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A THEORETICAL INVESTIGATION OF SYMPATRIC EVOLUTION OF TEMPORAL REPRODUCTIVE ISOLATION AS ILLUSTRATED BY MARINE BROADCAST SPAWNERS

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Recent theory suggests that frequency-dependent disruptive selection in combination with assortative mating can lead to the establishment of reproductive isolation in sympatry. Here we explore how temporal variation in reproduction might simultaneously generate both disruptive selection and assortative mating, and result in sympatric speciation. The conceptual framework of the model may be applicable to biological systems with negative frequency-dependent selection, such as marine broadcast spawners or systems with pollinator limitation. We present a model that is motivated by recent findings in marine broadcast spawners and is parameterized with data from the *Montastraea annularis* species complex. Broadcast spawners reproduce via external fertilization and synchronous spawning is required to increase the probability of successful fertilization, but empirical evidence shows that as density increases, so does the risk of polyspermy. Polyspermy is the fusion of multiple sperm with an egg at fertilization, a process that makes the embryo unviable. Synchrony can therefore also act as a source of negative density-dependent disruptive selection. Model analysis shows that the interaction between polyspermy and spawning synchrony can lead to temporal reproductive isolation in sympatry and that, more generally, increased density promotes maintenance of genetic variation.

KEY WORDS: **Broadcast spawners, *Montastraea*, negative density dependence, sympatric speciation, temporal reproductive isolation.**

Temporal reproductive isolation is one mechanism able to generate speciation by preventing gene exchange, but the evolution of temporal isolation in sympatry is thought to be unlikely (Coyne and Orr 2004, p. 208). Mayr regarded models of sympatric speciation via "seasonal isolation" as having serious to fatal weaknesses (Mayr 1963, p. 477). Yet examples of temporal isolation are known in insects like cicadas (Cooley et al. 2003), flowering plants (Blionis and Vokou 2002; Ellis et al. 2006), fish such as salmon (Aspinwall 1974; Quinn et al. 2000) or eel (Maes et al. 2006), and marine broadcast spawners such as corals (Knowlton et al. 1997) and algae (Clifton 1997). Generally, allopatry is used as a null hypothesis to explain a difference in breeding time. Two allopatric, or parapatric, populations may evolve at different breeding times as a result of different environmental conditions. In salmonides, for example, different environmental factors in different rivers, or in different areas of the river may lead to different optimal breeding times, and in plants, different pollinators in different areas could select for different flowering times (for review see Hendry and Day 2005).

A few models have been developed to study the possibility of reinforcement maintaining temporal isolation when populations come into secondary contact (Crosby 1970; Stam 1983;

Spirito 1987). Crosby (1970) introduced a multilocus model to investigate the dynamics of two subspecies that differed in flowering time, where hybrids had reduced viability, and found that temporal isolation in flowering time was possible. Spirito (1987) analyzed a one-locus version of the same model showing that the heterozygote equilibrium frequency (middle flowering time) was a decreasing function of the degree of assortative mating. In these models, however, heterozygote inferiority was explicitly assumed and was not a dynamic property of the system. To our knowledge, no models have been developed to study the possibility of evolving temporal isolation in sympatry.

The purpose of this article is to show that the evolution of temporal reproductive isolation in sympatry is possible based on a simple model motivated by the fertilization kinetics of marine broadcast spawners. Sessile broadcast spawners represent a suitable system in which to investigate such a possibility, because spawning is highly synchronized in many species (Babcock et al. 1986; Clifton 1997). The probability of fertilization during a spawning event is proportional to the degree of synchrony among different individuals of the same population (Levitan 2004). Spawning synchrony may be an adaptation to increase fertilization success or to swamp predators (Harrison et al. 1984), but it may also have costs. For example, predators may synchronize with the spawning event or the offspring may compete with each other. Another potential cost, which we will use to parameterize our model, is polyspermy, the fusion of multiple sperm with the egg at fertilization (Styan 1998; Franke et al. 2002; Levitan 2004). Normally, the egg avoids polyspermy by either a fast electrical block to excess sperm or by a slower physical block by raising of the vitelline envelope that separates the sperm from the egg membrane.

