### The evolution of conspecific gamete precedence and its effect on reinforcement

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### Abstract

Conspecific gamete precedence, the usage of conspecific sperm by a female that mates with both a conspecific and a heterospecific male, has been found in many taxa. We construct a population genetic model to examine the evolution of conspecific gamete precedence and its coevolution with premating isolation in the process of reinforcement. Our findings suggest that conspecific gamete precedence can evolve via a process very similar to reinforcement. We explore the nature of the selection against hybridization necessary to drive this evolution. Moreover, our results confirm the prediction of Marshall *et al.* (*Trends Ecol. Evol.* 2002;17:558–563) that conspecific gamete precedence will inhibit the evolution of reinforcement between two species. We further find that reinforcement will inhibit the evolution of conspecific gamete precedence. Both reinforcement and conspecific gamete precedence increase reproductive isolation and contribute to the process of speciation. We discuss factors that may affect which of these phenomena are likely to become predominant between incipient species.

### Introduction

The buildup of biological isolating mechanisms is an essential precursor to the coexistence of sister species in sympatry. These isolating mechanisms are classified by the stage of the life cycle in which they occur, either as premating, postmating-prezygotic, or postzygotic barriers. When sympatry is a consequence of secondary contact, speciation between partially isolated populations can proceed by reinforcement or the evolution of premating isolating mechanisms as a consequence of selection against hybridization. Selection against hybridization can occur either through the low fitness of hybrids (a postzygotic mechanism, e.g. Dobzhansky, 1937, 1940; Howard, 1993), or through lower fertility, or higher mortality of males or females that mate with heterospecifics (a postmatingprezygotic mechanism; Servedio, 2001; Servedio & Noor, 2003). There has been an increasing amount of empirical evidence for reinforcement accumulating over the past decade (e.g. Noor, 1995; Sætre et al., 1997; Rundle & Schluter, 1998; Nosil et al., 2003; see Coyne & Orr, 2004).

While most work on reinforcement to date has focused on premating isolation (e.g. mate choice) and postzygotic isolation (selection against hybrids), the role of postmating-prezygotic mechanisms is less well studied. One particularly interesting form of postmating-prezygotic isolation is conspecific gamete precedence, defined as the disproportional use of conspecific sperm or pollen by a female that has mated with both a conspecific and a heterospecific male. The phenomenon of conspecific gamete precedence has been gaining attention as evidence for it accumulates (Howard, 1999). Cases of conspecific gamete precedence have been found primarily in insects (such as flour beetles: e.g. Wade et al., 1993; Robinson et al., 1994, Drosophila: e.g. Price, 1997; Chang, 2004, flightless grasshoppers: Hewitt et al., 1989; Ritchie et al., 1989; Butlin, 1998; ladybird beetles: Nakano, 1985, and striped-ground crickets: e.g. Howard et al., 1998) and in freespawning marine invertebrates (such as sea urchins: e.g. Lillie, 1921; Palumbi & Metz, 1991; Metz et al., 1994; Geyer & Palumbi, 2005, and abalone: e.g. Vacquier et al., 1990; Kresge et al., 2000). Conspecific gamete precedence can also occur in plants (conspecific pollen precedence, e.g. Rieseberg et al., 1995; Carney et al., 1996).

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Marshall et al. (2002) point out that most studies of reinforcement, both empirical and theoretical, ignore the phenomenon of conspecific gamete precedence. Conspecific gamete precedence, however, may affect the process of reinforcement in two ways. First, conspecific gamete precedence itself has been hypothesized to evolve through a reinforcement-like process, where postmating-prezygotic isolation (as opposed to premating isolation) is driven by the presence of postzygotic isolation. This has been primarily supported by indirect evidence in freespawning marine invertebrates (in which gamete interactions occur at the post-spawning but prezygotic stage) where sperm-egg binding proteins are more modified when competition between conspecific and heterospecific sperm occurs (Lee et al., 1995; Metz & Palumbi, 1996; Metz et al., 1998; Gever & Palumbi, 2003; see Howard, 1999). Secondly, Marshall et al. (2002) suggested that the presence of conspecific gamete precedence may in turn negate the need for strong premating barriers; when conspecific sperm fertilize most of the female's eggs, female cost of mating with a heterospecific is reduced or eliminated. Indeed Marshall et al. (2002) showed in a preliminary comparative analysis that sympatric species pairs with significant conspecific gamete precedence tend to have very low premating isolation. They discuss, however, the need for additional data to be gathered to allow a more rigorous test of this hypothesis. Their suggested effect of conspecific gamete precedence on reinforcement had also not been explored theoretically.

Conspecific gamete precedence can potentially occur through three non-exclusive biological mechanisms. First, it can occur through male-male competition via interactions between male seminal components, mediated in part by the female (Price, 1997; Price et al., 2000). Secondly, it can arise, in the form of partial or complete incompatibility between sperm or seminal fluid of one species and the egg or female reproductive tract of another species, as a byproduct of divergent evolution of populations in allopatry (Chang, 2004; Fricke & Arnqvist, 2004). This non-adaptive form of conspecific gamete precedence will result in low fertility of a female that happens to mate only with heterospecifics. Finally, it can potentially arise as a form of cryptic female choice; females may effectively 'choose' the sperm of conspecifics over heterospecifics (see Eberhard, 1996). Regardless of how conspecific gamete precedence evolves, its presence may remove pressure on populations to evolve premating isolation, thus inhibiting reinforcement from occurring (as suggested by Marshall et al., 2002).

In this paper, we create a coevolutionary model of conspecific gamete precedence and reinforcement. We choose to limit the model to reinforcement via female choosiness, recognizing that while conspecific gamete precedence is expected to reduce the cost of heterospecific matings for females, it does not reduce such costs for males and may actually lead to increased male choosiness (Marshall *et al.*, 2002). Because we have chosen to model reinforcement using the most commonly considered form of premating isolation, female choice, our model does not apply directly to broadcast spawners, where premating isolation, if it occurs, is through characteristics such as spawning time [to parallel animals with internal fertilization, gamete interactions are best considered as postmating (post-spawning) but prezygotic interactions]. We expect that the model, which applies generally to internally fertilizing animals will be most relevant in insects where conspecific gamete precedence is known to occur and might interact with or prevent reinforcement.

