

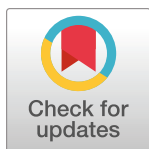
RESEARCH ARTICLE

Conflict over fertilization underlies the transient evolution of reinforcement

Catherine A. Rushworth^{1,2,3}, Alison M. Wardlaw^{1,4}, Jeffrey Ross-Ibarra^{2,5}, Yaniv Brandvain^{1*}

1 Department of Plant and Microbial Biology, University of Minnesota, St. Paul, Minnesota, United States of America, **2** Department of Evolution and Ecology and Center for Population Biology, University of California, Davis, California, United States of America, **3** Department of Biology, Utah State University, Logan, Utah, United States of America, **4** Canada Revenue Agency—Agence du revenu du Canada, Ottawa, Ontario, Canada, **5** Genome Center, University of California, Davis, California, United States of America

* ybrandva@umn.edu



OPEN ACCESS

Citation: Rushworth CA, Wardlaw AM, Ross-Ibarra J, Brandvain Y (2022) Conflict over fertilization underlies the transient evolution of reinforcement. PLoS Biol 20(10): e3001814. <https://doi.org/10.1371/journal.pbio.3001814>

Academic Editor: Michael D. Jennions, The Australian National University, AUSTRALIA

Received: December 9, 2021

Accepted: September 2, 2022

Published: October 13, 2022

Copyright: © 2022 Rushworth et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All code for our simulations are available on <https://github.com/carushworth/gameto-theory> and data summaries of simulation results are available on the dryad submission associated (<https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>) with this manuscript.

Funding: This work was funded by a collaborative National Science Foundation award (#1753632 to YB, #1754098 to JRI). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

When two species meet in secondary contact, the production of low fitness hybrids may be prevented by the adaptive evolution of increased prezygotic isolation, a process known as reinforcement. Theoretical challenges to the evolution of reinforcement are generally cast as a coordination problem, i.e., “how can statistical associations between traits and preferences be maintained in the face of recombination?” However, the evolution of reinforcement also poses a potential conflict between mates. For example, the opportunity costs to hybridization may differ between the sexes or species. This is particularly likely for reinforcement based on postmating prezygotic (PMPZ) incompatibilities, as the ability to fertilize both conspecific and heterospecific eggs is beneficial to male gametes, but heterospecific mating may incur a cost for female gametes. We develop a population genetic model of interspecific conflict over reinforcement inspired by “gametophytic factors”, which act as PMPZ barriers among *Zea mays* subspecies. We demonstrate that this conflict results in the transient evolution of reinforcement—after females adaptively evolve to reject gametes lacking a signal common in conspecific gametes, this gamete signal adaptively introgresses into the other population. Ultimately, the male gamete signal fixes in both species, and isolation returns to pre-reinforcement levels. We interpret geographic patterns of isolation among *Z. mays* subspecies considering these findings and suggest when and how this conflict can be resolved. Our results suggest that sexual conflict over fertilization may pose an understudied obstacle to the evolution of reinforcement.

Introduction

“Once the pollen grain has arrived at the stigma, it has made an irreversible move. There should be very intense selection for it to get around whatever barriers the female may erect.”—Janzen (1977)

Reproductive interactions present “different evolutionary interests of the two sexes” [1]. This sexual conflict (in the general sense, sensu [2]) stems from sex differences in the fitness

Competing interests: The authors have declared that no competing interests exist.

Abbreviations: LD, linkage disequilibrium; PME, pectin methylesterase; PMEI, PME inhibitor; PMPZ, postmating prezygotic.

consequences of mating. One sex (often the male) generally benefits from increasing their mating opportunities, while the other sex benefits from choosing mates and/or limiting mating—resulting in the evolution of more specific forms of sexual conflict (e.g., harmful mating tactics and mating evasion; [3]). Because mating and fertilization play a key role in mediating gene flow between divergent populations, sexual conflict can impact the process of speciation. Species boundaries may either be strengthened if sexual conflict poses a barrier to gene flow, or weakened if populations evolve mating tactics that can overcome heterospecific reproductive barriers [1,4–6].

The cost of producing low-fitness hybrid offspring can favor the evolution of enhanced reproductive isolation by a process known as reinforcement [7]. Reinforcement is generally modeled as the evolution of enhanced prezygotic isolation via female preference and male trait, or trait matching [8,9]. Such models generally include trade-offs between being attractive to conspecifics and heterospecifics (i.e., species evolve preferences for different trait values), and as such both sexes tend to benefit from assorting with conspecifics and avoiding the production of low-fitness hybrids. As such, most reinforcement theory aims to address the logistical challenge of maintaining a genetic association between trait and preference [10], rather than the strategic challenge posed by misaligned interests between the sexes. This is true even in models of polygyny (i.e., when females have multiple mates) because males who match a given female trait/preference are assumed to be less attractive to females with a different trait/preference [8,11]. However, if the trade-off between conspecific and heterospecific is less severe, missing conspecific mating opportunities can come at a cost for one sex (usually males), while for the other sex (usually females) the cost of producing a low-fitness hybrid often outweighs the marginal benefit of additional matings.

The imbalance between sexes in the opportunity costs of heterospecific mating sets the stage for sexual selection to impact the evolution of reinforcement. For example, Servedio and Bürger [12] showed that females with preferences for traits maladapted to their environment can favor males expressing these maladaptive traits, enabling persistence of such traits and thus inhibiting the evolution of reinforcement. Similarly, because a male preference results in more competition for mates than does indiscriminate mating [11], reinforcement by male mate choice is more constrained than reinforcement by female choice [8]. Likewise, Aubier and colleagues [9] found that male preference for conspecifics only evolved if potential male effort toward courting unpreferred females could be reallocated to preferred females. These differences in models of reinforcement by male and female mate choice suggest a conflict in the evolutionary interests of the sexes during the evolution of reinforcement; i.e., the benefit of siring low-fitness hybrids may exceed the opportunity cost for a male but not for a female, presenting an overall benefit only to males.

The sexual conflict over reinforcement described above is likely particularly severe for reinforcement of postmating prezygotic (PMPZ) isolation, because reproductive effort cannot be reallocated to preferred partners after mating has already occurred (Janzen’s “irreversible move”, above [13]). From the male perspective, gametes transferred to heterospecific females cannot be redirected, so universally compatible alleles in male gametes will be favored. Whereas from the female perspective, alleles that discriminate against heterospecific gametes in favor of conspecific gametes will be favored.

Motivated by the genetic basis of PMPZ isolation between hybridizing subspecies of *Zea mays* [14], we develop a population genetic model to evaluate how this sexual conflict over hybridization can alter the evolution of reinforcement. To clarify this process, we model one locally adapted “reinforcing” population in which an initially rare female-expressed fertilization barrier requires a population-specific male signal expressed in the pollen or sperm for effective fertilization, and another “non-reinforcing” population, which lacks this male signal

and is adapted to a different environment. This female barrier can increase in frequency in the reinforcing population, leading to the initial reinforcement of reproductive isolation. However, the male signal then adaptively introgresses across populations. Fixation of the male compatibility allele across the metapopulation renders the benefit of discerning female-expressed alleles neutral, ultimately eroding reinforcement. Notably, we find a similar outcome when two populations have their own unique incompatibilities (i.e., both species are reinforcing but do so by distinct male signals and female barriers), suggesting that this result is attributable to asymmetric costs and benefits experienced by the sexes, and not simply asymmetric cross-compatibility.

Results

Biological inspiration

Gametophytic factors—pairs of tightly linked loci expressed in pollen and styles—underlie the PMPZ barrier [15–19]. Counter to the classic reinforcement prediction that reproductive isolation will be highest in areas of sympatry and reduced in areas of allopatry [20], highland teosinte (*Z. m. subsp. mexicana*) growing in sympatry with domesticated maize landraces (*Z. m. subsp. mays*) shows elevated PMPZ isolation from allopatric maize populations, but no elevation in PMPZ isolation from sympatric maize [21,22]. Our model is inspired by the function of gametophytic factors and their puzzling biogeographic distribution.

Despite this inspiration, our model is neither specific nor fully faithful to the maize/teosinte system. We refer to the reinforcing population/species as *reinf* and the non-reinforcing population/species as *non-reinf*, which roughly represent *Z. m. subsp. mexicana* and *Z. m. subsp. mays*, respectively. Despite being inspired by a hermaphroditic system, we use the terms “male” and “female” to refer to male and female function, or sperm/pollen and female reproductive tract function, respectively.

Model overview

We deterministically iterate migration, gamete fusion, and selection, between two populations in secondary contact. See [S1 Text](#) for full description of this iteration, and <https://github.com/carushworth/gameto-theory> for the R code.

Population structure, migration, and pollination/mating. We model two demes (i.e., a two-island model) in two differing selective environments. We refer to these as populations or species/subspecies, as the populations represent two locally adapted (sub)species in secondary contact. We refer to the selective environment of each population when appropriate. Every generation, $g_{\text{non-reinf} \rightarrow \text{reinf}}$ of sperm/pollen in the *reinf* environment originates from the *non-reinf* population. Similarly, $g_{\text{reinf} \rightarrow \text{non-reinf}}$ sperm/pollen in the *non-reinf* environment originates from the *reinf* population (Fig 1). Within each environment, sperm/pollen and females meet at random.

Fertilization. Although mating/pollination within a deme is random, fertilization is controlled by a two-locus PMPZ incompatibility (*sensu* Lorch and Servedio [23], which is a specific form of a “preference/trait” model, with complete female choosiness [24]). The female-expressed locus $\mathcal{F}$ is under diploid control. We assume the incompatibility is dominant—i.e., females with one or two F alleles discriminate between sperm/pollen alleles, preferentially accepting those with the M allele (Fig 1A) at the male compatibility ($\mathcal{M}$) locus. Fertilization is random for females homozygous for the compatibility allele, f . This notation differs from that in the existing literature on gametophytic factors, which refer to gametophytic factors as haplotypes rather than pairs of genotypes (see [S2 Text](#)).