As the density of sperm increases, there is an increasing probability that a second sperm may enter the egg within the small window of time before these polyspermy blocks are effective. In many species this will cause death of the embryo (for review see Gould and Stephano 2003). Some taxa lack permanent blocks to polyspermy and are more tolerant of low levels of polyspermy, but still suffer from developmental failure as the number of sperm fusions increases (Goudeau and Goudeau 1993).

Polyspermy therefore acts as a negative density-dependent mechanism. At low gamete density synchronization is favored, but at high density, selection should favor individuals that spawn toward the tails of the spawning-time distribution. This can create the type of frequency-dependent disruptive selection that favors lineage branching and the evolution of reproductive isolation (Dieckmann and Doebeli 1999; Gavrilets 2004; Bürger et al. 2006), and follows the idea that sexual conflict may lead to sympatric speciation (Gavrilets and Waxman 2002).

Here we present a simple one-locus model to show how negative density dependence during a spawning event can generate stable lineage splitting. We will emphasize two questions: (1) Can reproductive temporal isolation evolve? (2) Under which conditions will temporal reproductive isolation be stable? We parameterize the model with experimental data on the effects of polyspermy in *Montastraea* corals, and suggest how at least two of the members of this species complex, *M. annularis* and *M. franksi*, could have arisen by sympatric speciation through temporal isolation.

Although our model is parameterized with broadcast spawners, the conceptual framework may also be applicable to the evolution of temporal reproductive isolation in biological systems with a restricted mating period, such as flowering plants with pollinator limitation.

Biology of Coral Spawning and Polyspermy

The *M. annularis* species complex consists of three named species *M. annularis*, *M. franksi*, and *M. faveolata* (Weil and Knowlton 1994). These corals reproduce via external fertilization by releasing their gametes in the water. Each polyp produces one gamete bundle containing approximately 100 eggs and one million sperm (Szmant et al. 1997; Levitan 2004). Bundles are released synchronously within a colony, rise to the water's surface, and then dissipate into individual gametes (van Veghel 1994). These hermaphroditic corals have a block to self fertilization; the gamete cloud from one coral colony must mix with the gametes from another genetic individual (Levitan 2004). All members of the species complex have massive spawning events during one or more nights, usually five to six days after the last full moon of August or September (Levitan 2004). Members of a particular species show a high degree of spawning synchrony, but one species, *M. franksi*, spawns an average of 100 min prior to the other two congeners (Fig. 1). This early-spawning species produces gametes that are completely cross-compatible with *M. annularis*, which spawns later in the evening (Levitan 2004). These two species spawn on the same evenings and can have overlapping spatial distributions on the same reefs (although *M. franksi* is often found in deeper water). Spawning times on any one night and reef indicate at least a 1 h gap in spawning times between the last *M. franksi* and the first *M. annularis* individuals (Levitan 2004). Field and laboratory studies indicate that this time gap is sufficient for sperm to age, dilute, and move sufficiently off the reef to make hybridization unlikely. Within species, corals that spawn at the tails of the spawning-time distribution have decreased reproductive success, likely caused by sperm limitation (Levitan 2004). Genetic and morphological analyses indicate regional differences in the degree to which these species are distinct, perhaps associated with regional differences in reef topography or water flow that influence patterns of gamete mixing and hybridization (Fukami

