifier locus, we additionally examined a FD-strategy implying that only r_{BC} (but not r_{AB}) depends on genotype fitness. This FD-strategy (hereafter referred to as 'direct-effect free' FD-strategy) seemed to be less advantageous than the corresponding 'standard' FD-strategy examined in the major part of simulations. Specifically, it was favored in ~46% of cases with zero optimal constant RR (compared to ~47% for the 'standard' FD-strategy), and in ~31% of cases with intermediate optimal constant RR (compared to ~92% for the standard FD-strategy). The difference grew with RR within the selected system (Fig. 3)

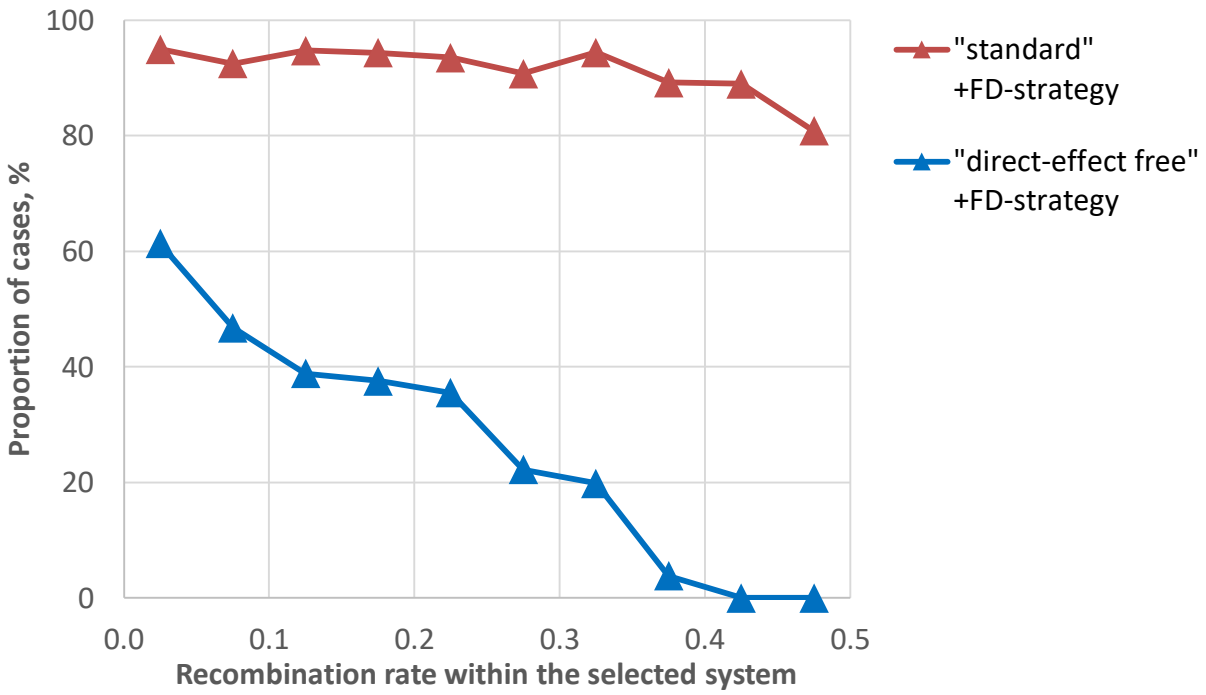

Figure 3. Effect of RR within the selected system on the proportion of cases in which different +FD-strategies ('standard' and 'direct-effect free') were favored over the corresponding OC-strategy with intermediate optimal constant RR.