We initially assume no direct fitness cost to either the female incompatibility F (e.g., there is no preference cost) or the male compatibility M , unless otherwise noted. Finally, we assume

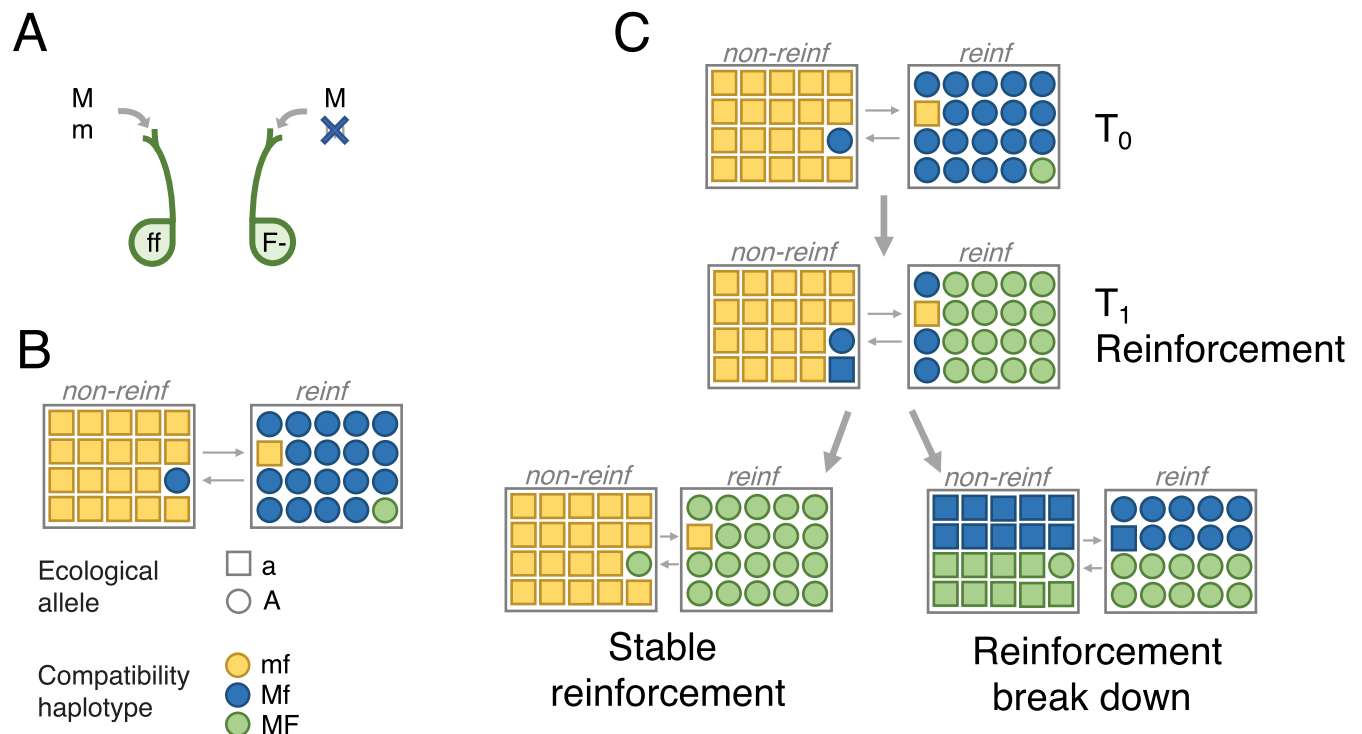


Fig 1. Model dynamics. (A) PMPZ incompatibility based on gametophytic factors. The dominant F allele at the female-expressed locus $\mathcal{F}$ encodes a fertilization barrier that can only be overcome by the male-expressed compatibility allele M at locus $\mathcal{M}$. (B) Male gametes disperse between two populations: one reinforcing (*reinf*) and one non-reinforcing (*non-reinf*), each of which face different selective pressures. Colors denote compatibility haplotypes (*mf*, *Mf*, *MF*), and shapes signify genotypes at the $\mathcal{A}$ locus, which underlies divergent ecological adaptation (a and A). Initially, *non-reinf* is fixed for the compatible f female-expressed allele, the incompatible sperm/pollen-expressed m allele, and a locally adaptive allele (haplotype *mfa*). *reinf* is initially fixed for the sperm/pollen compatibility allele M , and locally adapted allele A , with the female-expressed incompatibility F at an initially low frequency; i.e., haplotype *MfA* is initially common, and *MFA* is initially rare in *reinf*. (C) We run the model from the initial generation T_0 until the F allele reaches its equilibrium. If some reinforcement evolves by time T_1 (equivalent to the F allele increasing in frequency in *reinf*), two further outcomes are possible: The M allele may introgress onto the locally adaptive background and fix in both populations, leading to the breakdown of reinforcement (bottom right panel of C); or, the M allele may fail to spread in *non-reinf* while F continues to spread through *reinf*, completing reinforcement (bottom left panel of C).

<https://doi.org/10.1371/journal.pbio.3001814.g001>

that females expressing the incompatibility genotypes cannot be fertilized by incompatible sperm/pollen. Incomplete penetrance of the barrier results in expected quantitative differences in results but does not change the qualitative outcomes (S2 Fig).

Selection. We model isolation by local adaptation following [25] with extrinsic isolation driven by n local adaptation loci, each denoted as $\mathcal{A}_i$, where the subscript i is an arbitrary index of loci. Our primary analyses focus on the case of one locally adaptive locus (i.e., $n = 1$), an assumption that is relaxed where noted. Selection coefficients are $s_{\text{non-reinf}}$ and s_{reinf} in environments of the non-reinforcing and reinforcing populations, respectively. Fitness w is multiplicative within and among loci, $w = (1 - s_{\text{env}})^{\# \text{maladapted alleles}}$.

Initial allele frequencies. At the female-expressed $\mathcal{F}$ locus, we assume that the incompatibility allele, F , is initially rare in *reinf* (1% frequency) and absent in *non-reinf*.

At the male-expressed $\mathcal{M}$ locus, we assume that the male compatibility allele M is initially fixed in *reinf*, and absent in *non-reinf*, respectively. Variation in the initial frequency of M in *reinf*, however, has nearly no effect on the outcome (S1 Fig).

At the locally adaptive $\mathcal{A}$ locus, we assume that populations are initially fixed for the allele locally adapted to their environment (i.e., allele A is fixed in *reinf* and absent in *non-reinf*, and allele a is fixed in *non-reinf* and absent in *reinf*).

Recombination and genome structure. We initially assume a locus order of $\mathcal{A}_1\mathcal{M}\mathcal{F}$, with recombination rates $r_{\mathcal{A}_1\mathcal{M}}$ and $r_{\mathcal{M}\mathcal{F}}$. Local adaptation loci $\mathcal{A}_2$ through $\mathcal{A}_n$ are unlinked to one another and to the $\mathcal{M}$ and $\mathcal{F}$ loci. After presenting these results, we explore alternative marker orders.

A second gametophytic factor. To ensure that our results are not due to asymmetry of variation for female choice in only one population, we then introduce a model with a second unlinked incompatibility locus: $\mathcal{A}_z\mathcal{M}_z\mathcal{F}_z$. This barrier acts like the first, detailed above, but with initial frequencies in each population reversed. As such, each population is reinforcing and non-reinforcing at a different set of loci.

Sexual conflict leads to transient reinforcement

When reinforcement evolves, it is almost always transient. An example of the rise and fall of reinforcement is shown in Fig 2 (parameter values in legend). Fig 2A shows that the evolution of substantial reinforcement (Phase 1; Fig 2A) is ultimately fleeting. Reinforcement begins as the female incompatibility allele, F , increases in frequency in *reinf* (Fig 2B), preventing fertilization by locally maladapted immigrant haplotypes. This maintains both the high frequency of locally adapted (A) and male compatible (M) alleles in the environment of *non-reinf* (Fig 2C), and large nonrandom statistical association, a.k.a. linkage disequilibrium (hereafter, LD) between them (note the large value of r_{AM}^2 in Fig 2D and 2F).

Subsequently, however, the male compatibility allele M introgresses into *non-reinf*, eventually recombining onto the a background and undermining reinforcement (Phase 2 of Fig 2); i.e., migration of haplotypes from *reinf* into *non-reinf* enables recombination of M onto the locally adapted background. As the M_{fa} haplotype sweeps through *non-reinf* (Fig 2E), LD between M and A decreases in both populations (Fig 2D and 2F).

As M rises in frequency and eventually fixes across the metapopulation (Fig 2B), migrant sperm/pollen are no longer rejected, indicated by reinforcement approaching zero in Fig 2B. At this point, selection against F in *non-reinf* weakens until it is completely neutral (see discussion of Fig 3, below). From then on, F slowly equilibrates across populations (Phase 3 of Fig 2A) as continued migration and recombination between the $\mathcal{A}$ and $\mathcal{F}$ loci decreases LD between them (Fig 2D and 2F).

Allele frequency change across the life cycle

We now show how migration, fertilization, and selection drive changes in allele frequencies across the life cycle (Fig 3). See S3 Text for exact expressions.

Migration homogenizes allele frequencies. The change in allele frequency by migration is the difference in allele frequencies between populations weighted by the migration rate (Eq. S1). This homogenization of allele frequencies (Fig 3A) is seen as the decrease in frequency of all “local alleles” (A , M , and F always decrease in *reinf* and increase in *non-reinf*).

The stylar barrier F favors the male compatibility allele M and indirectly favors alleles in LD with it. The fertilization advantage of M depends on the proportion of incompatible females in the present generation (either heterozygous or homozygous for F). For a dominant female incompatibility, this equals $1 - p_{ff}$ where p_{ff} the frequency of females homozygous for the f allele, differs from p_f^2 due to nonrandom fertilization. The increase in frequency of allele M (derived in Eqs. S2 and S3) from sperm/pollen to paternally derived haplotypes equals

$$\Delta p_{\text{fertilization}} = (1 - p_{ff}) \frac{p'_M p'_m c}{1 - c p'_m} \quad (1)$$