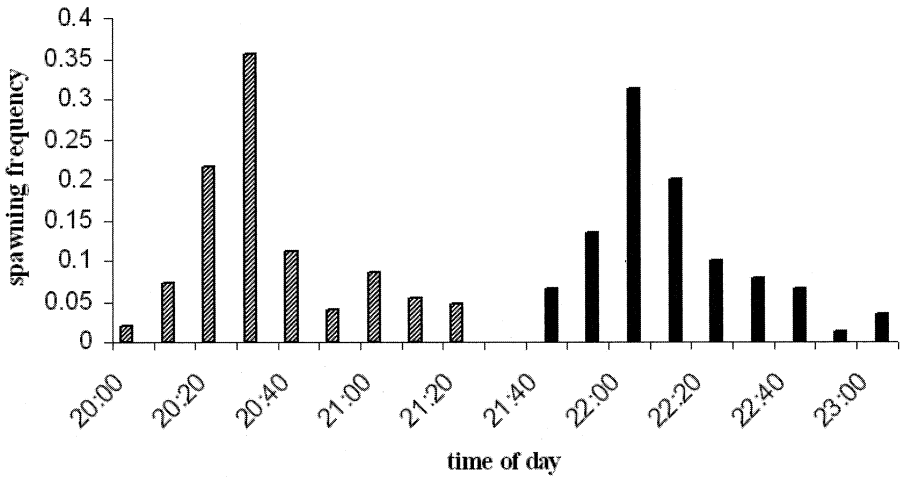

Figure 1. Spawning distributions. Time of day is shown on x-axis. Spawning frequency on y-axis. *Montastraea annularis* in black columns, *M. franksi* in striped columns (data from field observations of spawning times, methods and site descriptions; see Levitan 2004).

et al. 2004). In spite of the genetic differences, the species-specific differences in spawning time and gametic compatibility remain consistent across these regions (Levitan 2004).

Polyspermy may be an important factor in zygote survival for *Montastraea* corals. Laboratory studies of naturally spawning coral colonies indicate that fertilization success first increases with sperm concentration, but then decreases at high sperm concentration because of polyspermy (Fig. 2; see also Levitan 2004). Only a handful of studies have measured polyspermy under natural conditions in marine broadcast spawners, some on animals (Franke et al., 2002; Levitan 2004), and some on algae (Brawley 1992; Serrão et al. 1996). In the echinoid *Evechinus chloroticus*, polyspermy was shown to occur at fairly high rates even in the presence of a small degree of sperm limitation (Franke et al., 2002). This reflects the opposing constraints on eggs to facilitate fertilization when sperms are limiting but also to avoid polyspermy when sperms are abundant (Franke et al., 2002; Levitan 2004; Levitan et al. 2007).

Evidence for polyspermy in nature is not overwhelming, but data are difficult to collect, and there are in fact several lines of evidence that suggest that polyspermy might be an important selective force operating on broadcast-spawning taxa. Most obviously, the ubiquity of polyspermy blocks implies that, at least in times past, polyspermy has had a significant selective influence on egg traits. Second, variation in the effectiveness of these blocks varies among closely related species such that species facing higher levels of sperm competition have more effective blocks (Levitan et al. 2007). Third, the high levels of positive selection on gamete-recognition proteins in a diverse array of broadcast-spawning taxa are thought to be driven by sperm competition and the risk of polyspermy (Rice and Holland 1997), and field experiments with sea urchins have shown an interaction between density and frequency-dependent selection, such that females with rare genotypes are selected at high spawning densities, because they avoid the risk of polyspermy (Levitan and Ferrell 2006). Here we explore how polyspermy can act as a mechanism of both disruptive selection and assortative mating, and how it can lead to temporal reproductive isolation and sympatric speciation.

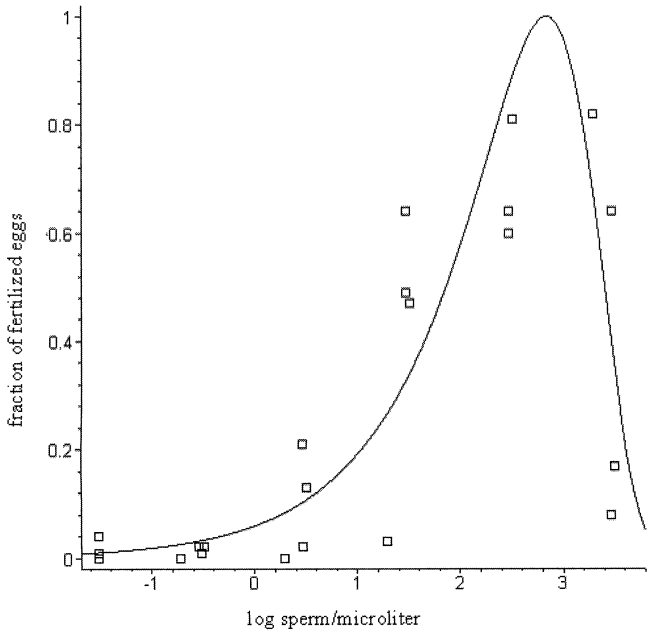

Figure 2. Fertilization success in *Montastraea franksi* as a function of sperm density in the laboratory. On the y-axis is the fraction of eggs successfully fertilized. On the x-axis is the log sperm/microliter. Data from Levitan (2004). Solid line is the best fit of a gamma distribution (eq. 2). The fitted parameter values are $k = 0.0596$, $a = 0.509$, $b = 0.000735$. $R^2 = 72\%$.