Additional criteria for comparison of recombination strategies

Where applicable, we tested for associations between different criteria used to compare recombination strategies. Specifically, we compared allele dynamics at the modifier locus (an individual-selection criterion, central to this study) with several group-selection criteria, such as population mean fitness, genetic dispersion, and time needed to reach mutation–selection balance. Typically, when ±FD-strategies were favored over the corresponding OC-strategies, they also increased population mean fitness, improving it, on average, by ~10^{-8}, and simultaneously decreased population genetic variation, by ~10^{-7}–10^{-6} (Fig. S2). However, such clear-cut associations were not always the case. For example, in cases with zero optimal constant RR, +FD-strategy always increased population mean fitness and always decreased population genetic variation, regardless of whether it was favored or rejected. Nevertheless, in cases in which +FD-strategy was rejected, the increase in population mean fitness and decrease in population genetic variation were much more pronounced (by 3–4 orders of magnitude) compared to cases in which it was favored. Population adaptation time did not seem to be affected by the evolutionary advantage of FD recombination.

DISCUSSION

Cases with non-zero optimal constant RR were quite numerous (Fig. S1). Their proportion was larger under higher dominance of deleterious mutations. In contrast, the proportion of cases with intermediate optimal constant RR was larger under lower dominance. Nevertheless, both results reflected the same pattern, discovered by Roze (2009): mutation dominance facilitates selection for recombination. Moreover, the proportion was larger with a linked modifier locus. It may be that in this case, modifier alleles remain associated with the selected-system haplotypes for a longer time, mitigating the constraints imposed on epistasis, as happens when a population practices less sex compared to panmixia (Otto 2009).

Several experiments with diploid organisms have demonstrated that variation in fitness among genotypes can affect RRs, including situations in which such variation originates from different genetic backgrounds of population members caused by deleterious mutations (Tucić et al. 1981; Tedman-Aucoin and Agrawal 2012). The results obtained herein indicate that FD recombination can be favored in diploids under mutation–selection balance. Previously, we showed the same for cyclical selection (Rybnikov et al. 2017b) and Red Queen dynamics (for preliminary results, see Rybnikov et al. 2017a). Thus, the evolutionary advantage of FD recombination seems to be general, rather than associated with a certain selection regime (although different regimes naturally differ in the proportion of cases favoring FD recombination).

In general, a modifier allele for one recombination strategy (regardless of whether it is constant or FD) can be favored over another one due to (*i*) an effect on RR between the modifier locus and the selected system (direct effect); (*ii*) an effect on RRs within the selected system (indirect effect); and (*iii*) a combination of both effects. A direct effect implies that the advantageous modifier allele creates a better association with preferred haplotypes of the selected system, whereas an indirect effect implies that this allele increases the proportion of preferred haplotypes. Potentially, RR between any pair of loci can depend on genotype fitness. If a modifier allele is capable of somehow ‘evaluating’ its current genetic environment and tends to recombine from the low-fitness chromosome (i.e., if it causes RR between itself and the selected system to be FD), then it will apparently increase its chances of surviving and spreading. Exploiting such benefits is commonly referred to as the ‘abandon-ship’ mechanism (Agrawal et al. 2005). On the other hand, if a modifier allele tends to disrupt low-fitness genotypes (i.e., if it causes RRs within the selected system to be FD), then it increases the proportion of fitter genotypes in the population and thereby minimizes, at least partially, the inherent costs of recombination. Yet, these benefits associated with RR plasticity within the selected system can be overshadowed to a certain extent, since the mean RR established within the selected system under FD recombination may deviate from the optimal RR. Thus, the FD

indirect effect is a trade-off, and it can drive the evolution of FD recombination only if its benefits surpass its costs.