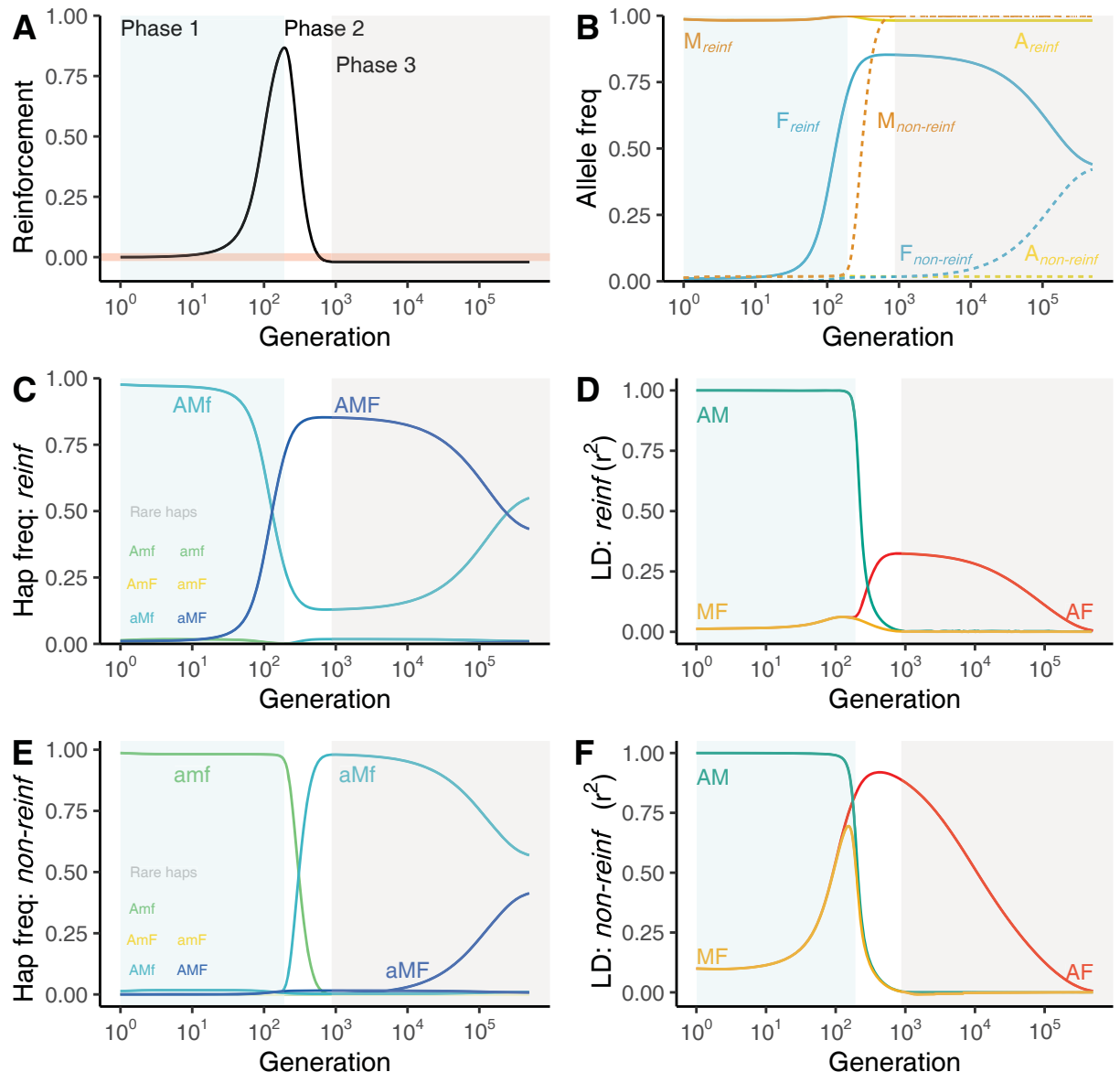


Fig 2. The rise and fall of reinforcement in 3 phases. A female barrier allele *F* preventing fertilization by *m* gametes spreads in the reinforcing population/species *reinf* (Phase 1: light blue). The compatible sperm/pollen allele, *M*, next introgresses into the non-reinforcing population/species *non-reinf* and spreads (Phase 2: white). After *M* fixes, the barrier allele *F* slowly disassociates from the reinforcing background, eventually equilibrating in both populations (Phase 3: light grey). (A) Reinforcement is transient, building in Phase 1 and breaking down in Phase 2. The pink line shows 0 reinforcement. (B) Allele frequencies in both populations, with solid and dashed lines showing frequencies in *reinf* and *non-reinf*, respectively. The *F* allele increases in *reinf* followed by the global fixation of *M* and subsequent neutrality of *F*. (C) Haplotype frequencies and (D) gametic linkage disequilibrium (LD) between all pairs of loci over time in the environment of *reinf*. (E) Haplotype frequencies and (F) gametic LD between all pairs of loci over time in the environment of *non-reinf*. LD is measured as *r*², and all measures describe populations after selection and before recombination. This figure illustrates a single set of parameter values with 1 adaptive locus. Selection: $s_{reinf} = s_{non-reinf} = 0.75$; Migration: $g_{non-reinf \rightarrow reinf} = g_{reinf \rightarrow non-reinf} = 0.1$; Recombination: $r_{AM} = r_{MF} = 0.0001$; Initial allele frequencies: $f_{M0, reinf} = 1, f_{M0, non-reinf} = 0, f_{F0, reinf} = 0.01, f_{F0, non-reinf} = 0$. The data underlying this figure can be found in <https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>.

<https://doi.org/10.1371/journal.pbio.3001814.g002>

where *c* is the intensity of incompatibility (or choosiness), and the superscript ' indicates that allele frequencies in sperm/pollen are taken after migration, while female frequencies lack ' because only sperm/pollen migrate.

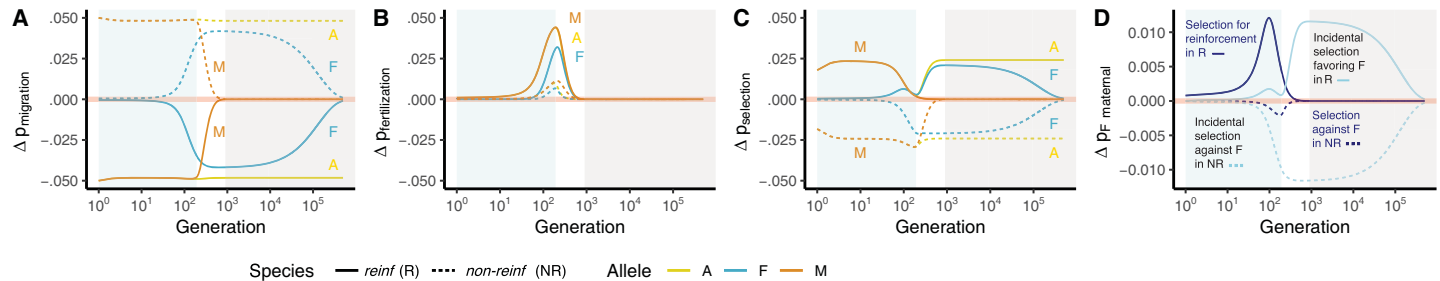


Fig 3. Allele frequency change across the life cycle. The change in frequency of alleles initially unique to *reinf* (A, M, and F) across the metapopulation. The transparent thick pink line at 0 denotes no change in allele frequency during this life phase. (A) During migration, alleles decrease in frequency in *reinf* (solid line, indicated by values below the 0 line) and increase in *non-reinf* (dotted line, indicated by values above the 0 line). (B) During fertilization, alleles native to *reinf* (A, M, and F) increase in both populations. This effect is strongest in the environment of *reinf*, due to a direct fertilization advantage of M and the benefit to alleles in LD with M. (C) Selection in *reinf* and *non-reinf* consistently acts to increase and decrease the frequency of A, respectively. Linkage between A and M results in overlapping lines during Phase 1. The transition from Phase 1 to Phase 2 is marked by a dip in the frequency of A, caused by near-fixation of F on the A background, as migrant haplotypes from *non-reinf* are unable to penetrate *reinf* at F's peak frequency. (D) Selection on F is decomposed into 2 components of allele frequency change. In dark blue, we show "selection for reinforcement" (the F allele frequency change attributable to preferential fusion with M), which enables avoidance of the maladapted allele in *reinf*. In light blue, we show the allele frequency change attributable to the incidental gametic phase linkage between F and A; see [Materials and methods](#) for more detail. **Parameter values:** One local adaptation locus with Selection: $s_{reinf} = s_{non-reinf} = 0.75$; Migration: $g_{non-reinf \rightarrow reinf} = g_{reinf \rightarrow non-reinf} = 0.1$; Recombination: $r_{AM} = r_{MF} = 0.0001$; Initial allele frequencies: $f_{M0, reinf} = 1, f_{M0, non-reinf} = 0, f_{F0, reinf} = 0.01, f_{F0, non-reinf} = 0$. Background shading marks Phase 1 (light blue), Phase 2 (white), and Phase 3 (grey) of transient reinforcement, as in Fig 2. The data underlying this figure can be found in <https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>.

<https://doi.org/10.1371/journal.pbio.3001814.g003>

In line with this result, Fig 3B shows that in both populations, the male compatibility allele, M, increases in frequency during fertilization until it reaches fixation. In addition to directly increasing the frequency of the M allele, selection indirectly favors alleles in LD with it (Eqs. S4 and S5). Because LD among alleles from *reinf* > 0 , the A and F alleles increase in frequency through a fertilization advantage to M in both populations (Fig 3B). This incompatibility system generates a *trans* association between maternally derived F and paternally derived M alleles (Eq. S6; [26]).

Allele frequency change by natural selection follows standard expectations. Selection increases the frequency of the locally adapted allele at locus A in each environment (Fig 3C; Eqs. S7 and S8). Likewise, linked selection on M and F alleles (Fig 3C) reflects LD with the locally adapted alleles (Fig 2D and 2F), with alleles in positive LD with A increasing in frequency in *reinf*, and decreasing in *non-reinf* (Eq. S9).

Selection favors the female incompatibility in the reinforcing population and disfavors it in the non-reinforcing population. The female incompatibility allele, F, which does not itself impact fitness, still deterministically changes in frequency due to its LD with alleles at other loci. This LD is generated by both the causal effect of the allele in mediating nonrandom fertilization, as well as population structure, historical events, etc. We partitioned the extent to which the increase in frequency of the female isolating barrier F is attributable to its causal effect on creating genotypic LD by imposing assortative fertilization (which we call "selection for reinforcement") versus "incidental selection" unrelated to the effect of F on preferential gamete fusion (see [Materials and methods](#) for details). "Selection for (or against) reinforcement" reflects the increase (or decrease) in frequency of F attributable to the *trans* LD it immediately generates with the locally (mal)adapted allele. "Incidental selection" reflects the change in frequency of F attributable to its LD (largely in *cis*) with the locally (mal)adapted allele generated by previous mating and/or historical population structure.

F initially rises in frequency in *reinf* because at the outset it preferentially fuses with sperm/pollen unlikely to contain a locally maladapted allele (Phase 1; Fig 3D). However, F's persistence once M has reached appreciable frequency in *non-reinf* is primarily attributable to incidental selection, in that it preferentially exists on locally adapted haplotypes (Fig 3D).

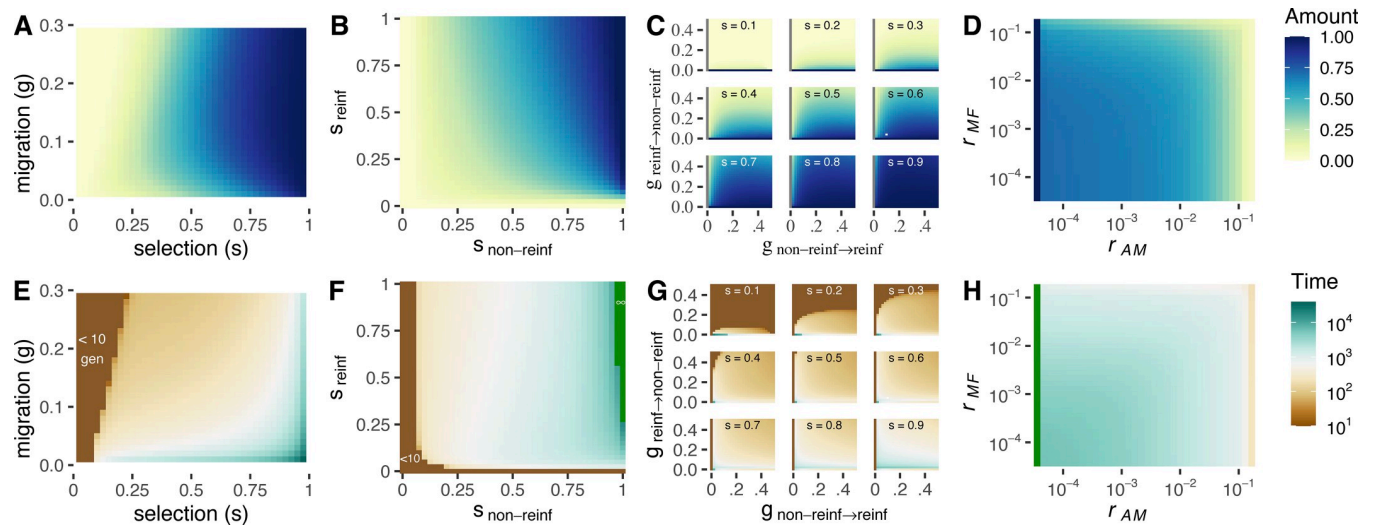


Fig 4. The maximum amount (A–D) and duration (E–H) of reinforcement. Reinforcement as a function of symmetric selection and migration (A and E) with $r_{AM} = r_{MF} = 10^{-4}$, different selection coefficients in reinforcing and non-reinforcing populations' environments (B and F), with $g_{non-reinf \rightarrow reinf} = g_{reinf \rightarrow non-reinf} = 0.03$ and with $r_{AM} = r_{MF} = 10^{-4}$, asymmetric migration rates across numerous selection coefficients (C and G, with $r_{AM} = r_{MF} = 10^{-4}$), and recombination rates (D and H) with a symmetric selection coefficient of 0.8 and $g_{non-reinf \rightarrow reinf} = g_{reinf \rightarrow non-reinf} = 0.03$. Complete or nontransient reinforcement is visible on the far right of figures F and H, indicated by the darkest green color, and the ∞ symbol in F. The amount of reinforcement is quantified as $(p_{[z, gen = x]} - p_{[z, gen = 0]}) / p_{[z, gen = 0]}$, where p_z equals the probability of being fertilized by nonmigrant sperm/pollen, scaled by the frequency of nonmigrant sperm/pollen. Time in generations is represented by "gen". The data underlying this figure can be found in <https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>.