The Model

Consider a large constant population of N diploid sessile marine broadcast spawners, where population regulation takes place during recruitment to the adult population. The phenotypic trait under selection is the time of spawning during a night, t, which is controlled by a single locus with two alleles, 1 and 2 at frequencies, p and q, respectively. Each individual will release gametes over a time interval, the spawning event. Assume $g_{ij}(t)$ is the temporal gamete distribution of genotype ij at time t. We assume that this function has a Gaussian form with parameters that are discussed further below. The number of gametes present at time t carrying alleles of type i will be

$$N_i(t) = N_{ii}g_{ii}(t) + N_{ij}g_{ij}(t)/2,$$

where N_{ii} and N_{ij} are the numbers of parental individuals of genotypes ii and ij, respectively. The total number of gametes present in the water at time t will be $N(t) = N_1(t) + N_2(t)$. We assume that these gametes unite at random, but the probability of successful zygote formation depends on the number of gametes in the water according to a function $W(N(t))$ to be discussed below. The number of successful zygotes of the three genotypes at time t will thus be

$$N_{11}(t)' = p(t)^2N(t)W(N(t)),$$

$$N_{12}(t)' = 2p(t)q(t)N(t)W(N(t)),$$

$$N_{13}(t)' = q(t)^2N(t)W(N(t)),$$

where $p(t) = N_1(t)/N(t)$ is the frequency of the i allele present at time t, and $q(t) = 1 - p(t)$. To complete we need to integrate all the newly formed zygotes over the entire spawning event to find the total number of zygotes of each type that enter the next generation. The frequency of each genotype entering the next generation becomes $p'_{11} = \frac{\int N'_{11}(t)dt}{\int N'_{11}(t)dt + \int N'_{12}(t)dt + \int N'_{22}(t)dt}$; and so forth for other genotypes.

We now have a set of recursive equations that describes the model.

The Temporal Distribution of Gametes Per Genotype

Polyps of the same coral genet, whether physically connected or not, tend to spawn at the same time over an approximately 5-min period (D. R. Levitan pers. obs. 2004). This within-genet variation is approximately Gaussian and much smaller than differences among genetic individuals (40–60 min; D. R. Levitan pers. obs. 2004). Released gamete bundles rise to the surface and slowly disperse into individual eggs and sperm over a period of approximately 10 min (D. R. Levitan, pers. obs.). These gametes then disperse and move off the reef dependent on flow conditions (Levitan 2004). The probability of encountering a gamete from this bundle in the water therefore first increases as the gamete bundle disperses to become available in a larger area, and then decreases as the gametes get diluted or move off the reef. We thus use a Gaussian to represent the temporal distribution of gametes in the water per genotype.

$$g_{ij}(t) = G_{ij}\frac{e^{-\frac{1}{2}\left(\frac{t-\delta_{ij}}{\sigma_{ij}}\right)^2}}{\sqrt{2\pi\sigma_{ij}^2}}, \quad (1)$$

where δ and σ are parameters controlling the mean and the standard deviation of the distribution, respectively. The parameter G_{ij} represents the total number of gametes released by each genotype over the spawning event. For the purposes of this article we assume that the three genotypes only differ in the mean spawning time, and thus that $G = G_{11} = G_{12} = G_{22}$, and $\sigma = \sigma_{ii} = \sigma_{ij} = \sigma_{jj}$. Unless otherwise mentioned we assume that the alleles have additive effects on the mean spawning time (i.e., $\delta_{ij} = (\delta_{ii} + \delta_{jj})/2$). Different dominance relations can be explored by varying the relationship of the heterozygote phenotype relative to the homozygote phenotypes.