Sometimes, the 'abandon-ship' mechanism is viewed as the only explanation for the evolutionary advantage of FD recombination, perhaps due to its self-evidence (e.g., Griffiths and Bonser 2013). This can be the case, but not always. In haploids, 'selfish' modifier alleles have indeed been shown to be favored, even if they do not affect RRs within the selected system at all; even if the selected system consists of only a single locus (Hadany and Beker 2003b; Agrawal et al. 2005). However, in diploids, the 'abandon-ship' mechanism is inefficient. The intuitive explanation is that in diploids, modifier alleles cannot 'recognize' which of the two homologous chromosomes causes lower fitness, unless a cis/trans effect (a rather specific phenomenon) is assumed. Thus, modifier alleles make 'right' and 'wrong' decisions with equal probabilities and, therefore, with no effect on their own frequencies in the next generation. The inefficiency of the 'abandon-ship' mechanism in diploids was shown analytically under directional selection (Agrawal et al. 2005). In our previous model with cyclical selection (Rybnikov et al. 2017b), we tested for efficiency of the 'abandon-ship' mechanism in diploids numerically. For this purpose, in simulations with a linked modifier locus, we examined a strategy implying that only RR between the modifier locus and the selected system (but not RRs within the latter) depends on genotype fitness. We showed that such an FD-strategy was always neutral in relation to the corresponding OC-strategy, i.e., allele frequencies at the modifier locus did not change, regardless of their initial frequencies. Moreover, two arbitrary recombination strategies were neutral if they implied equal effects on RR within the selected system, regardless of the effects on RR between the modifier locus and the selected system. Based on those results, we inferred that the FD direct effect does not work in diploids, and therefore ascribed the evolutionary advantage of FD recombination, whenever it was observed, to only FD indirect effect (Rybnikov et al. 2017b). In the current model with mutation–selection balance, we conducted analogous simulations and obtained the same result: modifier alleles with equal effects on RR within the selected system always remained neutral, regardless of differences in their effects on the modifier linkage to the selected system. However, as we showed above (see section: Relative roles of direct and indirect effects of the modifier), FD recombination can give rise to a more sophisticated interplay between direct and indirect effects. This happens if (*i*) the selected system consists of at least three selected loci, and (*ii*) the modifier locus is linked to the selected system (Supplementary text S4).

Thus, in our simulations with an *unlinked* modifier locus, the revealed evolutionary advantage of FD recombination (~26–30% of cases with zero optimal constant RR, depending on amplitude of RR plasticity) should be ascribed to FD indirect effect alone, i.e., to benefits associated

with RR plasticity within the selected system. The proportion of cases in which FD recombination was favored tended to grow with the amplitude of RR plasticity, suggesting that the benefits of large amplitude (higher RR plasticity) are stronger that its costs (higher deviation from the optimal, in this case zero, RR). However, in some cases, starting from a certain value of amplitude, the costs did outweigh the benefits, implying the existence of intermediate optimal amplitude (Fig. S3). This further confirms the conclusion that the evolutionary advantage of FD recombination is a trade-off between benefits of RR plasticity and costs of deviation from the optimal RR. In simulations with a *linked* modifier locus, the evolutionary advantage of FD recombination seemed to be even more pronounced (~47% of cases with zero optimal constant RR, and ~92% of cases with intermediate optimal constant RR). However, in these situations, the evolutionary advantage of FD recombination should be ascribed to both indirect and direct effects. The difference between 'standard' and 'direct-effect free' +FD-strategies (Fig. 3) elucidates, in fact, the contribution of direct effect to the overall evolutionary advantage of FD recombination. Importantly, 'direct-effect free' +FD-strategy was still favored in a remarkable proportion of cases (~46% of cases with zero optimal constant RR, and ~31% of cases with intermediate optimal constant RR), which confirms the conclusion that indirect effect alone can drive the evolution of FD recombination in diploids.

In situations with intermediate optimal constant RR, +FD-strategy was favored much more often (in ~92% of cases) than ±FD-strategy (in ~43% of cases). This intriguing discrepancy reflects the above considered trade-off between the benefits associated with RR plasticity and the costs associated with deviation from the optimal RR. Under purifying selection, most of the population is represented by the wild-type genotype, while mutant genotypes exist at extremely low frequencies (in our simulations, mutant allele frequencies never exceeded 0.2%). Thus, ±FD-strategy which, by definition, decreases RRs in fitter genotypes and increases RRs in less fit ones, inevitably moves the population mean RR strongly downward. This shift appears to be critical, resulting in a relatively modest favoring of ±FD-strategy. Mitigating the 'punishment' imposed upon fitter genotypes immediately improved the picture. In the marginal situation, when FD recombination was presented in the form of +FD-strategy (which does not affect the predominant fitter genotypes at all and only increases RR in less fit ones), its success became much more salient.