<https://doi.org/10.1371/journal.pbio.3001814.g004>

In *non-reinf*, *F* is disfavored through both selection against this incompatibility (because *F* is preferentially fertilized by *A*-bearing sperm/pollen) and incidental selection acting on the *A* locus (because *F* is in gametic phase LD with the locally maladapted *A* allele). As recombination erodes LD between *M* and *A*, selection weakens against the incompatibility *F* in *non-reinf* due to its effects on nonrandom fertilization. Incidental selection against *F* in *non-reinf* similarly weakens as recombination erodes LD between *F* and *A* (Fig 3D).

Determinants of the strength and duration of reinforcement. We now show how varying parameter values influence the maximum amount (Fig 4A–4D) and duration (Fig 4E–4H) of reinforcement in the face of this conflict.

Reinforcement often requires strong selection. As selection on the locally adaptive allele intensifies, both the maximum extent (Fig 4A) and total duration (Fig 4E) of reinforcement increase. With symmetric selection and symmetric migration, selection on the local adaptation locus must be exceptionally strong for any reinforcement to evolve—e.g., even with $s = 0.3$, only very subtle reinforcement evolves for a very short time. However, other parameter choices—such as asymmetric migration or selection—can mediate the strength of selection required for reinforcement to evolve (see below).

With symmetric migration and asymmetric selection, the strength of selection in *non-reinf* (the population without the stylar incompatibility) generally has a greater effect on the extent and duration of reinforcement than does the strength of selection in *reinf* (Figs 4B and 4F and S3). This is because strong selection in *non-reinf* removes the migrant *MA* haplotype, minimizing opportunities for *M* to recombine onto the locally adapted *a* background.

The extent and symmetry of migration mediates reinforcement. With symmetric migration, intermediate migration rates always maximize the extent of reinforcement (Figs 4A and S3A), while the duration of reinforcement decreases with the migration rate (Figs 4E and S3B), regardless of the selection coefficient.

The effect of asymmetric migration on the extent of reinforcement highlights how migration mediates this sexual conflict. Migration from *non-reinf* to *reinf* favors reinforcement by increasing the number of maladapted immigrants available for heterospecific matings (Fig 4C). By contrast, increasing migration from *reinf* to *non-reinf* accelerates the introgression of the *M* allele into *non-reinf*, especially at higher migration rates, rapidly undermining reinforcement (Fig 4G). With unidirectional migration from *non-reinf* to *reinf*, substantial reinforcement can persist for prolonged time periods (Fig 4C and 4G).

Linkage between female barrier and male (in)compatibility alleles does not strongly impact the amount or duration of reinforcement. Contrary to classic results of reinforcement theory [10], linkage between the male and female (in)compatibility alleles, r_{MF} , has only a modest effect on the evolution of reinforcement. This result is seen across most selection coefficients and most values of r_{AM} (Fig 4D and 4H; reproduced in S5A and S5D Fig). Reordering the loci in the model does not alter this outcome—i.e., the extent and duration of reinforcement is largely insensitive to r_{MF} in models with loci in $\mathcal{MFA}$ order (S5B and S5E Fig).

Instead, linkage between the local adaptation locus, $\mathcal{A}$, and either $\mathcal{M}$ or $\mathcal{F}$ loci are critical to the evolution of reinforcement. Marker order $\mathcal{MAF}$ highlights the impact of recombination between the components of the PMPZ incompatibility complex on both the duration and intensity of reinforcement (S5C and S5F Fig). While both r_{AM} and r_{FA} modulate the level of reinforcement (S5C Fig), the duration of reinforcement is independent of r_{FA} (S5E Fig), and nearly completely determined by recombination between the male compatibility $\mathcal{M}$ and local adaptation locus $\mathcal{A}$, r_{AM} . When $\mathcal{A}$ and $\mathcal{M}$ are tightly linked, substantial reinforcement can evolve and last for some time. The strength and duration of reinforcement drops, initially modestly, and then quite precipitously, as the recombination rate increases, with nearly no reinforcement evolving when $\mathcal{A}$ and $\mathcal{M}$ are unlinked (Fig 4D and 4H). Selection modulates this effect of recombination (S4 Fig); when selection is very strong (e.g., $s > 0.6$) some reinforcement can evolve, even when $\mathcal{A}$ and $\mathcal{M}$ are separated by up to a centiMorgan (i.e., $r_{AM} = 0.01$).

This result suggests that the rate of recombination between the local adaptation and male compatibility loci, r_{AM} , underlies the sexual conflict over reinforcement. When r_{AM} is high, meaning $\mathcal{A}$ and $\mathcal{M}$ are loosely linked, *M* can more easily recombine onto the locally adapted *a* background, which facilitates its introgression into *non-reinf*. By escaping from the *A* background, *M* has greater long-term viability in *non-reinf* than it would if it remained associated with this locally maladaptive allele, increasing the male benefit to overcoming the incompatibility.

The presence of multiple unlinked local adaptation loci allows for (transient) reinforcement. Our results so far suggest that transient reinforcement by PMPZ incompatibilities requires tight linkage between loci underlying incompatibility and a single locus under divergent selection. However, the genetic architecture of local adaptation is often polygenic [27].

We therefore investigate if weaker selection at more unlinked loci can allow reinforcement to transiently evolve by setting r_{A_1M} to 0.5 and introducing up to 4 additional unlinked local adaptation $\mathcal{A}$ loci. Fig 5 shows that reinforcement can evolve when alternate alleles at numerous unlinked loci experience divergent selection in the 2 populations. This is consistent with recent work showing that, when numerous loci underlie reproductive isolation, selection on early-generation hybrids acts not against isolated loci, but on phenotypes underlain by pedigree relationships [28]. While the selection coefficients displayed are still quite large, this suggests that weaker selection at many loci could likely result in the transient evolution of reinforcement.

An opposing gametophytic factor does not stabilize reinforcement. To explore the possibility that the collapse of reinforcement could be prevented by the presence of distinct incompatibilities expressed in each population, we included a model in which both

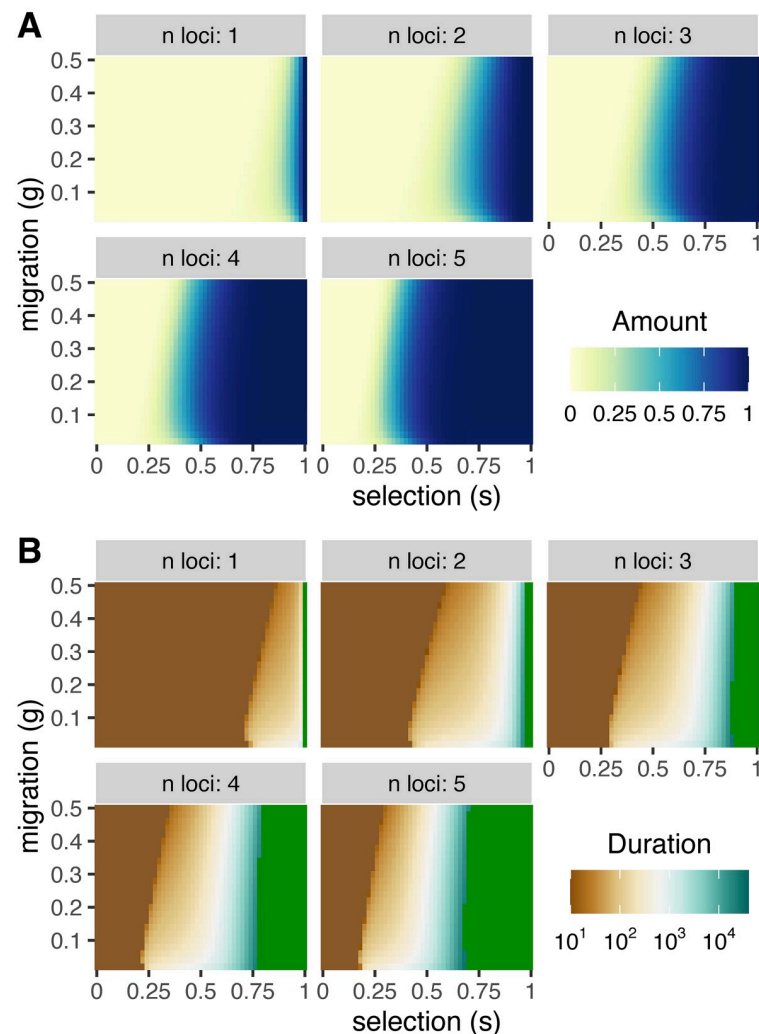


Fig 5. Oligogenic ecological selection. The maximum strength (A) and duration (B) of reinforcement with ecological selection at n loci, where all ecologically selected loci are unlinked to one another and to the gametophytic factor. The selection coefficient s against a maladaptive allele is multiplicative within and among loci—e.g., the fitness of an individual homozygous for the locally maladaptive allele at all n loci is $(1-s)^{2n}$. Migration rate g is symmetric and recombination rate $r_{MF} = 10^{-4}$. The data underlying this figure can be found in <https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>.

<https://doi.org/10.1371/journal.pbio.3001814.g005>

populations are “reinforcing” but by an independent set of loci. Although a second complementary incompatibility allows reinforcement to begin at lower selection intensities and slightly expands the parameter space for which reinforcement stably evolves (S6A Fig), reinforcement usually remains transient (S6B Fig).

A large cost of the male compatibility allele can stabilize reinforcement. We next ask how a cost to the male compatibility allele impacts the evolution of reinforcement; ignoring the question of how such a costly allele could have spread in *reinf*. For illustrative purposes, we limit our focus to exploration of parameters presented in Fig 2 and described in its legend.

Assigning a cost s_M to the M allele can lead to one of three qualitatively different outcomes (S7 Fig). If its cost is sufficiently small ($s_M < 0.01$ in our example), M rapidly and adaptively

introgresses and undermines reinforcement as shown above. A larger cost to M ($0.01 < s_M < 0.08$) results in an equilibrium level of reinforcement (i.e., partial reproductive isolation [29]) wherein the cost of M is balanced by a benefit of heterospecific fertilization to M -bearing pollen/sperm. An even larger cost to M ($s_M > 0.08$ in our example) prevents the introgression of this allele altogether, resulting in stable reinforcement. In sum, a sufficiently large cost to the male compatibility allele can stabilize the evolution of reinforcement, but a small cost does not.