The Fitness Function

In our model, fitness is determined by the probability of an egg being fertilized given a density of sperm at time t. To obtain the shape of the fitness function, we need to incorporate both positive and negative density dependence into a function that increases with sperm availability, attains a maximum, and then decreases with increasing levels of polyspermy. Based on data presented in Figure 2, we used a gamma distribution to represent this function.

$$W(N(t)) = kN(t)^a e^{-N(t)b};$$
$$a, b > 0; k = \left(\frac{eb}{a}\right)^a, \quad (2)$$

where a and b describe the shape of the gamma distribution and k rescales the function so that the maximum is 1 (i.e., 100% fertilization). More complex fertilization functions that consider polyspermy exist (Styan 1998). Although these models are more satisfying from a mechanistic perspective, they are also less tractable in the present case and do not substantially increase the fit to empirical data. With the data from Figure 2, the R^2 value increases from 72% to 83% using Styan's model, which incorporates considerably more parameters.

Model Dynamics

INVASION CRITERIA

Rare mutant invasion

We start by investigating the conditions for a rare mutant spawning-time allele to invade a monomorphic population in

which the common allele frequency is 99.9%. Without loss of generality we assume that the mean spawning time of the resident population is 0 ($\delta_{11} = 0$) and ask when a mutant with heterozygote mean spawning time δ ($\delta_{12} = \delta$) is able to invade. In Appendix A we show that this occurs if and only if the following criterion is fulfilled:

$$\int_{-\infty}^{\infty} e^{-\frac{at^2}{2\sigma^2}} e^{\frac{-\alpha e^{-\frac{t^2}{2\sigma^2}}}{\sqrt{2\pi\sigma^2}}} \left[e^{-\frac{(t-\delta)^2}{2\sigma^2}} - e^{-\frac{t^2}{2\sigma^2}} \right] dt > 0. \quad (3)$$

We are thus left with four parameters that may affect the invasion dynamics (a, α, σ, δ). The parameter α = bGN, is a measure of the strength of negative density dependence. The parameter α is thus a composite measure that combines population size (N), number of gametes released (G), and the speed of the polyspermy block (b). Small values of α correspond to weak negative density dependence. The parameters σ and δ determine the amount of gene exchange among and within genotypes. The parameter σ is a measure of the duration of the spawning event. A small σ means that the gametes of a particular genotype are released in the water during a short interval of time and thus that gene exchange among genotypes is restricted unless all the genotypes spawn close in time with each other. The parameter δ represents the mean of the spawning event for the heterozygote genotype. If δ is negative then the mutant heterozygote spawns before the resident, the opposite is true if δ is positive. We will treat the case with δ positive, the other case being identical because of the symmetry of the model. If δ is small then the mutant heterozygote spawns immediately after the resident and gene exchange among genotypes is large. If δ is large compared to σ then gametes from different genotypes will rarely encounter each other (strong assortative mating). With complete recessivity there were no qualitative changes on the invasion dynamics.

Negative density dependence (cost of polyspermy)

We start by investigating the effects of density dependence (α) on the invasion dynamics. First in equation (3) no polyspermy cost corresponds to $\alpha = 0$ (i.e., b = 0), the second exponential factor in (3) then becomes a constant equal to one. This exponential decay function is a measure of how fast the probability of fertilization decreases with increasing density. If there were no cost associated with density (i.e., no polyspermy) then we would predict that no mutant could invade. Figure 3 shows the value of (3) in white and the zero plane in black. Invasion is possible in portions of the black surface (different combinations of σ and δ) above the white plane. Not surprisingly, invasion is not possible if there is no risk of polyspermy (Fig. 3a). In the absence of polyspermy the system only experiences positive density dependence and selection favors synchronous spawning. With polyspermy the strength of negative density dependence makes invasion possible (Fig. 3b). In this particular case, invasion is possible if the temporal gamete distribution is rather narrow (i.e., all gametes are released within a short period corresponding to small values of σ) and the mutant heterozygote phenotype is not too different from the resident. Different choices of fitness function may hold different results, but if the resident phenotype is under negative density-dependent selection then invasion should still be possible for some density levels. For all the parameter combinations discussed here numerical simulations have been carried out showing that the system always evolves toward an equilibrium where the frequency of the