The results presented herein do not contradict those previously obtained by Agrawal et al. (2005). In both models, the 'abandon-ship' mechanism alone cannot drive the evolution of FD recombination. At the same time, in the selected two-locus system examined by Agrawal et al. (2005), FD recombination could not manifest its potential benefits, associated with RR plasticity within the selected system, due to the absence of variation in fitness among recombination-responsive genotypes. Our explicit tests with a similar selected system gave the same results.

CONCLUSION

Numerical analysis of the model presented herein with mutation–selection balance suggests, together with our previous models with cyclical selection and Red Queen dynamics, that FD recombination can be evolutionarily advantageous in diploids under various selection regimes. Remarkably, FD recombination can even be favored when any constant non-zero recombination is rejected, including the entire class of situations with no linkage between the recombination modifier and its affected genomic regions. This indicates that non-zero recombination can be favored more often than predicted by a spectrum of classical models with constant RRs (Barton 1995; Otto and Barton 1997, 2001; Otto and Feldman 1997), under much milder constraints on key parameters such as selection intensity, epistasis, population size, modifier linkage, etc. This amazing 'recombination-favoring' potential of FD recombination was first demonstrated by Gessler and Xu (2000) in their haploid selection models, and then extended to a diploid cyclical selection regime (Rybnikov et al. 2017b). Moreover, the same pattern was also shown for the rate of sex, which has long been known to exhibit fitness dependence (for recent review, see Ram and Hadany 2016). Specifically, FD sex seemed to often be favored over asexual reproduction in cases where any constant rate of sex was rejected (Mostowy and Engelstädter 2012).

The evolution of FD recombination in diploids is driven primarily by RR plasticity within the selected system (indirect effect). The 'abandon-ship' mechanism (direct effect) is inefficient in diploids when acting alone. Yet, our results indicate that the direct effect does matter in the presence of an indirect effect. An indirect effect can also drive the evolution of FD recombination in haploids, as was demonstrated in models with unlinked modifier locus (Hadany and Beker 2003b). All of this allows us to suggest that benefits associated with RR plasticity within the selected system represent a more universal mechanism than the 'abandon-ship' benefits. However, it is likely that FD recombination first appeared in haploids. There, it could indeed have been 'invented' by some 'selfish' modifier genes, which spread by exploiting the 'abandon-ship' benefits (Gessler and Xu 2000; Otto 2009). Later, such genes probably expanded this ability to other genome regions, becoming recombination-modifying genes *per se*. Once this happened, FD recombination stopped being entirely dependent on the 'abandon-ship' benefits, and could also evolve in diploids, where the 'abandon-ship' mechanism does not work.

We are aware of the limitations of simulation modeling, and believe that analytical treatment will allow clarifying the underlying mechanisms. Still, we think that the results presented herein, showing that the evolution of FD recombination in diploids is not a dead end problem, warrant further experimental and theoretical studies.