The model uses six loci, the fewest possible to consider the basic biology of conspecific gamete precedence and reinforcement. It is therefore not intended to be biologically realistic, but to be sufficient to assess the hypotheses present in the literature regarding the interaction of these forces. Specifically we examine the following questions: (i) Can conspecific gamete precedence evolve via a reinforcement-like process? and (ii) When conspecific gamete precedence is present, does it inhibit the evolution of reinforcement? The model considers the evolution of conspecific gamete precedence by the third mechanism above, cryptic female choice. We do this in order to render the phenomenon as parallel as possible to our mechanism of premating isolation during reinforcement, female mate choice. We complete our analysis by examining how conspecific gamete precedence and reinforcement might coevolve with one another if neither is initially present in a population.

### Model

We study the evolution of conspecific gamete precedence and reinforcement using a population genetic model of two populations exchanging migrants (a two-island model). The model, which contains six haploid, diallelic loci, is a modification and extension of previous reinforcement models (Servedio, 2000, 2004).

One of the primary factors affecting whether or not speciation will occur in population genetic models is whether nonrandom mating operates via a 'one-allele' or a 'two-allele' mechanism (e.g. Felsenstein, 1981). In a 'one-allele' model, reproductive isolation evolves by the same allele spreading in both populations (e.g. an allele for positive assortative mating, or for reduced migration). A 'two-allele' model, in contrast, creates reproductive isolation through the establishment of different, population-specific alleles, one characteristic of each of the incipient species (e.g. separate preference alleles for each of two population-specific traits). Theoretical studies have consistently demonstrated that speciation occurs more easily via a one-allele mechanism than a two-allele mechanism (reviewed in Kirkpatrick & Ravigné, 2002. Servedio & Noor, 2003, see Felsenstein, 1981). While

two-allele models are probably more biologically realistic than one-allele self-imprinting models (but see Ortiz-Barrientos et al., 2004), one-allele models retain value in theoretical studies. We use one-allele models for both reinforcement and conspecific gamete precedence for two primary reasons: (i) they allow ancestral conditions to be random mating, rather than an established mate preference, and yet have isolation evolve in a single step by the spread of one allele, and (ii) because alleles causing reproductive isolation spread to fixation in oneallele models (e.g. Servedio, 2000, 2004), they present an easy metric, the rate of spread of the isolating allele, for comparisons between closely related models (i.e. reinforcement vs. conspecific gamete precedence). We discuss predictions that can be made from our conclusions to two-allele models at the end of the paper.

We present the six-locus model by breaking it down into two subsystems, each involving three loci (Fig. 1). The first of these leads to reinforcement whereas the second leads to conspecific gamete precedence. Throughout the paper upper case letters denote the names of the alleles and loci, lower case letters denote the frequency of alleles, and subscripts denote the identity of alleles.

## Reinforcement: postzygotic isolation and assortative mating

The first set of three-loci model reinforcement by describing premating and postzygotic interactions. The first two



**Fig. 1** Diagram of the six-locus model. The loci in each subsystem are shown. The isolation loci are the loci that evolve during reinforcement (*P*) and conspecific gamete precedence (*Q*). The incompatibility loci consist of either loci causing postzygotic incompatibilities (*M* and *N*) or gametic incompatibilities (*B* and *C*). The arrows represent some of the genetic associations (linkage disequilibrium) between loci that lead to evolution at the isolation loci. The solid arrows represent associations that form within each subsystem or if the subsystems are evolving in isolation from one another. The dashed arrows represent associations that will form only when the starred (\*) association exists because of selection at both of the sets of incompatibility loci (*s*<sub>E</sub> and *s*<sub>I</sub>0) or if phase 1 of the simulations were skipped.

loci, *M* and *N*, contain population specific alleles (subscripted 1 and 2 for populations 1 and 2 respectively) that determine species identity. Hybrid genotypes at these loci  $(M_1N_2 \text{ and } M_2N_1)$  result in low fitness with a selection coefficient  $s_{\rm E}$  (relative fitness  $1 - s_{\rm E}$ ). The relative fitness of  $M_1N_1$  and  $M_2N_2$  genotypes is 1. These types of epistatic interactions (Dobzhansky–Muller incompatibilities) causing postzygotic isolation have been found in several empirical studies (e.g. Palopoli & Wu, 1994; Lamnissou *et al.*, 1996; Orr & Irving, 2001; Presgraves, 2003).

The third locus, P, determines whether assortative mating occurs, based on the genotype that males carry at the M and N loci (see Servedio, 2004 for a full description of mating in this subsystem). Females with the  $P_{\rm N}$  allele mate at random.  $P_{\rm C}$  females (choosy females, those with the assortative mating allele) that have matching alleles at their M and N loci (i.e.  $M_1N_1$  or  $M_2N_2$  females) are  $d_{\text{Assort}}$  ( $d_{\text{Assort}} > 1$ ) times more likely to mate upon encountering males which share these same alleles at their *M* and *N* loci. We thereby created a mating table where the frequency of matings is determined by the encounter frequencies of each genotype of female and male (encounters occur at random), times  $d_{Assort}$  in the crosses of  $M_1N_1$  females by  $M_1N_1$  males and of  $M_2N_2$ females by  $M_2N_2$  males. Hybrid males at the M and N loci (i.e.  $M_1N_2$  or  $M_2N_1$ ) are therefore not preferred by females, and hybrid P<sub>C</sub> females have no mating preference. This situation, where there is a pleiotropic effect of incompatibilities on both fitness and appearance of males, may occur when hybrids are undesirable mates because they appear unhealthy or because there is condition dependence of mating traits. It can also result when mate recognition traits are affected by extrinsic postzygotic incompatibilities (for more discussion, see Servedio, 2004).

# Gametic incompatibility and conspecific gamete precedence

The last three loci control fitness effects that occur between mating and zygote formation (postmating-prezygotic effects). The first two of these loci, *B* and *C*, code for population-specific traits expressed by males and females after mating. Specifically, locus *B* controls the expression of a trait in the egg or reproductive tract of the female, whereas locus *C* controls the expression of a trait in the sperm or seminal fluid of the male. Each of these loci contains two alleles, with  $B_1$  and  $C_1$  being characteristic of population 1, and  $B_2$  and  $C_2$  being characteristic of population 2.

We assume that females mate twice, each time following the rules for mating preferences described in the reinforcement susbsystem above. The mating table, after two matings, is normalized so that each female genotype has the same mating success (e.g. Kirkpatrick, 1982). This is carried out, for a given female genotype, by dividing the frequency of each double mating by the sum

of each male genotype times the strength by which it is preferred by that female genotype.