Discussion

For decades, researchers have presented theoretical and empirical challenges to the process of reinforcement, starting with a foundational paper by Felsenstein that identified recombination as a critical challenge to reinforcement [10]. Since then, a large body of theory has investigated the circumstances that permit or hinder the evolution of reinforcement (reviewed in [30]). Despite its potential role in hampering speciation [1], however, sexual conflict over hybridization has received relatively little attention in the literature. When it is mentioned, sexual conflict over hybridization is included only as a brief aside in papers concerning the role of sexual conflict in speciation more broadly [1,5,6].

Here, we identify the transient dynamics generated by sexual conflict over reinforcement and the evolutionary traces it leaves behind—namely the adaptive spread of female barriers in one species/population and the adaptive introgression of male compatibility alleles into the other. These results provide a rich set of predictions and interpretations of empirical patterns that were absent from previous game theoretic [1,5] and verbal [31] models.

In our model, sexual selection favors sperm/pollen traits that overcome a heterospecific female barrier. This poses a conflict: Females are selected to avoid the production of maladapted hybrids, while sperm (or pollen) that increase their fertilization success will generally be favored. The breakdown of reproductive isolation is marked by the rapid adaptive introgression of the male compatibility trait into *non-reinf*, following recombination onto the locally adapted haplotype. Back-migration of this allele into *reinf* hastens its fixation across populations. The final step in the model is the slow homogenization of the female barrier allele across demes, as the male compatibility allele fixes in both, rendering the female choice allele ineffective. Such homogenization of female preference does not occur when reinforcement is stable, and the male trait does not spread across both populations. Ultimately, we show that barriers acting at different stages of hybridization can affect how reinforcement proceeds. Below, we discuss the relationship of our results to previous theory, implications for the process of reinforcement, and empirical implications for hybridizing taxa, including *Z. mays* subspecies.

Theoretical context and predictions for reinforcement

Previous models of reinforcement treated the sexes interchangeably [10] or assumed assortative mating under female control [32–34] (but see [8,9,35] for models with male choice) either by “matching” or “preference/trait” mechanisms of assortative mating [24]. Both matching and preference/trait models induce a trade-off between heterospecific and conspecific mating: a male with a trait favored by heterospecific females will have limited mating success with conspecific females.

While numerous studies have addressed the role of introgression in reinforcement (e.g., [36–40]), a distinguishing feature of our model is the mechanism of nonrandom fertilization, which we model as a PMPZ incompatibility functioning as a gametophytic factor in *Z. mays* [41] and PMPZ barriers in broadcast spawners (e.g., [42]). In this special class of preference/trait model, introgression of a male compatibility allele is facilitated by the fact that it does not

prevent heterospecific mating—i.e., by definition, our model lacks a trade-off between con- and heterospecific mating (i.e., no reallocation *sensu*; [9]). As such, (in)compatibility-type mating interactions can result in the transient evolution of reinforcement, while other matching or preference/trait models cannot.

Implications for reinforcement by pre- versus postmating barriers

Our model assumes that being attractive to one species does not come at a cost of being attractive to one's own species. How does this assumption likely map onto cases of reinforcement by pre- and postmating isolation mechanisms?

Postmating prezygotic isolation. To the extent that PMPZ barriers function similarly to those in our study, our model suggests transient reinforcement by PMPZ barriers. However, physical and/or biochemical properties of PMPZ interactions may minimize the opportunity for interspecific sexual conflict by enforcing a trade-off between overcoming a heterospecific barrier and successfully fertilizing conspecifics. This would allow for the evolutionary stability of reinforcement, consistent with our results from our model that showed that a sufficiently large cost to pollen/sperm compatibility could stabilize reinforcement. For example, as Howard [43] argued, and Lorch and Servedio [23] showed, a preference for conspecific sperm can stably evolve to minimize heterospecific fertilization. Thus, conspecific sperm precedence in competitive fertilization likely allows stable reinforcement by PMPZ isolation (as shown by, e.g., [44]). Likewise, mechanistic features of noncompetitive fertilization can also induce a trade-off between inter- and intraspecific crossing success. For example, if pollen must grow an optimal distance to fertilize an ovule (as observed in interspecific *Nicotiana* crosses) [45], success on both hetero- and conspecific styles is impossible.

Premating isolation. In principle, our model could apply to the reinforcement of premating isolation, as well as postmating isolation, so long as there is no trade-off in attractiveness to each species. There are numerous examples of premating traits that may increase heterospecific reproductive success without trading off conspecific attractiveness. For example, divergence in male competitive ability between two lineages of the common wall lizard (*Podarcis muralis*) results in asymmetric hybridization [46,47]. Likewise, loci underlying plumage traits appear to be asymmetrical and adaptively introgress from one to another subspecies of the red-backed fairywren, *Malurus melanocephalus* [48], presumably because this trait confers an advantage in extra-pair copulation due to sensory bias [49]. In a classic example of this phenomenon, plumage of the golden-collared manakin *Manacus vitellinus* appears to adaptively introgress into *Manacus candei* upon secondary contact [50], and this spread is likely mediated by female choice [51]. However, it does not appear that the female preference in any of these cases initially arose as a mechanism of reinforcement.

Sexual conflict and sexual selection undermine reinforcement

Our model shows that the common phenomenon of sexual conflict (see [3] for examples), wherein male and female interests are misaligned, can erode reinforcement by PMPZ incompatibilities. This role for sexual conflict in removing species boundaries runs counter to the conventional role it is thought to play in speciation [1]. Previous theory [52] and experiments [53], as well as natural patterns of reproductive isolation [54,55] and diversification rates [56] suggest that independent coevolutionary arms races between male and female traits in two incipient species can pleiotropically result in behavioral or mechanical isolation. In this manner, intraspecific sexual conflict was thought to be an “engine of speciation” [57]. By contrast, we show that interspecific conflict between the sexes over fertilization hampers speciation. This highlights an underappreciated challenge to reinforcement by PMPZ barriers. Broadly,

our results align with studies suggesting that incompatibilities, especially those with a transmission advantage [58], can adaptively introgress across species boundaries [59].

Servedio and Bürger [12] found that Fisherian sexual selection can undermine reinforcement. Specifically, they found that migration of female preference alleles provides a mating advantage to otherwise locally maladaptive heterospecific male traits by sexual selection, undermining reinforcement. In contrast, our model shows that the benefit of siring low fitness hybrids, when the alternative is missed fertilization opportunities that result in no offspring, can also undermine the evolution of reinforcement. Counter to Servedio and Bürger, we find that the female incompatibility remains at low frequency in the non-reinforcing species for a long time. As such, our models make contrasting predictions: Servedio and Bürger's model predicts that alternative female preferences will coexist in both populations early in the evolutionary process, while our model predicts that female preference for the "wrong" species signal will only become common in both populations very late in the process of genetic exchange.

Our model can be seen as a specific instance of the lek paradox [60], as female preference ultimately erodes variation for a male trait. Following previously proposed resolutions of the lek paradox (e.g., [61]), Proulx [62] suggested that a female preference for an indicator of paternal fitness (e.g., sperm competitiveness) could act as a "one allele" mechanism of reinforcement (sensu; [10]). Under our model of sexual conflict, however, the lek paradox is ultimately unresolved. Rather, our model results in a male trait fixed in both species, which does not ultimately aid in assortative mating but is a mark of the "ghost of reinforcement past".

Empirical implications, predictions, and interpretation of current observations

Our model, based on a well-characterized PMPZ incompatibility, shows that reinforcement by such mechanisms is precarious. As such, to the extent that such barriers do not incur a trade-off between conspecific and heterospecific fertilization success, we predict that reinforcement by PMPZ barriers should be rare. Thus, the finding that gametic isolation in broadcast spawners is not the product of reinforcement [63], as well as meta-analyses showing that PMPZ isolation does not differ between sympatric and allopatric species pairs in *Drosophila* [64] or across 3 angiosperm genera [65], are consistent with our model. Still, negative evidence is not necessarily evidence for the negative.

As such, the few documented cases in which PMPZ barriers are reinforced allow for better evaluation of our predictions. Specifically, we predict that reinforcement by PMPZ barriers should often involve certain characteristics, which are consistent with the empirical literature. These include the following: (1) recent sympatry, so that the male barrier has not yet increased in frequency (e.g., [66]); (2) a trade-off between male success in overcoming inter- and intra-specific postmating barriers, as is found in preference/trait mechanisms (e.g., [44]); (3) unidirectional gene flow (e.g., [67]); and/or (4) exceptionally strong postzygotic isolation, such that gene flow is very rare, as seen in *Drosophila yakuba* and *Drosophila santomea* (e.g., [64]). However, reinforcement by PMPZ isolation in *D. yakuba* and *D. santomea* is difficult to reconcile with our model, as the pair have a stable hybrid zone, no evidence of conspecific sperm precedence, and bidirectional hybridization [68]. Nonetheless, our model suggests a plausible evolutionary mechanism for existing cases of reinforcement by PMPZ isolation and generates specific hypotheses to be tested.

Predictions for maize and teosinte

Zea mays subsp. *mays* and *Zea mays* subsp. *mexicana* grow in close sympatry and hybridize [69,70]. Evidence for genome-wide selection against admixture, despite adaptive introgression

of some teosinte loci into maize [71], is consistent with the idea that hybrids are often disfavored—perhaps because hybrids are removed from maize fields by anthropogenic weeding, and maize traits expressed in teosinte environments are likely maladaptive [72,73] (although clear cases of adaptive introgression and deliberate hybridization by farmers exist).

This system has all the ingredients necessary for reinforcement—the occurrence of gene flow, the presence of a stylar incompatibility in teosinte sympatric with maize, and the reduced but nonzero fitness of hybrids. However, the elevated pollen discrimination exhibited by highland teosinte sympatric with maize [15–18] is surprisingly ineffective in preventing fertilization by sympatric maize landraces [21,22], against whom selection for reinforcement should be strongest.

Our model explains this observation as the initial evolution of reinforcement (i.e., a stylar barrier sweeps through teosinte) followed by the adaptive introgression of teosinte pollen compatibility alleles into maize. Notably, alternative explanations for this pattern are insufficient. For example, this pattern is not simply attributable to the loss of isolation upon secondary contact, because allopatric teosinte do not reject maize pollen [21,22]. Nor can this be explained by complex speciation, in which teosinte sympatric with maize would be more recently diverged from maize than are allopatric teosinte, as this is incompatible with both genetic evidence and the history of maize domestication [74]. We suggest that in most sympatric populations, at most gametophytic factors, the stylar fertilization barrier (the *F* allele) rapidly swept through teosinte (Phase 1 in Fig 2), and the pollen compatibility allele (*M*) adaptively introgressed into sympatric highland maize landraces (Phase 2 in Fig 2).

Caveats

Our model made many simplifications and abstractions for tractability and generality. Most notably, we assumed only two populations, a single gametophytic factor, and a simple multiplicative fitness function across a small number of divergently selected loci. Our results show that the architecture of adaptive differentiation and the linkage between locally adaptive alleles and PMPZ incompatibilities modulate the rise and fall of reinforcement. Across taxa, adaptive differentiation can be controlled by few [75–77] or many [78] loci, and linkage between locally adaptive alleles and PMPZ incompatibilities is biologically variable and rarely known. In maize, evidence is mixed—one gametophytic factor *tcb1* is tightly linked to a domestication locus *su1* [79,80], which likely experiences divergent selection. However, two other known gametophytic factors are far from loci under strong divergent selection.