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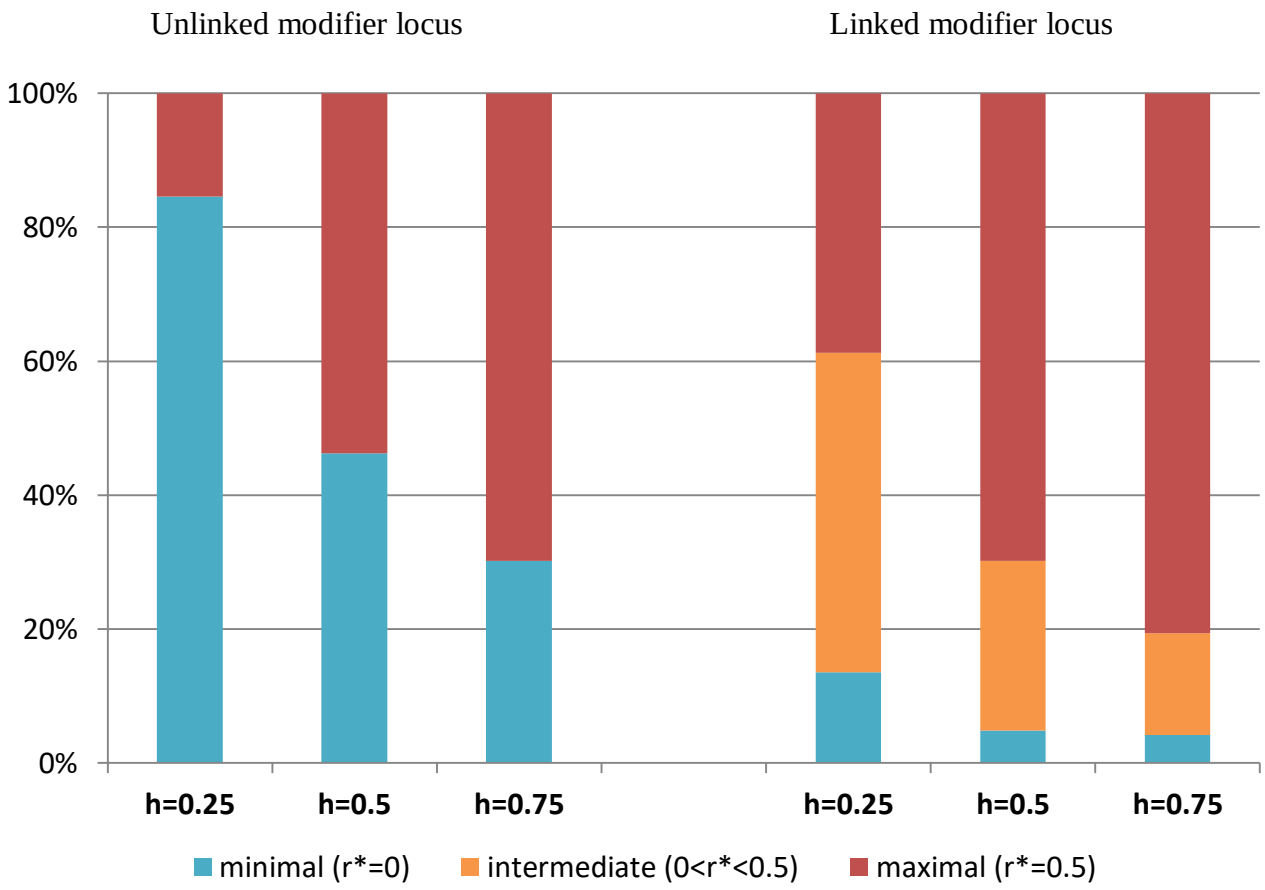

Figure S1. Effect of dominance of deleterious mutations on distribution of cases with respect to optimal constant RR within the selected system.