Mating twice has two consequences. First, if a female mates with two males from her own population (conspecific males, e.g. a  $B_1$  female mating with two  $C_1$ males), the cross has higher fertility (relative fitness of 1) than when she mates with two heterospecific males (relative fitness for this mating type:  $1 - s_I$ ), where population identity is determined by the B and C loci (see Table 1). Successful fertilization is divided evenly between each of the two conspecific or two heterospecific males in these crosses, no first or last male advantage is assumed. If a female mates with one conspecific and one heterospecific male and there is no evolved conspecific gamete precedence (e.g. a  $B_1$  female mating with a  $C_1$  and a  $C_2$  male), then offspring are formed in the proportion of 1 to  $1 - s_I$  being fertilized by conspecific and heterospecific males respectively, resulting in a lower number of hybrid offspring. Mismatches between the *B* and *C* loci therefore cause postmating-prezygotic incompatibilities (see Servedio, 2001). While these postmating-prezygotic incompatibilities technically cause conspecific gamete precedence, arising as a byproduct of divergence in allopatry (females disproportionally use conspecific sperm), we are primarily concerned in this study with the evolution of conspecific gamete precedence via adaptive mechanisms. We therefore refer to interactions between the *B* and *C* loci as 'gametic incompatibilities' throughout the rest of the paper, and reserve the term 'conspecific gamete precedence' for adaptive interactions as described below.

The second consequence of females mating twice is that it provides the opportunity for adaptive conspecific gamete precedence. Whether or not this conspecific gamete precedence occurs is controlled by locus Q, where  $Q_{\rm C}$  represents the conspecific gamete precedence allele and  $Q_{\rm N}$  the allele for no precedence. Conspecific gamete precedence, when it occurs ( $q_{\rm C} \neq 0$ ), is based on species identity at the *B* and *C* loci. Specifically, when a female mates with both a conspecific and a heterospecific male, conspecific sperm are used for fertilization  $d_{\rm CGP}$ ( $d_{\rm CGP} \ge 1$ ) times more than heterospecific sperm ( $d_{\rm CGP}$ can be directly compared to the strength of assortative mating  $d_{\rm Assort}$ ). This effect combines with the gametic incompatibility described above, so that  $d_{\rm CGP}/(1 + d_{\rm CGP})$  zygotes are formed from conspecific sperm vs.  $[1/(1 + d_{CGP})](1 - s_I)$  zygotes formed from heterospecific sperm in this type of cross (see Table 1).

#### Life cycle

Each generation, both populations go through the following life cycle stages:

- **1** Migration occurs symmetrically between the two populations with a low rate of 1% of the population migrating per generation.
- **2** Natural selection occurs at the *M* and *N* loci, as described for the reinforcement subsystem above.
- **3** Females mate twice, either randomly or assortatively, depending on their genotype at the *P* locus.
- **4** Conspecific gamete precedence (if any) occurs, and females suffer potential fertility effects of mismatches of their allele at the *B* locus with their mates' alleles at the *C* locus, as described for the conspecific gamete precedence subsystem above.
- **5** Free recombination occurs between the six loci (*QBCPMN*).

This six-locus population genetic model leads to a large number of recursion equations and is intractable analytically. Deterministic simulations, consisting of iterating through the exact recursion equations in C, were used to explore the evolutionary dynamics of this system. These simulations tracked changes in the frequencies of each genotype through the life cycle, assuming an infinite population size. The computer code is available from the authors on request.

### **Simulations**

We explain a general approach for the simulations here; variants to this basic form are described below. We begin with the assumption that each population has evolved for a while in allopatry, and has diverged at the M, N, B, and C loci. There is initially random mating and no conspecific gamete precedence. Each simulation therefore starts with the  $Q_N B_1 C_1 P_N M_1 N_1$  genotype set at a frequency near fixation on island 1, whereas island 2 starts with a frequency near fixation of the genotype  $Q_N B_2 C_2 P_N M_2 N_2$ . Simulations were run in two phases. As in previous models (Servedio, 2000), phase 1 simulated

<b>Table 1</b> Relative fertility of crosses with each type	of	male.
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	Relative fertility of cross for each type of male when females mate with						
	Two conspecific males		One conspecific and one heterospecific male		Two heterospecific males		
	Male 1	Male 2	Conspecific male	Heterospecific male	Male 1	Male 2	
With no conspecific male precedence With conspecific male precedence	$\frac{1}{2}$ $\frac{1}{2}$	$\frac{1}{2}$ $\frac{1}{2}$	$\frac{1}{2}$ $\frac{d_{cGP}}{1+d_{cGP}}$	$\frac{\frac{1-s_{I}}{2}}{\frac{1-s_{I}}{1+d_{CGP}}}$	$\frac{\frac{1-s_I}{2}}{\frac{1-s_I}{2}}$	$\frac{\frac{1-s_I}{2}}{\frac{1-s_I}{2}}$	

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evolution after the initiation of secondary contact, when the ancestral alleles  $Q_N$  and  $P_N$  remain fixed in both island populations. The life cycle was iterated until an initial equilibrium was reached in each population for the frequency of the *B*, *C*, *M* and *N* alleles. This was considered to have occurred when the relative change in the frequency of each of the eight genotypes [(current – previous)/previous] was < 10<sup>-11</sup> per generation. If variation at any of the loci was lost at this point, the run was not pursued further and reinforcement and/or the evolution of conspecific gamete precedence were considered not to have occurred. See Servedio (2000) for further description of this phase.

In phase 2, we introduced either  $Q_{\rm C}$ ,  $P_{\rm C}$ , or both alleles (depending on the specific run, see below) at a frequency of 0.001 in both island populations. Introductions were performed in a way that established linkage equilibrium between the Q and/or P locus and all of the other loci in the system. Simulations continued by iterating the life cycle for at least 1500 generations. Reinforcement was considered to have occurred if  $P_{\rm C}$  spread, and conspecific gamete precedence was considered to have evolved if  $Q_{\rm C}$ spread. To compare the separate and combined effects of assortative mating and conspecific sperm precedence on reproductive isolation, we calculated an index of reproductive isolation as the proportion of purebred females from population 1 that mate with males that share their alleles at the *B*, *C*, *M* and *N* loci ( $\Theta_{B_1C_1M_1N_1}$  = proportion of  $B_1C_1M_1N_1$  females mating with like males; Servedio, 2004). Unless otherwise noted, simulation results at 1500 generations are reported as a common time point, so that rate of evolution of *P* and *Q* alleles can be compared between simulations. In all cases where simulations were run until the phase 2 equilibrium was reached (i.e. either P or *Q* allele frequencies changed  $< 10^{-11}$  in one generation),  $P_{\rm C}$  and/or  $Q_{\rm C}$  fixed and the index of reproductive isolation always increased.