We further assume that the male compatibility allele is initially common and stylar incompatibility is initially rare in *reinf*, and we do not address the origins of gametophytic factors. While the initial divergence of these alleles is outside the scope of our model, it could be explained by pleiotropy, selection to prevent polyspermy (a known risk to embryo viability in maize) [81], or Fisherian runaway selection (as proposed for gametophytic factors by Jain) [82]. Pleiotropy may explain the initial evolution of gametophytic factors in *Z. mays*, as they are members of the multifunction pectin methylesterase (PME) and PME inhibitor (PMEI) gene families [15–17,83] and could be favored by mechanisms related to other functions. Notably, a subclass of PMEs contain both PME and PMEI domains, providing a potential explanation for tight linkage of the *M* and *F* alleles [84].

Conclusions

We find that considering the role of sexual conflict—a mismatch between optimal fertilization outcomes of each sex—in reinforcement generates novel predictions and may explain numerous patterns in nature. Our results are particularly relevant to potential cases of reinforcement

by gamete recognition in plants, as well as broadcast spawners (e.g., Lysin/VERL in abalone [85] or Bindin/EBR1 in sea urchins [86]), and even to cases of internal fertilization in which premating isolation is inefficient, costly, or otherwise unpreferred [87]. In these situations, we predict that reinforcement by PMPZ will be rare, transient, or involve a trade-off between heterospecific and conspecific fertilization (i.e., some mechanism of reallocation). Finally, although our model is developed specifically for interactions between haploid male gametes and diploid females, similar dynamics could arise for premating barriers with a similar genetic architecture and lacking reallocation of male reproductive effort.

Materials and methods

Quantifying reinforcement and its duration

We summarized our results by quantifying the duration and maximum extent of reinforcement. We quantified the amount of reinforcement at generation g as $(p_{[z, \text{gen}=g]} - p_{[z, \text{gen}=0]}) / p_{[z, \text{gen}=0]}$. Where p_z equals the probability of being fertilized by nonmigrant sperm/pollen, scaled by the frequency of nonmigrant sperm/pollen. We quantified the duration of reinforcement as the number of generations for which the amount of reinforcement was greater than 0.05.

Partitioning selection

All selection for or against the female incompatibility allele, F , is indirect, as it does not itself impact fitness; i.e., selection impacts the frequency of an allele at the $\mathcal{F}$ locus, not because of its effect on fitness, but because of its genetic background (i.e., linkage disequilibrium between F and A). Each generation, some of the LD between F and A is immediately attributable to either (a) population structure, historical events, etc. (primarily by *cis*-LD), which we call “incidental selection” or (b) to the causal effect of the F allele in generating genetic associations by the gametes permitting fertilization (primarily via *trans*-LD; see subsection The generation of trans linkage disequilibrium during fertilization in the [Mathematical Appendix S3](#)), which we call “selection for reinforcement”. See [24] for a discussion of how LD in *cis* and *trans* contribute to Fisherian sexual selection. We developed this new terminology because “linked selection” and “indirect selection” are insufficient in distinguishing these causal forces.

Thus, the change in frequency in F is attributable to both its circumstance (“incidental selection”) and its causal effect on generating a nonrandom association (“selection for reinforcement”). We aim to partition total selection for (or against) the F allele into incidental selection (unrelated to the effect of F on nonrandom fertilization, $\Delta p_{F, \text{Incidental}}$) and selection for reinforcement (the causal effect of F on its selective trajectory, $\Delta p_{F, \text{Reinforcement}}$). In this exercise, we ignore the change in frequency of paternally derived genotypes (which includes migration, fertilization, and selection), as none of this change is plausibly attributable to the F allele. We include the subscript *mat* with each variable to remind readers we are focused on the maternally derived F alleles.

We first compute the difference in allele frequency between maternally derived haplotypes in offspring after versus before selection as $\Delta p_{F, \text{mat}}$ directly from our results: $\Delta p_{F, \text{mat}} = p_{F, \text{mat-derived after sel}} - p_{F, \text{mat-derived before sel}}$. We then decompose $\Delta p_{F, \text{mat}}$ into components of reinforcing and incidental selection:

$$\Delta p_{F, \text{mat}} = \Delta p_{F, \text{mat, Reinforcement}} + \Delta p_{F, \text{mat, Incidental}} \quad (2)$$

Each generation, we find $\Delta p_{F,\text{mat, Incidental}}$ by calculating $\Delta p_{F,\text{mat}}$ under the counterfactual case of random fertilization. We then find the change in frequency of F by selection for reinforcement $\Delta p_{F,\text{mat, Reinforcement}}$ by rearranging Eq 2.

Computer code. All code is written in R [88] and is available at <https://github.com/carushworth/gameto-theory>. We generated figures with the ggplot2 and cowplot packages [89,90] and used the dplyr package to process numeric results [91].

Dryad DOI

[10.5061/dryad.rjdfn2zf8](https://doi.org/10.5061/dryad.rjdfn2zf8) [92]

Supporting information

S1 Text. Pseudocode for our iteration. A detailed description of our model.
(DOCX)

S2 Text. Traditional notation for gametophytic factors. Our model is inspired by gametophytic factors in that underlie PMPZ barriers between *Zea mays* subspecies. We connect our model to the traditional terminology that describes gametophytic factors.
(DOCX)

S3 Text. Mathematical appendix. We derive key analytical results from our model.
(DOCX)

S1 Fig. The initial frequency of M in the reinforcing species does not influence qualitative results. The impact of variability in the initial frequency of sperm/pollen compatibility allele, M , in *reinf*, on the transient reinforcement of postmating prezygotic isolation. All lines overlap. Parameter values: Selection — $s_{\text{reinf}} = s_{\text{non-reinf}} = 0.75$. Migration — ($g_{\text{non-reinf} \rightarrow \text{reinf}} = g_{\text{reinf} \rightarrow \text{non-reinf}} = 0.1$). Recombination — $r_{\mathcal{AM}} = r_{\mathcal{MF}} = 0.0001$. Allele frequencies — $f_{M0,\text{reinf}} = 0.01$, $f_{M0,\text{non-reinf}} = 0$, $f_{F0,\text{reinf}} = 0.01$, $f_{F0,\text{non-reinf}} = 0$. The data underlying this figure can be found in <https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>.
(EPS)

S2 Fig. Female choosiness alters strength of reinforcement. We allow for an imperfect barrier (i.e., variation in female choice) by allowing females with fertilization barrier genotypes to be fertilized by a given haplotype, k , with probability $x_k = \frac{p_k(1-\delta_k c)}{\sum x_k}$, where p_k is the frequency of haplotype k in pollen after fertilization. δ_k equals 0 for compatible sperm/pollen grains and 1 for incompatible sperm/pollen grains. c , the efficacy of the barrier, is colored in the plot above. Parameter values: Selection — $s_{\text{reinf}} = s_{\text{non-reinf}} = 0.75$. Migration — ($g_{\text{non-reinf} \rightarrow \text{reinf}} = g_{\text{reinf} \rightarrow \text{non-reinf}} = 0.1$). Recombination — $r_{\mathcal{AM}} = r_{\mathcal{MF}} = 0.0001$. Allele frequencies — $f_{M0,\text{reinf}} = 1$, $f_{M0,\text{non-reinf}} = 0$, $f_{F0,\text{reinf}} = 0.01$, $f_{F0,\text{non-reinf}} = 0$. The data underlying this figure can be found in <https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>.
(EPS)

S3 Fig. The impact of asymmetric selection on the extent (A) and duration (B) of reinforcement. Reinforcement strength and duration are estimated over a range of symmetric migration rates with $r_{\mathcal{AM}} = r_{\mathcal{MF}} = 10^{-4}$. The data underlying this figure can be found in <https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>.
(EPS)

S4 Fig. The impact of linkage on the extent (A) and duration (B) of reinforcement. Reinforcement strength and duration are estimated over a range of symmetric selection

coefficients, $g_{\text{non-reinf} \rightarrow \text{reinf}} = g_{\text{reinf} \rightarrow \text{non-reinf}} = 0.03$. The data underlying this figure can be found in <https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>. (EPS)

S5 Fig. Locus order impacts the amount and duration of reinforcement. Top row (A–C) is reinforcement amount; bottom row (D–F) is duration, as estimated under different marker orders. Default marker order is $\mathcal{AMF}$: amount (A); duration (D). Marker order $\mathcal{MFA}$: amount (B); duration (E). Marker order $\mathcal{MAF}$: amount (C); duration (F). Shown are results with a symmetric selection coefficient of 0.8 and migration $g_{\text{non-reinf} \rightarrow \text{reinf}} = g_{\text{reinf} \rightarrow \text{non-reinf}} = 0.01$. The data underlying this figure can be found in <https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>. (EPS)

S6 Fig. Asymmetrical variation in female preference does not underlie transience of reinforcement. Incorporating a second gametophytic factor in the *non-reinf* does not qualitatively change the amount (A) or duration of reinforcement (B), although reinforcement begins at lower intensities of selection and reaches completion across more selection coefficients. The data underlying this figure can be found in <https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>. (EPS)

S7 Fig. Introducing a cost (s_M) to the male compatibility allele impacts the evolution of conflict over reinforcement. (A) The frequency of the M allele in *non-reinf* over time as a function of the additive cost of the allele (s_M noted by color, with several values labeled for clarity). The frequency of the M allele in *non-reinf* (B) and the extent of reinforcement (C) after 10,000 generations (taken to be the equilibrium value, as evidenced by S7A Fig). Results are plotted on a $\log_{10}$ scale for clarity, though numbers are nontransformed. Dashed grey lines in (B) and (C) note the values (0.0125 and 0.08) at which we transition from transient reinforcement to a polymorphic equilibrium, and then to complete and stable reinforcement, respectively. All measures describe populations after selection and before recombination. This figure illustrates a single set of parameter values with 1 adaptive locus. Selection: $s_{\text{reinf}} = s_{\text{non-reinf}} = 0.75$; Migration: $g_{\text{non-reinf} \rightarrow \text{reinf}} = g_{\text{reinf} \rightarrow \text{non-reinf}} = 0.1$; Recombination: $r_{\mathcal{AM}} = r_{\mathcal{MF}} = 0.0001$; Allele frequencies: $f_{M0,\text{reinf}} = 1$, $f_{M0,\text{non-reinf}} = 0$, $f_{F0,\text{reinf}} = 0.01$, $f_{F0,\text{non-reinf}} = 0$. The data underlying this figure can be found in <https://datadryad.org/stash/dataset/doi:10.5061/dryad.rjdfn2zf8>. (EPS)

Acknowledgments

We are grateful to Robin Hopkins and Maria Servedio, whose comments greatly improved the manuscript. We also thank Jerry Kermicle for helpful conversation. We would like to acknowledge Felix Andrews for the smorgasbord of statistical advice, although we did not follow it.

Author Contributions

Conceptualization: Alison M. Wardlaw, Jeffrey Ross-Ibarra, Yaniv Brandvain.

Data curation: Catherine A. Rushworth, Yaniv Brandvain.

Formal analysis: Yaniv Brandvain.

Funding acquisition: Jeffrey Ross-Ibarra, Yaniv Brandvain.