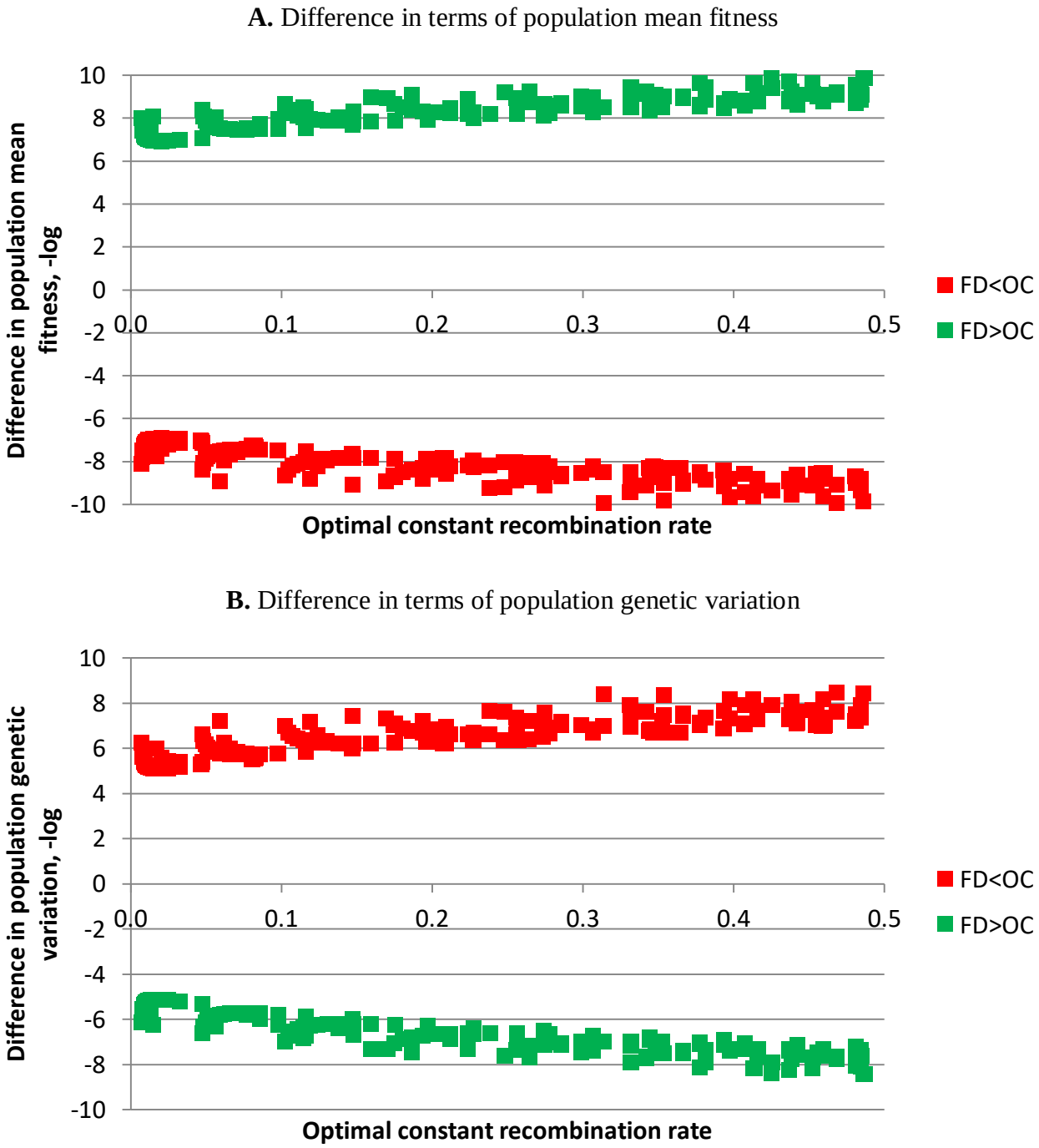

Figure S2. Changes in population mean fitness (A) and population genetic variation (B) in cases with opposite outcomes (favored/non-favored FD recombination).

Values on the ordinate axes represent –log of absolute difference when this difference is positive, and log of absolute difference when this difference is negative. The data refer to cases with intermediate optimal constant RR, when ±FD-strategy is favored (green) and disfavored (red).