Several kinds of simulations were performed that differed in whether conspecific gamete precedence or assortative mating were already established at the start of the simulation or whether they were introduced after the population reached its initial (phase 1) equilibrium. We do not attempt a thorough analysis of all of the parameter space of this complex model. Instead we concentrate on addressing three specific questions related to the evolution of conspecific gamete precedence and its effect on reinforcement. We describe these three sets of simulations here. Over 500 simulation runs were performed in total.

# What forces can drive the evolution of conspecific gamete precedence?

We first examine the evolution of conspecific gamete precedence in the absence of assortative mating ( $P_{\rm N}$  remains fixed in both populations throughout the simulation), by exploring rates of evolution given

postzygotic isolation, gametic incompatibility, or both, as a driving force. Specifically we examined how far an allele for conspecific gamete precedence,  $Q_{\rm C}$ , evolved both with and without postzygotic isolation ( $s_{\rm E}$ ) and gametic incompatibilities ( $s_{\rm I}$ ), given different degrees of conspecific gamete precedence ( $d_{\rm CGP}$ ). In addition we also examined how this evolution affected reproductive isolation using our index of reproductive isolation ( $\Theta_{B,C,M,N_1}$ ).

# Does conspecific gamete precedence prevent reinforcement?

To answer this question, we compared the rate of evolution of the assortative mating allele with and without conspecific gamete precedence present. We did this by comparing the spread of the  $P_{\rm C}$  allele in simulations with  $Q_{\rm N}$  held fixed in both populations to that in simulations with  $Q_{\rm C}$  held fixed. Separate simulations were run for several different strengths of assortative mating ( $d_{\rm Assort}$ ) and conspecific gamete precedence ( $d_{\rm CGP}$ ).

### How do conspecific gamete precedence and reinforcement coevolve?

To examine how conspecific gamete precedence and assortative mating coevolve, we compared the rate of evolution of the  $Q_{\rm C}$  and  $P_{\rm C}$  alleles, and concomitant development of reproductive isolation, when these alleles are introduced simultaneously in phase 2 of the simulations. Simulations were conducted with different levels of postzygotic isolation ( $s_{\rm E}$ ,), gametic incompatibility ( $s_{\rm I}$ ), conspecific gamete precedence ( $d_{\rm CGP}$ ), and assortative mating preference strength ( $d_{\rm Assort}$ ).

### Results

We do not report the results from all the runs we simulated because of lack of space. Instead we report subsets of the runs that provided answers to the three questions above. All additional results obtained were consistent with the general principles described in our findings below.

# What forces can drive the evolution of conspecific gamete precedence?

The conspecific gamete precedence allele evolves fastest at intermediate levels of gametic incompatibility  $(s_1 \approx 0.3;$  Fig. 2a). Very high levels of gametic incompatibility lead to reduced levels of genetic variation at the gametic incompatibility loci (*B* and *C*) in each population at the end of phase 1 of the simulations (i.e. the frequencies of the population-specific alleles in each population approach 1). Low genetic variation will lead to slow responses to further selection at the *B* and *C* loci.



**Fig. 2** Evolution of a conspecific gamete precedence allele (frequency of the  $Q_C$  allele at 1500 generations). (a) Different levels of gametic incompatibilities ( $s_i$ ; when  $s_E = 0.5$ ). The highest rate of evolution occurs at intermediate  $s_I$  (0.3). (b) Different strengths of conspecific gamete precedence,  $d_{CGP}$ . Given selection against heterospecific matings ( $s_I = 0.3$ ), the conspecific gamete precedence allele evolves faster when there is no selection against hybrid offspring ( $s_E = 0$ , solid line) than when there is some selection against hybrids ( $s_E = 0.5$ , dashed line).

Because the evolution of  $Q_{\rm C}$  relies both upon selection through epistasis with the *B* and *C* loci and upon indirect selection acting through linkage disequilibrium with the *B* and *C* loci, low variation at *B* and *C* should ultimately also slow the evolution of  $Q_{\rm C}$ . In order to test this hypothesis, we artificially started runs at phase 2 using a single set of allele frequencies from one sample run of phase 1. By comparing the first few generations of phase 2 in these runs with the original runs, we were able to discern whether the original drop in  $Q_{\rm C}$  evolution with strong selection was (i) because of different selective conditions (in the test runs we should again get slower evolution of  $Q_{\rm C}$  with stronger selection) or was (ii) a byproduct of differences in the amount of genetic variation left from the initial equilibrium of phase 1 (in the test runs we should get faster evolution of  $Q_{\rm C}$  with stronger selection). We found that in the test runs early generations of  $Q_{\rm C}$  evolution, started in phase 2, are more rapid with a higher s<sub>I</sub> throughout the entire curve, in contrast to the original runs (shown in Fig. 2a). We therefore conclude that it is not a very high s<sub>1</sub> per se that causes slow evolution of  $Q_{\rm C}$  in this part of the curve, but that lower genetic variation at the B and C loci is the cause.

We next explored the effects of the strength of conspecific gamete precedence ( $d_{CGP}$ ) and the strength of selection against hybrids ( $s_E$ ), assuming that  $s_I = 0.3$ . Figure 2b shows that the conspecific gamete precedence allele evolves faster with stronger conspecific gamete precedence ence also evolves more rapidly when there is no postzygotic

selection ( $s_E = 0$ ) vs. when hybrids are at a fitness disadvantage ( $s_E = 0.5$ ). Additional simulations show that this decrease is monotonic with increasing  $s_E$  (data not shown.).