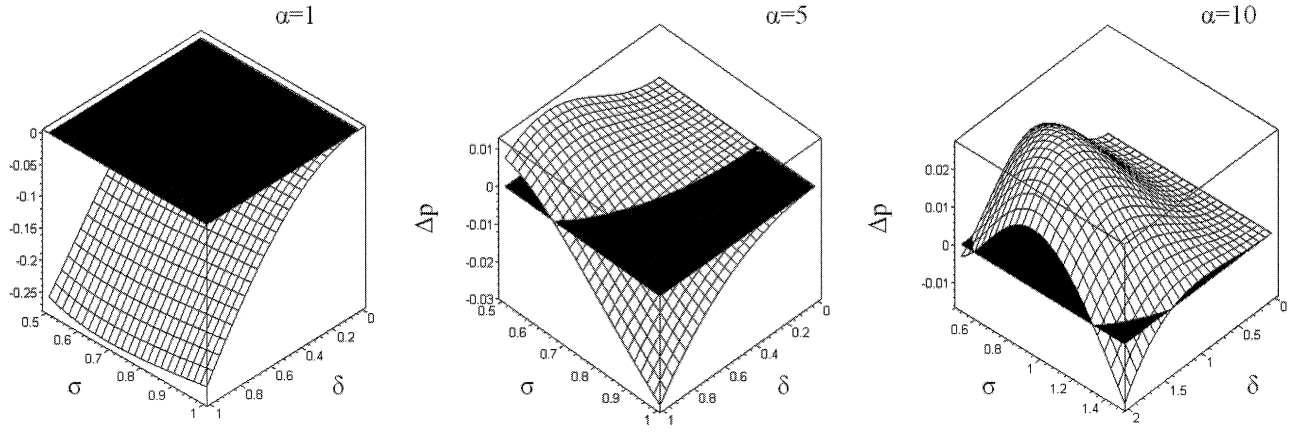

Figure 3. **Invasion criteria and negative density dependence. The x-axis shows the value of the mean spawning time of the heterozygote, δ. The y-axis shows the value of the duration of the spawning event, σ. The z-axis shows the fitness of a rare mutant (left side of equation 3); invasion occurs when this is above the black plane. From left to right, α, the strength of negative density dependence increases making invasion of a rare allele possible for a wider combination of δ and σ. (a) Very low strength of negative density dependence (no risk of polyspermy). (b) Increasing the value of α makes invasion possible for certain combination of σ and δ. (c) Increasing α invasion becomes possible over a larger combination of parameters.**