Investigation: Catherine A. Rushworth, Alison M. Wardlaw, Yaniv Brandvain.

Methodology: Yaniv Brandvain.

Project administration: Yaniv Brandvain.

Supervision: Jeffrey Ross-Ibarra, Yaniv Brandvain.

Visualization: Catherine A. Rushworth, Yaniv Brandvain.

Writing – original draft: Catherine A. Rushworth, Alison M. Wardlaw, Yaniv Brandvain.

Writing – review & editing: Catherine A. Rushworth, Jeffrey Ross-Ibarra, Yaniv Brandvain.

References

1. Parker GA, Partridge L. Sexual Conflict and Speciation. *Philos Trans R Soc B Biol Sci.* 1998; 353:261–274. <https://doi.org/10.1098/rstb.1998.0208> PMID: 9533125
2. Parker GA. Sexual Conflict over Mating and Fertilization: An Overview. *Philos Trans R Soc B Biol Sci.* 2006; 361:235–259. <https://doi.org/10.1098/rstb.2005.1785> PMID: 16612884
3. Arnqvist G, Rowe L. Sexual Conflict. Princeton: Princeton University Press; 2005.
4. Parker GA. Sexual Selection and Sexual Conflict. In: Blum MS, Blum NA, editors. *Sexual Selection and Reproductive Competition in Insects.* Cambridge: Academic Press; 1979. p. 123–166.
5. Gavrillets S, Hayashi T. Speciation and Sexual Conflict. *Evol Ecol.* 2005; 19:167–198.
6. Gavrillets S. Is Sexual Conflict an "Engine of Speciation"? *Cold Spring Harb Perspect Biol.* 2014; 6: a017723. <https://doi.org/10.1101/cshperspect.a017723> PMID: 25395295
7. Dobzhansky T. *Genetics and the Origin of Species.* New York: Columbia University Press; 1937.
8. Servedio MR. Male Versus Female Mate Choice: Sexual Selection and the Evolution of Species Recognition via Reinforcement. *Evolution.* 2007; 61:2772–2789. <https://doi.org/10.1111/j.1558-5646.2007.00247.x> PMID: 17924955
9. Aubier TG, Kokko H, Joron M. Coevolution of Male and Female Mate Choice Can Destabilize Reproductive Isolation. *Nat Comm.* 2019; 10(1):5122. <https://doi.org/10.1038/s41467-019-12860-9> PMID: 31719522
10. Felsenstein J. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution.* 1981; 35:124–138. <https://doi.org/10.1111/j.1558-5646.1981.tb04864.x> PMID: 28563447
11. Servedio MR. Population Genetic Models of Male and Mutual Mate Choice. *Evolution.* 2006; 2006(60):674–685. PMID: 16739450
12. Servedio MR, Bürger R. The Counterintuitive Role of Sexual Selection in Species Maintenance and Speciation. *Proc Natl Acad Sci U S A.* 2014; 2014(111):8113–8118. <https://doi.org/10.1073/pnas.1316484111> PMID: 24821767
13. Janzen DH. A Note on Optimal Mate Selection by Plants. *Am Nat.* 1977; 111:365–371.
14. Mangelsdorf PC, Jones DF. The Expression of Mendelian Factors in the Gametophyte of Maize. *Genetics.* 1926; 11:423–455. <https://doi.org/10.1093/genetics/11.5.423> PMID: 17246465
15. Moran Lauter AN, Muszynski MG, Huffman RD, Scott MP. A Pectin Methylesterase ZmPme3 Is Expressed in Gametophyte factor1-s (Ga1-s) Silks and Maps to that Locus in Maize (*Zea mays* L.). *Front Plant Sci.* 2017; 1926:8.
16. Lu Y, Kermicle JL, Evans MMS. Genetic and Cellular Analysis of Cross-Incompatibility in *Zea mays*. *Plant Reprod.* 2014; 27:19–29.
17. Lu Y, Hokin SA, Kermicle JL, Hartwig T, Evans MMS. A pistil-expressed pectin methylesterase confers cross-incompatibility between strains of *Zea mays*. *Nat Comm.* 2019; 10:2304.
18. Wang M, Chen Z, Zhang H, Chen H, Gao X. Transcriptome Analysis Provides Insight into the Molecular Mechanisms Underlying Gametophyte Factor 2-Mediated Cross-Incompatibility in Maize. *Int J Mol Sci.* 2018; 19(6):1757. <https://doi.org/10.3390/ijms19061757> PMID: 29899298
19. Chen Z, Zhang Z, Zhang H, Li K, Cai D, Zhao L, et al. A pair of non-Mendelian genes at the *Ga2* locus confer unilateral cross-incompatibility in maize. *Nat Comm.* 2022; 13:1993.
20. Coyne JA, Orr HA. Patterns of Speciation in *Drosophila*. *Evolution.* 1989, 1989; 43(2):362–381.
21. Kermicle J, Taba S, Evans MMS. The Gametophyte-1 Locus and Reproductive Isolation Among *Zea mays* subspecies. *Maydica.* 2006; 51:219–225.

22. Kermicle J, Evans MMS. The *Zea mays* Sexual Compatibility Gene *Ga2*: Naturally Occurring Alleles, Their Distribution, and Role in Reproductive Isolation. *J Hered*. 2010; 101:737–749.
23. Lorch PD, Servedio MR. The Evolution of Conspecific Gamete Precedence and Its Effect on Reinforcement. *J Evol Biol*. 2007; 20:937–949. <https://doi.org/10.1111/j.1420-9101.2007.01306.x> PMID: 17465905
24. Kopp M, Servedio MR, Mendelson TC, Safran RJ, Rodr RL, Hauber ME, et al. Mechanisms of Assortative Mating in Speciation with Gene Flow: Connecting Theory and Empirical Research. *Am Nat*. 2018; 191:1–20. <https://doi.org/10.1086/694889> PMID: 29244561
25. Kirkpatrick M. Reinforcement During Ecological Speciation. *Proc R Soc B Biol Sci*. 2001; 268:1259–1263. <https://doi.org/10.1098/rspb.2000.1427> PMID: 11410152
26. Veller C, Muralidhar P, Haig D. On the Logic of Fisherian Sexual Selection. *Evolution*. 2020; 74:1234–1245. <https://doi.org/10.1111/evo.13944> PMID: 32128812
27. Barghi N, Hermisson J, Schlötterer C. Polygenic Adaptation: A Unifying Framework to Understand Positive Selection. *Nat Rev Genet*. 2020; 21:769–781. <https://doi.org/10.1038/s41576-020-0250-z> PMID: 32601318
28. Veller C, Edelman NB, Muralidhar P, Nowak MA. Recombination, variance in genetic relatedness, and selection against introgressed DNA. *BioRxiv* [Preprint]. 2019 bioRxiv 846147 [posted 2019 Nov 18; revised 2021 Sep 4]: [31 p.]. Available from: <https://www.biorxiv.org/content/10.1101/846147v2>. <https://doi.org/10.1101/846147>
29. Servedio MR, Hermisson J. The Evolution of Partial Reproductive Isolation as an Adaptive Optimum. *Evolution*. 2020; 2020(74):4–14. <https://doi.org/10.1111/evo.13880> PMID: 31721186
30. Kirkpatrick M, Ravigné V. Speciation by Natural and Sexual Selection: Models and Experiments. *Am Nat*. 2002; 202(2):159(Suppl 3):S22–S35. <https://doi.org/10.1086/338370> PMID: 18707367
31. Coyne JA, Orr HA. Speciation. Sunderland: Sinauer; 2004.
32. Lande R. Models of Speciation by Sexual Selection on Polygenic Traits. *Proc Natl Acad Sci U S A*. 1981; 78:3721–3725. <https://doi.org/10.1073/pnas.78.6.3721> PMID: 16593036
33. Kelly JK, Noor MA. Speciation by Reinforcement: A Model Derived from Studies of *Drosophila*. *Genetics*. 1996; 143:1485–1497.
34. Servedio MR, Kirkpatrick M. The Effects of Gene Flow on Reinforcement. *Evolution*. 1997; 51:1764–1772. <https://doi.org/10.1111/j.1558-5646.1997.tb05100.x> PMID: 28565111
35. Spencer HG, McArdle BH, Lambert DM. A Theoretical Investigation of Speciation by Reinforcement. *Am Nat*. 1986; 128:241–262.
36. Sanderson N. Can Gene Flow Prevent Reinforcement? *Evolution*. 1989; 1989(43):1223–1235. <https://doi.org/10.1111/j.1558-5646.1989.tb02570.x> PMID: 28564502
37. Liou LW, Price TD. Speciation by Reinforcement of Premating Isolation. *Evolution*. 1994; 48:1451–1459. <https://doi.org/10.1111/j.1558-5646.1994.tb02187.x> PMID: 28568419
38. Kirkpatrick M, Servedio MR. The Reinforcement of Mating Preferences on an Island. *Genetics*. 1999; 151:865–884. <https://doi.org/10.1093/genetics/151.2.865> PMID: 9927476
39. Servedio MR. Reinforcement and the Genetics of Nonrandom Mating. *Evolution*. 2000; 54:21–29. <https://doi.org/10.1111/j.0014-3820.2000.tb00003.x> PMID: 10937179
40. Matute DR. Reinforcement can overcome gene flow during speciation in *Drosophila*. *Curr Biol*. 2010; 20:2229–2233.
41. Kermicle JL. A Selfish Gene Governing Pollen-Pistil Compatibility Confers Reproductive Isolation Between Maize Relatives. *Genetics*. 2006; 172:499–506. <https://doi.org/10.1534/genetics.105.048645> PMID: 16157680
42. Lessios H. Reproductive Isolation Between Species of Sea Urchins. *Bull Mar Sci*. 2007; 81:191–208.
43. Howard DJ. Reinforcement: Origin, Dynamics, and Fate of an Evolutionary Hypothesis. In: Harrison RG, editor. *Hybrid Zones and the Evolutionary Process*. Oxford: Oxford University Press; 1993. p. 46–69.
44. Castillo DM, Moyle LC. Conspecific sperm precedence is reinforced, but postcopulatory sexual selection weakened, in sympatric populations of *Drosophila*. *Proc R Soc B Biol Sci*. 2019; 286:20182535.
45. Lee CB, Page LE, McClure BA, Holtsford TP. Post-pollination hybridization barriers in *Nicotiana* section *Alatae*. *Sex Plant Reprod*. 2008; 21:183–195.
46. While GM, Michaelides S, Heathcote RJP, MacGregor HEA, Zajac N, Beninde J, et al. Sexual Selection Drives Asymmetric Introgression in Wall Lizards. *Ecol Lett*. 2015; 18:1366–1375. <https://doi.org/10.1111/ele.12531> PMID: 26468006