We tested the hypothesis that this second result also occurs because of a lack of genetic variation; specifically that postzygotic selection  $(s_{\rm E})$  reduces genetic variation at the loci on which conspecific gamete precedence acts (B and C). When  $s_{\rm E}$  and  $s_{\rm I}$  are both positive, the gametic incompatibility loci (B and C) are in linkage disequilibrium with the hybrid incompatibility loci (*M* and *N*), because of divergence that has occurred in allopatry and selection against mismatched alleles in both sets of loci. Therefore during phase 1 of the simulations when postzygotic selection acts to reduce the frequency of the  $M_1$  (and  $N_1$ ) allele in population 2, for example,  $B_1$  (and  $C_1$ ) allele frequency decreases as well, reducing the genetic variation at these loci in each population. This effect does not occur when  $s_{\rm E} = 0$ . To tease apart the effects of this lack of variation and the strength of selection  $(s_{\rm E})$  we compared the frequency of the  $B_1$  allele with and without hybrid incompatibility ( $s_E = 0$  vs. 0.5) after one generation of phase 2 simulations using fixed starting genotype frequencies from a sample run of phase 1 (with  $s_1 = 0.3$ ). In population 1 (where subscript 1 alleles predominate) we found that the  $B_1$  allele frequency is higher with hybrid incompatibility ( $s_{\rm E} = 0.5$ ) than without it ( $s_E = 0$ ) after the viability selection stage of the life cycle. Likewise, in population 2 (where subscript 2 alleles predominate)  $B_1$  frequency is lower with hybrid incompatibility than without it. These patterns are the reverse of those found in Fig. 2b, indicating that the lowered genetic variation with high  $s_{\rm E}$  must have been responsible for our original results. We then observed that the elevated frequency differences under postzygotic selection lead to a higher increase in the frequency of the  $Q_{\rm C}$  allele during the conspecific gamete precedence stage of the life cycle, confirming the effect of this reduced variation on the evolution of  $Q_{\rm C}$ .

We found throughout our simulations that conspecific gamete precedence did not evolve if there were no gametic incompatibilities ( $s_{I} = 0$ ), even if there was postzygotic isolation ( $s_{\rm E} > 0$ ). We hypothesized that this was due to the fact that linkage disequilibrium between the gametic incompatilibility loci (B and C) and the postzygotic isolation loci (M and N) erodes to zero under these conditions in phase 1 of the simulations; the cues used by females in conspecific gamete precedence (B and C) therefore have no association with fitness (note that this is not the case if both  $s_{\rm I}$  and  $s_{\rm E}$  are positive). If this is the cause of our results, then conspecific gamete precedence should evolve under these selection conditions if linkage disequilibrium were artificially set between the gametic incompatibility and postzygotic isolation loci. We accomplished this by skipping phase 1 of the simulations (the desired linkage equilibrium was present in our

# Does conspecific gamete precedence prevent reinforcement?

If we assume that strong conspecific gamete precedence  $(d_{CGP} = 5)$  is fixed  $(Q_C = 1)$  in both populations at the start of the simulations, the evolution of reinforcement is dramatically slowed. Figure 3a shows the evolution of the assortative mating allele  $P_C$  (reinforcement) under different strengths of female preference  $(d_{Assort})$  when conspecific gamete precedence is present vs. absent.

When the conspecific gamete precedence allele is fixed  $(Q_{\rm C} = 1)$ , reinforcement is slower with stronger conspecific gamete precedence (higher  $d_{\rm CGP}$ , Fig. 3b). It is worth noting, however, that even when conspecific gamete precedence is fixed and strong  $(d_{\rm CGP} = 5)$ ,  $P_{\rm C}$  still evolves, although very slowly (Fig. 3b,  $P_{\rm C} = 0.0124$ ).

Even when there is no assortative mating, reproductive isolation evolves to relatively high levels (Table 2)



**Fig. 3** Evolution of the assortative mating allele (reinforcement) with different strengths of assortative mating ( $d_{Assort}$ ). The figures show the frequency of the allele for assortative mating reached at 1500 generations after its introduction (with  $d_{CGP} = 5$ ), given both selection against heterospecific matings ( $s_I = 0.3$ ) and selection against hybrids ( $s_E = 0.5$ ). (a) Assortative mating evolves more rapidly when there is no conspecific gamete precedence ( $Q_N$  fixed, solid line) than when conspecific gamete precedence is present ( $Q_C$  fixed, dashed line). (b) Assortative mating evolves more slowly when an allele for stronger conspecific gamete precedence ( $Q_C$ , with a higher  $d_{CGP}$ ) is fixed (dashed line). The solid line shows the evolution of assortative mating with no conspecific gamete precedence ( $Q_N$  fixed).

**Table 2** The frequency of an assortative mating allele  $[f(P_C)]$  and the index of reproductive isolation  $(\Theta_{B_1C_1M_1N_1})$  for different strengths of assortative mating  $(d_{Assort})$  and or conspecific gamete precedence  $(d_{CGP})$  given different evolutionary scenarios. In the first two scenarios, either assortative mating or conspecific gamete precedence were fixed, whereas in the third they coevolved. All values are after 1500 generations.

$d_{\rm Assort}$ and/or $d_{\rm CGP}$	$f(P_{\rm C})$	$\Theta_{B_1C_1M_1N_1}$
Assortative mating with no CGI	D	
$d_{\rm Assort} = 1.1$	0.00281	0.86514
$d_{\rm Assort} = 2$	0.23472	0.88318
$d_{Assort} = 3$	0.64352	0.92223
$d_{Assort} = 4$	0.80310	0.93968
$d_{\rm Assort} = 5$	0.86776	0.94799
CGP with random mating		
$d_{\rm CGP} = 1.1$	0	0.86511
$d_{\rm CGP}=2$	0	0.86951
$d_{CGP} = 3$	0	0.89145
$d_{\rm CGP}=4$	0	0.90860
$d_{CGP} = 5$	0	0.91779
Coevolution of CGP and assort	tative mating	
$d_{\text{Assort}} = d_{\text{CGP}} = 1.1$	0.00281	0.86517
$d_{\text{Assort}} = d_{\text{CGP}} = 2$	0.22992	0.88638
$d_{\text{Assort}} = d_{\text{CGP}} = 3$	0.59894	0.93117
$d_{\text{Assort}} = d_{\text{CGP}} = 4$	0.72601	0.94986
$d_{\rm Assort} = d_{\rm CGP} = 5$	0.77049	0.95802

due to the effects of conspecific gamete precedence. However, reproductive isolation evolves more rapidly in response to the introduction of assortative mating than to the introduction of conspecific gamete precedence (Table 2, compare first two sections).