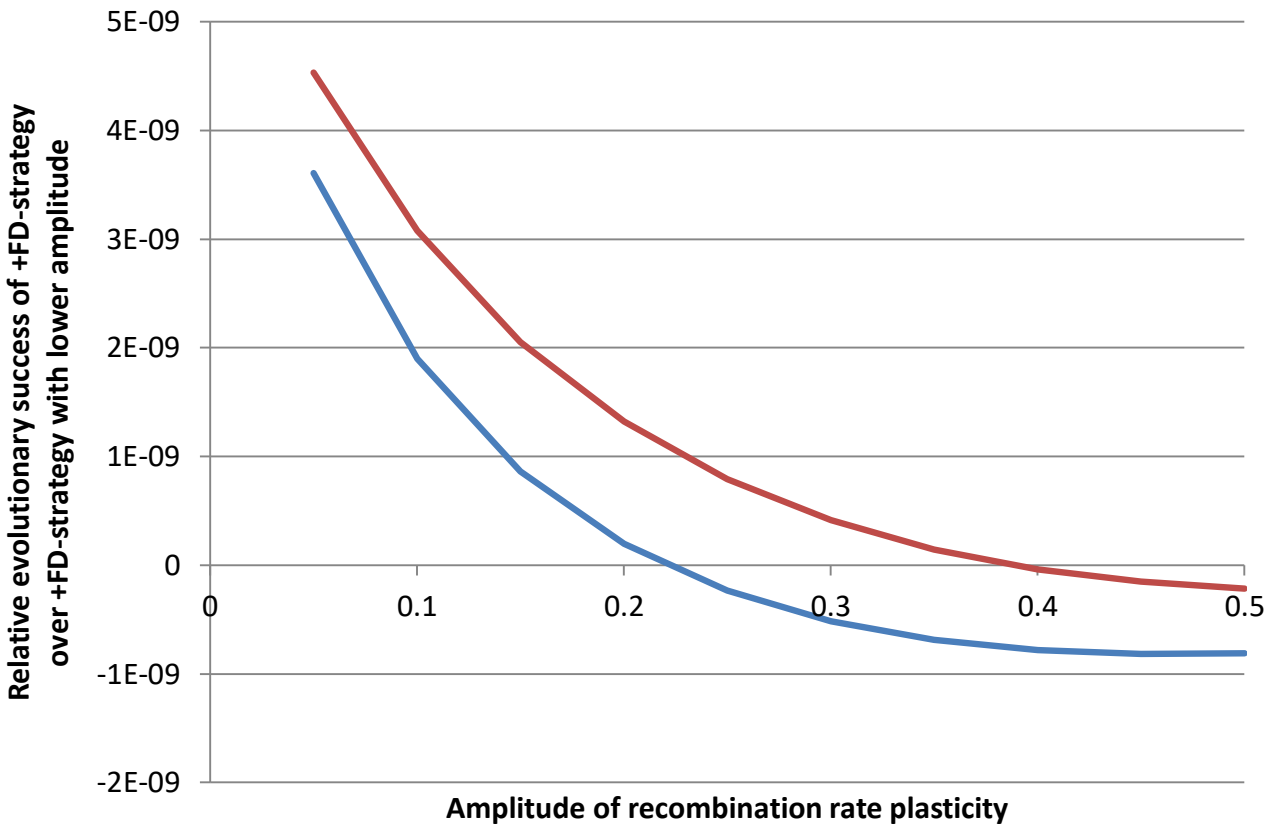

Figure S3. Results of competition between +FD-strategies that differ in amplitude of RR plasticity (for cases with zero optimal constant RR).

The ordinate axis represents the relative evolutionary advantage of +FD-strategy with a given amplitude over +FD-strategy with one step ($\delta = 0.05$) smaller amplitude (the advantage is expressed as the difference between speed of direct invasion and speed of 'backward invasion', per 10,000 generations). Note that below a certain amplitude value, the advantage is positive (i.e., +FD-strategy with such an amplitude is *better* than +FD-strategy with a smaller amplitude), whereas above this value, the advantage becomes negative (i.e., +FD-strategy with such an amplitude is *worse* than +FD-strategy with a smaller amplitude). This critical value of amplitude (~0.22 and ~0.38 for cases depicted by the blue and orange curves, respectively) corresponds to the *optimal* amplitude of RR plasticity.

S4. Interplay between the indirect and direct effects of the modifier

Let us first consider a genetic system with modifier locus (*M*) and two loci (*A* and *B*) subject to selection against deleterious mutations (here, we consider the same model as in the main text, but locus *C* is made selectively neutral). Let r_{AB}^{*} be the optimal constant RR between the selected loci for a given r_{MA}^{0} between the modifier locus and the adjacent selected locus. Consider competition between two modifier alleles, M^1 and M^2 in several situations:

1. <u>Indirect effect alone</u>: Allele M^1 confers $r_{MA}^{1} = r_{MA}^{0}$ and $r_{AB}^{1} = r_{AB}^{*}$. Allele M^2 also confers $r_{MA}^{2} = r_{MA}^{0}$, but $r_{AB}^{2} \neq r_{AB}^{*}$. Expectedly, in this situation, allele M^2 was always rejected, since r_{AB}^{*} is, by definition, the optimal constant RR within the selected system for the given modifier linkage (see cases 1 and 2 in Table S1).
2. <u>Direct effect alone</u>: Allele M^1 confers $r_{MA}^{1} = r_{MA}^{0}$ and $r_{AB}^{1} = r_{AB}^{*}$. Now, allele M^2 confers $r_{AB}^{2} = r_{AB}^{*}$, but $r_{MA}^{2} \neq r_{MA}^{0}$. In this situation, alleles M^1 and M^2 were always neutral (see cases 3 and 4 in Table S1).
3. <u>Indirect and direct effects together</u>: Allele M^1 confers $r_{MA}^{1} = r_{MA}^{0}$ and $r_{AB}^{1} = r_{AB}^{*}$. Now, allele M^2 affects pleiotropically both intervals, conferring $r_{AB}^{2} \neq r_{AB}^{*}$ and $r_{MA}^{2} \neq r_{MA}^{0}$. In this situation, allele M^2 can be favored even despite the non-optimality of r_{AB}^{2}, since r_{AB}^{*}, which is optimal for modifier linkage r_{MA}^{0}, does not necessarily remains optimal for modifier linkage r_{MA}^{2} (in Table S1, in cases 5 and 6 allele M^2 was favored, while in cases 7 and 8 it was still rejected).

Thus, direct effect can matter only in the presence of indirect effect; otherwise, it remains neutral. The above consideration does not specifically assume FD recombination; yet, such assumption did not change the results. Namely, under no indirect effect, modifier alleles still remained neutral, even if one of them caused FD recombination between itself and the selected system. One can expect that indirect effect will favor modifier allele causing FD recombination within the selected system, due to the above discussed benefits of RR plasticity. However, this did not happen in the two-locus selected system, as we explicitly tested both in the current model with mutation–selection balance and in the previous model with cyclical selection. The reason is that in the two-locus selected systems the benefits of RR plasticity cannot be manifested, since there exists only one 'recombination-responsive' genotype (double heterozygote *AaBb*), which means, in fact, no variation in fitness among genotypes where recombination matters. At the same time, costs of deviation from the optimal RR do exist even in two-locus selected systems.

Table S1. Influence of indirect and/or direct effects on the result of competition between modifier alleles (a numerical example)

Effects	Case	Competing modifier alleles				Result of competition	Explanation
		M^1		M^2			
		r^1_{MA}	r^1_{AB}	r^2_{MA}	r^2_{AB}		
Indirect alone	1	0.05	0.068	0.05	0.058	M^2 is rejected	For $r^0_{MA} = 0.05$, $r^*_{AB} = 0.068$
	2	0.05	0.068	0.05	0.078		
Direct alone	3	0.05	0.068	0.04	0.068	M^2 is neutral	If $r^1_{AB} = r^2_{AB}$, then r_{MA} does not matter
	4	0.05	0.068	0.06	0.068		
Indirect and direct together	5	0.05	0.068	0.06	0.058	M^2 is favored	For $r^0_{MA} \neq 0.05$, $r^*_{AB} \neq 0.068$
	6	0.05	0.068	0.04	0.078		
	7	0.05	0.068	0.04	0.058	M^2 is rejected	
	8	0.05	0.068	0.06	0.078		

Now, let us take into consideration the third selected locus (*C*). In this system, contrary to the two-locus selected system, modifier allele causing FD recombination within the selected system can be favored due to the benefits of RR plasticity. Indeed, mean RRs established within the selected system ($\overline{r_{AB}}$ and $\overline{r_{BC}}$) may still deviate from the optimal RRs (r^*_{AB} and r^*_{BC}), but as shown in the main text, the benefits of RR plasticity in such system can overcome these costs. Besides, what is especially important, FD recombination within the three-locus selected systems inevitably creates direct effect on r_{MB}, even if no direct effect on r_{MA} is assumed. This 'hidden' direct effect emerges since r_{MB} depends not only on r_{MA}, but also on r_{AB}, and the latter does vary among genotypes.