47. MacGregor HEA, While GM, Barrett J, P G, Carazo P, Michaelides S, et al. Experimental Contact Zones Reveal Causes and Targets of Sexual Selection in Hybridizing Lizards. *Funct Ecol*. 2017; 31:742–752.
48. Baldassarre DT, White TA, Karubian J, Webster MS. Genomic and morphological analysis of a semi-permeable avian hybrid zone suggests asymmetrical introgression of a sexual signal. *Evolution*. 2014; 68:2644–2657. <https://doi.org/10.1111/evo.12457> PMID: 24889818
49. Baldassarre DT, Webster MS. Experimental Evidence That Extra-Pair Mating Drives Asymmetrical Introgression of a Sexual Trait. *Proc R Soc B Biol Sci*. 2013; 280:20132175. <https://doi.org/10.1098/rspb.2013.2175> PMID: 24089341
50. Parsons TJ, Olson SL, Braun MJ. Unidirectional Spread of Secondary Sexual Plumage Traits Across an Avian Hybrid Zone. *Science*. 1993; 260:1643–1646. <https://doi.org/10.1126/science.260.5114.1643> PMID: 17810207
51. Stein AC, Uy JAC. Unidirectional introgression of a sexually selected trait across an avian hybrid zone: A role for female choice? *Evolution*. 2006; 60(7):1476–1485. PMID: 16929664
52. Gavrilits S, Waxman D. Sympatric Speciation by Sexual Conflict. *Proc Natl Acad Sci U S A*. 2002; 99:10533–10538. <https://doi.org/10.1073/pnas.152011499> PMID: 12149438
53. Palopoli MF, Peden C, Woo C, Akiha K, Ary M, Cruze L, et al. Natural and experimental evolution of sexual conflict within *Caenorhabditis* nematodes. *BMC Evol Biol*. 2015; 15:93.
54. Brandvain YJ, Haig D. Divergent Mating Systems and Parental Conflict as a Barrier to Hybridization in Flowering Plants. *Am Nat*. 2005; 166:330–338. <https://doi.org/10.1086/432036> PMID: 16224688
55. Ting JJ, Woodruff GC, Leung G, Shin N-R, Cutter AD, Haag ES. Intense Sperm-Mediated Sexual Conflict Promotes Reproductive Isolation in *Caenorhabditis* Nematodes. *PLoS Biol*. 2014; 12(7):e1001915. <https://doi.org/10.1371/journal.pbio.1001915> PMID: 25072732
56. Arnqvist G, Edvardsson M, Friberg U, Nilsson T. Sexual Conflict Promotes Speciation in Insects. *Proc Natl Acad Sci U S A*. 2000; 97(19):10460–10464. <https://doi.org/10.1073/pnas.97.19.10460> PMID: 10984538
57. Rice WR, Linder JE, Friberg U, Lew TA, Morrow EH, Stewart AD. Inter-Locus Antagonistic Coevolution as an Engine of Speciation: Assessment with Hemiclinal Analysis. *Proc Natl Acad Sci U S A*. 2005; 102 (Suppl 1):6527–6534. <https://doi.org/10.1073/pnas.0501889102> PMID: 15851669
58. Meiklejohn CD, Landeen EL, Gordon KE, Rzatkiwicz T, Kingan SB, Geneva AJ, et al. Gene Flow Mediates the Role of Sex Chromosome Meiotic Drive During Complex Speciation. *eLife*. 2018; 7 (e35468). <https://doi.org/10.7554/eLife.35468> PMID: 30543325
59. Bank C, Bürger R, Hermisson J. The limits to parapatric speciation: Dobzhansky-Muller incompatibilities in a continent-island model. *Genetics*. 2012; 191:845–863. <https://doi.org/10.1534/genetics.111.137513> PMID: 22542972
60. Borgia G. Sexual Selection and the Evolution of Mating Systems. In: Blum MS, Blum NA, editors. *Sexual Selection and Reproductive Competition in Insects*. Cambridge: Academic Press; 1979. p. 19–80.
61. Rowe L, Houle D. The Lek Paradox and the Capture of Genetic Variance by Condition Dependent Traits. *Proc R Soc B Biol Sci*. 1996; 263:1415–1421.
62. Proulx SR. Female Choice via Indicator Traits Easily Evolves in the Face of Recombination and Migration. *Evolution*. 2001; 55:2401–2411. <https://doi.org/10.1111/j.0014-3820.2001.tb00755.x> PMID: 11831656
63. Geyer L, Palumbi S. Reproductive Character Displacement and the Genetics of Gamete Recognition in Tropical Sea Urchins. *Evolution*. 2003; 57:1049–1060. <https://doi.org/10.1111/j.0014-3820.2003.tb00315.x> PMID: 12836822
64. Turissini DA, Matute DR. Fine scale mapping of genomic introgressions within the *Drosophila yakuba* clade. *PLoS Genet*. 2017; 13(9):e1006971. <https://doi.org/10.1371/journal.pgen.1006971> PMID: 28873409
65. Moyle LC, Olson MS, Tiffin P. Patterns of Reproductive Isolation in Three Angiosperm Genera. *Evolution*. 2004; 58:1195–1208. <https://doi.org/10.1111/j.0014-3820.2004.tb01700.x> PMID: 15266970
66. Poikela N, Kinnunen J, Wurdack M, Kauranen H, Schmitt HT, Kankare M, et al. Strength of Sexual and Postmating Prezygotic Barriers Varies Between Sympatric Populations with Different Histories and Species Abundances. *Evolution*. 2019; 73:1182–1199. <https://doi.org/10.1111/evo.13732> PMID: 30957216
67. Kay KM. Reproductive Isolation Between Two Closely Related Hummingbird-Pollinated Neotropical Gingers. *Evolution*. 2006; 60:538–552. PMID: 16637499
68. Matute DR. Reinforcement of Gametic Isolation in *Drosophila*. *PLoS Biol*. 2010; 8(3):e1000341. <https://doi.org/10.1371/journal.pbio.1000341> PMID: 20351771

69. Hufford MB, Lubinsky P, Pyhäjärvi T, Devengenzo MT, Ellstrand NC, Ross-Ibarra J. The Genomic Signature of Crop-Wild Introgression in Maize. *PLoS Genet.* 2013; 9(5):e1003477. <https://doi.org/10.1371/journal.pgen.1003477> PMID: 23671421
70. Hufford MB, Lubinsky P, Pyhäjärvi T, Devengenzo MT, Ellstrand NC, Ross-Ibarra J. Correction: The Genomic Signature of Crop-Wild Introgression in Maize. *PLoS Genet.* 2013; 9(9). Available from: <https://doi.org/10.1371/annotation/2eef7b5b-29b2-412f-8472-8fd7f9bd65ab>
71. Calfee E, Gates D, Lorant A, Perkins MT, Coop G, Ross-Ibarra J. Selective Sorting of Ancestral Introgression in Maize and Teosinte Along an Elevational Cline. *PLoS Genet.* 2021; 17(10):e1009810. <https://doi.org/10.1371/journal.pgen.1009810> PMID: 34634032
72. Wilkes HG. Hybridization of Maize and Teosinte in Mexico and Guatemala and the Improvement of Maize. *Econ Bot.* 1977; 31:254–293.
73. Hufford MB, Xu X, Van Heerwaarden J, Pyhäjärvi T, Chia J-M, Cartwright RA, et al. Comparative Population Genomics of Maize Domestication and Improvement. *Nat Genet.* 2012; 44(7):808–811. <https://doi.org/10.1038/ng.2309> PMID: 22660546
74. Ross-Ibarra J, Tenaillon MI, Gaut BS. Historical Divergence and Gene Flow in the Genus *Zea*. *Genetics.* 2009; 181:1399–1413. <https://doi.org/10.1534/genetics.108.097238> PMID: 19153259
75. Selby JP, Willis JH. Major QTL controls adaptation to serpentine soils in *Mimulus guttatus*. *Mol Ecol.* 2018; 27:5073–5087.
76. Lowry DB, Willis JH. A Widespread Chromosomal Inversion Polymorphism Contributes to a Major Life-History Transition, Local Adaptation, and Reproductive Isolation. *PLoS Biol.* 2010; 8(9):e1000500. <https://doi.org/10.1371/journal.pbio.1000500> PMID: 20927411
77. Lowry DB, Willis JH. Correction: A Widespread Chromosomal Inversion Polymorphism Contributes to a Major Life-History Transition, Local Adaptation, and Reproductive Isolation. *PLoS Biol.* 2012; 10(1): <https://doi.org/10.1371/annotation/caa1b7dd-9b6d-44db-b6ce-666954903625>
78. Yeaman S. Genomic Rearrangements and the Evolution of Clusters of Locally Adaptive Loci. *Proc Natl Acad Sci U S A.* 2013; 110:E1743–E1751. <https://doi.org/10.1073/pnas.1219381110> PMID: 23610436
79. Wang H, Nussbaum-Wagler T, Li B, Zhao Q, Vigouroux Y, Faller M, et al. The Origin of the Naked Grains of Maize. *Nature.* 2005; 436:714–719. <https://doi.org/10.1038/nature03863> PMID: 16079849
80. Whitt SR, Wilson LM, Tenaillon MI, Gaut BS, Buckler ES. Genetic Diversity and Selection in the Maize Starch Pathway. *Proc Natl Acad Sci U S A.* 2002; 99:12959–12962. <https://doi.org/10.1073/pnas.2024769999> PMID: 12244216
81. Grossniklaus U. Polyspermy Produces Tri-Parental Seeds in Maize. *Curr Biol.* 2017; 27(24):R1300–R1302. <https://doi.org/10.1016/j.cub.2017.10.059> PMID: 29257958
82. Jain SK. Population Dynamics of a Gametophytic Factor Controlling Selective Fertilization. *Genetica.* 1967; 38:485–503.
83. Lu Y, Moran-Lauter AN, Makkena S, Scott MP, Evans MMS. Insights into the molecular control of cross-incompatibility in *Zea mays*. *Plant Reprod.* 2020; 33:117–128.
84. Tian G-W, Chen M-H, Zaltsman A, Citovsky V. Pollen-Specific Pectin Methylesterase Involved in Pollen Tube Growth. *Dev Biol.* 2006; 294:83–91. <https://doi.org/10.1016/j.ydbio.2006.02.026> PMID: 16564517
85. Swanson WJ, Vacquier VD. Concerted Evolution in an Egg Receptor for a Rapidly Evolving Abalone Sperm Protein. *Science.* 1998; 281:710–712. <https://doi.org/10.1126/science.281.5377.710> PMID: 9685267
86. Metz EC, Kane RE, Yanagimachi H, Palumbi SR. Fertilization Between Closely Related Sea Urchins Is Blocked by Incompatibilities During Sperm-Egg Attachment and Early Stages of Fusion. *Biol Bull.* 1994; 187:23–34. <https://doi.org/10.2307/1542162> PMID: 29281311
87. Turissini DA, McGirr JA, Patel SS, David JR, Matute DR. The rate of evolution of postmating-prezygotic reproductive isolation in *Drosophila*. *Mol Biol Evol.* 2017; 35:312–334.
88. R Core Team. R: A Language and Environment for Statistical Computing; 2020. Available from: <https://www.R-project.org/>.
89. Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag; 2016. ISBN. Available from: <https://ggplot2.tidyverse.org>.
90. Wilke CO. Cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'; 2020. Available from: <https://CRAN.R-project.org/package=cowplot>.
91. Wickham H, François R, Henry L, Müller K. dplyr: A Grammar of Data Manipulation; 2020. Available from: <https://CRAN.R-project.org/package=dplyr>.
92. Rushworth CA, Wardlaw AM, Ross-Ibarra J, Brandvain Y. Conflict over fertilization underlies the transient evolution of reinforcement. *Dryad Repos.* 2022. <https://doi.org/10.5061/dryad.rjdfn2zf8>