### How do conspecific gamete precedence and reinforcement coevolve?

When alleles for assortative mating and conspecific gamete precedence are introduced at the same time, they can coevolve. As expected, when there is gametic incompatibility ( $s_I = 0.3$ ) causing the conspecific gamete precedence allele ( $Q_C$ ) to increase in frequency, the assortative mating allele ( $P_C$ ) evolves more slowly than when there is no gametic incompatibility ( $s_I = 0$ ) and conspecific gamete precedence does not evolve (Fig. 4, dashed lines vs. solid thin lines). Likewise, stronger assortative mating (larger  $d_{Assort}$ ) results in slower evolution of the conspecific gamete precedence allele, and hence more and more rapid spread of the  $P_C$  allele (Fig. 4, dashed thick line).

Even though evolution of an assortative mating allele  $(P_C)$  is slowed by the coevolution of a conspecific gamete preference allele  $(Q_C)$ , reproductive isolation builds up faster than when either allele evolves alone (Table 2, compare third section with the other two). Evolution in both the *P* and *Q* subsystems affects the amount of reproductive isolation.



**Fig. 4** Coevolution of an assortative mating allele and a conspecific gamete precedence allele given different strengths of assortative mating ( $d_{Assort}$ ). The figure shows the frequency of the alleles for assortative mating ( $P_{C_r}$  thin lines) and conspecific gamete precedence ( $Q_C$ , thick lines) reached at 1500 generations after their introduction with  $s_E = 0.5$ . Solid lines represent  $s_I = 0$  and dashed lines represent  $s_I = 0.3$ . Evolution of the conspecific gamete precedence allele is not affected by the strength of assortative mating when there is no selection against heterospecific matings ( $s_I = 0$ ). However, strong assortative mating decreases the rate of evolution of the conspecific gamete precedence allele when there is selection against heterospecific matings ( $s_I = 0.3$ ).

Conspecific gamete precedence can evolve, although its rate of evolution is slowed considerably (Fig. 5), even when some degree of assortative mating ( $d_{Assort} = 2.0$ ) is fixed in both populations.



**Fig. 5** Evolution of the conspecific gamete precedence allele with different strengths of conspecific gamete precedence ( $d_{CGP}$ ) with and without established assortative mating. The figure shows the frequency of the allele for conspecific gamete precedence reached at 1500 generations after its introduction. Given selection against heterospecific matings ( $s_I = 0.3$ ) and selection against hybrids ( $s_E = 0.5$ ), assortative mating evolves more rapidly when there is no assortative mating ( $P_N$  fixed, solid line) than when assortative mating is present ( $P_C$  fixed, dashed line).

#### Discussion

Conspecific gamete precedence is increasingly being recognized to play an important role in speciation (e.g. Howard et al., 1998, 2002; Civetta & Singh, 1999). Previous authors have hypothesized both that (i) conspecific gamete precedence should be able to evolve through a reinforcement-like process, because of selection against incompatibilities in heterospecific matings (e.g. Howard, 1999), and that (ii) the presence of conspecific gamete precedence should inhibit reinforcement (Marshall et al., 2002). We find support for both of these hypotheses, as well as an inhibitory effect of reinforcement on the evolution of conspecific gamete precedence. We also find that as both the evolution of assortative mating and conspecific gamete precedence increase the amount of reproductive isolation, both contribute to the process of speciation.

# What forces can drive the evolution of conspecific gamete precedence?

We find that conspecific gamete precedence can indeed evolve through a reinforcement-like process. Whether this evolution will take place, however, depends on the nature of selection against heterospecific matings. Specifically, we find that conspecific gamete precedence can evolve when females recognize conspecific vs. heterospecific sperm using traits that accurately predict whether there are incompatibilities that lower the number of offspring produced in heterospecific crosses. In our model, these traits are expressed at the gametic incompatibility loci, B and C. The loci B and C possess alleles that both cause epistatic incompatibilities when mismatched (specifically, lower fertility) and are used by females as cues in conspecific gamete precedence through cryptic female choice (see Servedio, 2004 for a description of a similar effect). We find that conspecific gamete precedence does not evolve when the only source of selection is caused by postzygotic incompatibilities, represented in our model by *M* and *N* (i.e.  $s_{I} = 0$ ,  $s_{\rm E} > 0$ ).

It is not known whether traits involved in gametic incompatibilities can also, through pleiotropy, act as cues in conspecific gamete precedence. For example, cell surface proteins that affect sperm–egg interactions may or may not also be used by females for discriminating between conspecific and heterospecific sperm. If this kind of pleiotropy does not exist, then either conspecific gamete precedence must evolve (i) in a non-adaptive fashion (e.g. even the presence of gametic incompatibilities causes a type of conspecific gamete precedence), (ii) adaptively through a process unlike female choice, (iii) adaptively through a mechanism akin to female choice but the cues used for it are physically linked to, or in linkage disequilibrium with, loci causing gametic incompatibilities, or (iv) adaptively through a mechanism akin to female choice but the loci involved in gametic incompatibilities are not associated with the cues for conspecific sperm choice. In order to distinguish between the last two explanations we can consider the evidence that when there is postzygotic isolation ( $s_{\rm F} > 0$ ) but no gametic incompatibilities ( $s_{I} = 0$ ), conspecific gamete precedence cannot evolve. In this situation, there is no linkage disequilibrium between the loci involved in incompatibilities (N and M) and the loci used as cues in conspecific gamete precedence (B and C); any linkage disequilibrium present at the start of the simulation has eroded, under these conditions, by the end of phase 1 of the simulations. This suggests that the fourth explanation above cannot work. Indeed it is difficult to imagine what mechanism could possibly allow conspecific gamete precedence to evolve if the cue used for sperm choice did not in any way predict whether the mating were likely to produce either more offspring or offspring with higher fitness. If conspecific gamete precedence does work by a mechanism like female choice, it is therefore very likely that the cues used by females are also involved in, physically linked to, or in linkage disequilibrium with, traits that are used as cues in conspecific gamete precedence.

Our results show that postzygotic incompatibilities alone cannot drive the evolution of conspecific gamete precedence. When the loci used as cues in conspecific gamete precedence (B and C) are selectively neutral, alleles at these loci quickly become randomly distributed between the two incipient species because of gene flow in phase 1 of our simulations. No genetic association is therefore maintained between these loci and the loci that cause hybrid incompatibilities (M and N), preventing selection on M and N from driving the evolution of conspecific gamete precedence. In nature, however, genetic variation for conspecific gamete precedence may be segregating in the population upon the initiation of secondary contact; this can be represented in our model by skipping phase 1 of the simulations. In this case, there is some genetic association (linkage disequilibrium) between the *B* (and *C*) and *M* (and *N*) loci due to their history of divergence in allopatry. When *B* and *C* are selectively neutral, this linkage disequilibrium lingers upon initiation of secondary contact until, without any force maintaining it, it eventually erodes to zero. We show that while linkage disequilibrium is still present in the population, selection on the *M* and *N* loci can drive the evolution of conspecific gamete precedence, because the cues B and C would be, to some degree, accurately associated with a high fitness offspring genotype. In other words, a female mating with a 'conspecific' on the basis of a male's trait at the C locus would also be likely to be accurately matching her M and N alleles to those of the male. Evolution of conspecific gamete precedence by this mechanism will not be expected to proceed as far or as rapidly, however, as it would if it were caused by

postmating-prezygotic incompatibilities pleiotropically used as cues in conspecific gamete precedence.

As long as there is both selection against gametic incompatibility and variation at the gametic incompatibility loci, some level of conspecific gamete precedence appears to be able to evolve in our model. This occurs for two reasons. First, gametic incompatibilities have less opportunity to occur when the conspecific gamete precedence allele,  $Q_{\rm C}$ , is present. The  $Q_{\rm C}$  allele is therefore directly favoured by selection during the conspecific gamete precedence phase of the life cycle. (Note that this selection would not occur if the cues used for conspecific gamete precedence were not associated with incompatibilities.) This selection, which can be thought of as an epistatic interaction between the Q, B and C loci, is described as direct because the frequency of alleles at the Q locus would change even if there were no linkage equilibrium between the Q locus and the B and C loci. Similar direct selection does not, in contrast, occur at the assortative mating locus, P; evolution at this locus occurs solely through indirect selection resulting from linkage disequilibrium between the P locus and the other loci in the system (e.g. Kirkpatrick, 1982, Kirkpatrick & Barton, 1997). The presence of direct selection at the *Q* locus can be attributed to the fact that it acts at the same stage of the life cycle as fertility selection; it therefore directly determines the amount of fertility selection that will occur. This is a phenomenon that will occur, by definition, in any model of conspecific gamete precedence.

The second reason that conspecific gamete precedence evolves so readily is because of our assumption that conspecific gamete precedence occurs via a one-allele mechanism of speciation (Felsenstein, 1981). Two-allele mechanisms of speciation are inhibited by recombination occurring between the locus causing reproductive isolation and other loci with population-specific beneficial effects (Felsenstein, 1981); one-allele models avoid this effect. The universal spread of an allele leading to speciation in some one-allele models, such as this one, is also aided by a positive genetic association forming between the allele causing speciation and the alleles favoured by selection in each population (see Servedio, 2000).

Unexpectedly, when both gametic incompatibility  $(s_I)$  and selection against hybrids  $(s_E)$  are present in the system, conspecific gamete precedence can evolve more slowly than when only gametic incompatibilities are present. There are two phenomena with opposing effects that occur when there is selection against hybrids. First, selection against hybrids can help to drive the evolution of conspecific gamete precedence when the loci controlling postzygotic incompatibilities (*M* and *N*) are genetically associated with the gametic incompatibility loci that serve as conspecific gamete precedence cues (*B* and *C*). In our model, this will occur whenever selection against both types of incompatibilities is present in the system (or if phase 1 of our simulations were skipped, as discussed

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above). Secondly, due to this same genetic association, strong selection against hybrids will not only deplete genetic variation at the postzygotic isolation loci, but also erode genetic variation at the gametic incompatibility loci. Stronger selection against hybrids can therefore tend to slow the evolution of conspecific gamete precedence. Very strong gametic incompatibilities also erode genetic variation in this way, leading to a unimodal relationship between this selection and the spread of the allele for conspecific gamete precedence.

## Does conspecific gamete precedence prevent reinforcement?

Our results confirm the prediction of Marshall *et al.* (2002) that when conspecific gamete precedence is present in a population, reinforcement will be inhibited. Furthermore, the degree of this inhibition depends on the strength of the conspecific gamete precedence. Specifically, when females are more likely to choose conspecific sperm, the rate of spread of an allele causing reinforcement quickly declines.

We find, however, that reinforcement can still evolve, although very slowly, even in the face of strong conspecific gamete precedence. This evolution has two causes in our model. First, conspecific gamete precedence does not prevent hybrid formation by females that happen to mate with two heterospecifics. If heterospecific migrants are rare in a natural population, mating with them twice will be unlikely, but could still happen with some small frequency, hence potentially driving reinforcement at a slow rate. Secondly, an individual's alleles in the conspecific gamete precedence subsystem in our model are not completely predictive of alleles in the reinforcement subsystem. During reinforcement in our model, for example, purebred  $(M_1N_1 \text{ or } M_2N_2)$  females with the  $P_C$ allele are more likely to mate with males that share their genotype at the *M* and *N* loci. Conspecific gamete precedence, on the other hand, acts to ensure a match of a female's genotype at the *B* locus with the male's genotype at the C locus. Unless linkage disequilibrium between *B* (and *C*) and *M* (and *N*) is perfect (i.e. complete physical linkage), a female that is insured of choosing males with the proper *C* alleles may still be forming low fitness hybrid offspring if its mate does not match its M and N alleles. This separation between alleles driving conspecific gamete precedence and reinforcement is also likely in nature unless there is a high degree of pleiotropy. The formation of low fitness hybrids, although rare, may therefore also drive reinforcement at a slow rate even when conspecific gamete precedence is complete.

Reinforcement, like conspecific gamete precedence, occurs ubiquitously in our model under standard conditions. This is due to the fact that like conspecific gamete precedence, reinforcement occurs in our model via a one-allele mechanism (the 'assortative mating' mechanism of Servedio, 2000, with the mating cue modified as in Servedio, 2004). This shared assumption allows us to compare the evolution of conspecific gamete precedence and reinforcement more easily.

## How do conspecific gamete precedence and reinforcement coevolve?

Genetic variation for both conspecific gamete precedence and reinforcement may often co-occur in populations. In order to determine the potential course of speciation in this circumstance, we examined the coevolution of these two phenomena. We found that when variation for conspecific gamete precedence and reinforcement are introduced into a population simultaneously, the evolution of each of these forces negatively affects the other. Furthermore, just as reinforcement is inhibited by previously established conspecific gamete precedence, the presence of previously established assortative mating between populations decreases the rate of spread of a conspecific gamete precedence allele.

These results have interesting implications for speciation. Marshall *et al.* (2002) argued that the presence of conspecific gamete precedence could account for the fact that reinforcement may not be as common in nature as some researchers expect. Our results suggest that the opposite may be true as well; the presence of reinforcement in some species may inhibit the evolution of conspecific gamete precedence. Specifically, our findings predict that reinforcement and conspecific gamete precedence may rarely co-occur at significant levels because whichever one happens to evolve first will inhibit the evolution of the other. It should be noted that male choice is not expected to interact with conspecific gamete precedence in the same way, and more work needs to be done to determine how these processes interact.

There is not enough comparative data to evaluate these predictions at this time. Marshall *et al.* (2002) presented a preliminary analysis that suggests that the presence of conspecific gamete precedence can inhibit reinforcement, but argued for a more appropriate test that is beyond current data. Patterns of occurrence of conspecific gamete precedence and reinforcement may be complicated by the fact that each phenomenon merely inhibits the evolution of the other, but will not prevent it altogether. Both phenomena, may, therefore, co-occur in some pairs of species that have been in secondary contact for a long enough period of time.

Some taxa may possess other characteristics, beyond the ones examined in this paper, which may predispose them towards the evolution of either conspecific gamete precedence or reinforcement. Many known cases of conspecific gamete precedence, for example, occur in the Orthoptera (e.g. Hewitt *et al.*, 1989; Ritchie *et al.*, 1989; Butlin, 1998; Howard *et al.*, 1998), although there is not enough data to know whether this prevalence is the result of sampling bias. Flies in the genus *Drosophila*, on the other hand, show evidence of both reinforcement (e.g. Noor, 1995; Ortiz-Barrientos *et al.*, 2004) and conspecific gamete precedence (e.g. Price, 1997; Price *et al.*, 2000; Chang, 2004), although these phenomena occur in different species pairs. Interestingly, Orthoptera generally possess a single expandable sperm storage organ and sperm mixing [precedence of second male sperm (P2) ranging from 0.39 to 0.62 for four of the species in the above citations]. This may encourage interactions between heterospecific sperm and lead to conspecific gamete precedence being more common. *Drosophila*, on the other hand, have three rigid spermathecae into which females may sort sperm. They also have stronger last male sperm precedence (P2 ranging from 0.74 to 0.83 for four of the species in the above citations).

A final factor that may affect whether conspecific gamete precedence or reinforcement is more likely to evolve is whether they operate via a one-allele or twoallele mechanism (*sensu* Felsenstein, 1981). In this study, one-allele mechanisms were used to model both of these processes, but it is not known whether one-allele or twoallele mechanisms are more common in nature. If we had used a two-allele model for just one of our subsystems (reinforcement or conspecific gamete precedence), evolution in the one-allele subsystem would doubtless have been easier than in the two-allele subsystem.

The more interesting question is whether any of our primary results would have differed if we had used twoallele models for both subsystems. While it is not possible to definitively answer this question without making these models, logic tells us that our primary results should be robust to the type of model used. Conspecific gamete precedence, for example, should not be able to evolve if the cues used for sperm selection do not adequately reflect the potential for incompatibilities. This means that regardless of the type of model, conspecific gamete precedence should not be able to evolve if the only incompatibilities are postzygotic (i.e.  $s_I = 0$ ), unless there is some linkage or pleiotropy between these loci and loci used as cues for sperm precedence. Similarly, it is hard to imagine a mechanism whereby the presence of established conspecific gamete precedence could inhibit the evolution of reinforcement (or vice versa) in our oneallele model but not affect it in a two-allele model. Finally, because the allele for conspecific gamete precedence interacts epistatically with the B and C loci to determine fitness, it will be under direct selection (vs. indirect selection) in both a one-allele and two-allele model. A two-allele model would, however, be worth exploring in the future to explicitly address these predictions.

Our results include a measure of the amount of reproductive isolation that will accumulate between the populations due to the evolution of conspecific gamete precedence and/or reinforcement. We find that reinforcement is somewhat more effective in isolating the populations than conspecific gamete precedence. We further find that although conspecific gamete precedence and reinforcement inhibit each other when they are both allowed to evolve, reproductive isolation accumulates more quickly when these phenomena are coevolving than when just one or the other is allowed to evolve. It is unclear, however, whether these results would be robust to changes in the assumptions of the model. Both conspecific gamete precedence and reinforcement do, however, positively contribute to reproductive isolation in all cases tested.

Throughout the paper our models are formulated specifically to address conspecific gamete precedence and reinforcement in internally fertilizing animals. As mentioned above, there is also accumulating evidence for conspecific gamete precedence in freespawning marine invertebrates and in plants. The lysin/VERL (e.g. Swanson & Vacquier, 1998) and bindin/EBR1 (Metz et al., 1994; Kamei & Glabe, 2003) sperm/egg incompatibility systems of freespawning marine invertebrates may be better modelled by considering the evolution of the sperm protein or egg receptor per se (rather than considering the evolution of a gene that causes female choice). Pollen tube growth down a stigma and style can be modelled in a similar way. Such a model would differ substantially from the one presented here, making it difficult to speculate what it would predict. Marine invertebrates and plants can also, however, be isolated by differences in spawning and flowering time respectively. Whether the present model could encompass the evolution of assortative mating via temporal isolation depends on whether it occurs via a oneallele or a two-allele mechanism (both are technically possible). These additional systems may be fruitful avenues for future modelling efforts.

### Conclusions

Our results test several predictions regarding the evolution of conspecific gamete precedence and its effects on reinforcement. First, we demonstrate that conspecific gamete precedence can evolve by a reinforcement-like process, which may be most likely to be driven by incompatibilities acting at the postmating–prezygotic stage. Secondly, we confirm predictions (Marshall *et al.*, 2002) that the presence of conspecific gamete precedence in a population is expected to inhibit the evolution of reinforcement. We further find that reinforcement has a similar inhibitory effect upon the evolution of conspecific gamete precedence. Which of these phenomena is likely to evolve in a population may depend largely on specifics of the biology of species pairs that allows one process to evolve more quickly than the other.

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