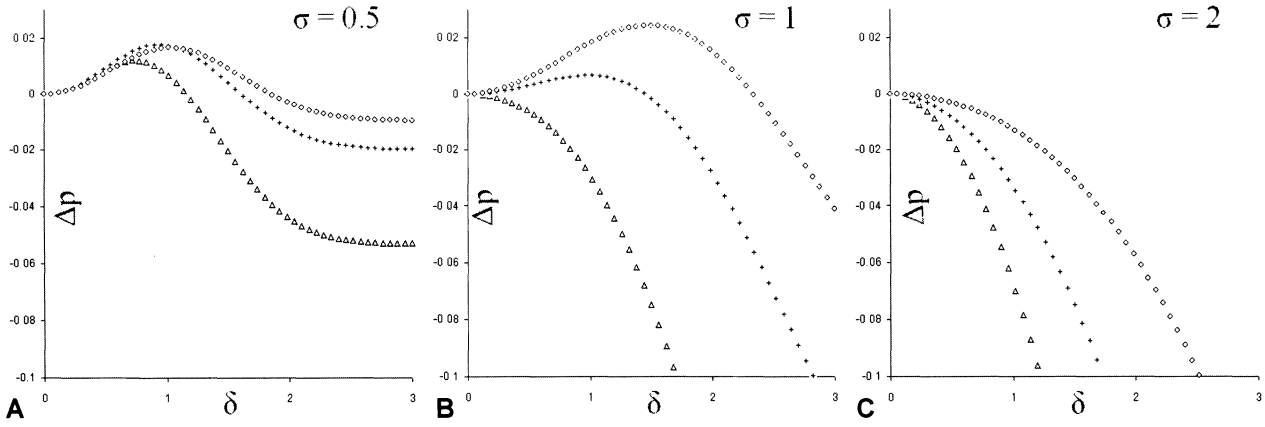

Figure 4. Invasion criteria and mutant-to-resident spawning-time difference (δ). The x-axis shows the value of the mean spawning time of the heterozygote, δ. The y-axis shows the fitness of a rare mutant (left side of equation 3). From left to right the duration of the spawning event increases (σ). Open triangles (α = 5). Crosses (α = 7.5). Open circles (α =10). a) Short duration of the spawning event (σ small). As expected stronger negative density dependence (crosses and circles) allows the invasion of a wider range of mutants. (b–c) Increasing σ even more, fewer or no mutants can invade. When σ is increased, and δ and α are left constant, invasion curves are moved down.

two alleles is 0.5. This reflects the symmetry of the model in which the alleles only have additive effects on the mean spawning time.

Gene exchange among and within genotypes

In the previous section we saw how density affects the invasion dynamics. Now we discuss the impact of gene exchange among and within genotypes. The gene exchange is controlled by the width of a genotype's temporal gamete distribution (σ) and the difference between mutant and resident in their mean spawning time (δ). It is not the absolute values of these parameters that matter, but rather the relation between them. For instance, invasion may be possible if a mutant that spawns very close to the resident occurs in a population with a very short spawning duration.

We therefore focus on how a change in the difference between the spawning times of mutant and resident (δ) affects the invasion dynamics. A successful mutant is one that spawns to avoid polyspermy. A mutant will invade if it spawns before or after most of the resident individuals have spawned, in this way the mutant's eggs will not suffer from polyspermy. If the duration of the spawning event is short it follows that a "good" mutant needs to spawn relatively close to the mean of the population (δ small). Conversely if a mutant spawns too far from the population mean then most of its gametes would not be fertilized due to the absence of their counterpart from the resident population. The exact details of invasion rely heavily on the strength of polyspermy. In Figures 4 and 5 the mutant-to-resident invasion criteria are shown for different parameter combinations.

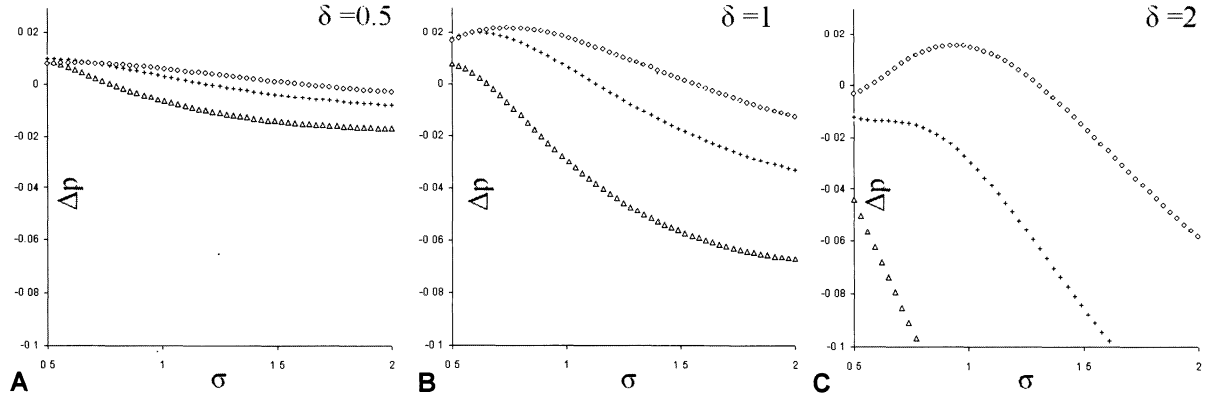

Figure 5. Invasion criteria and spawning event duration (σ). The x-axis shows the value of the duration of the spawning event, σ. The y-axis shows the fitness of a rare mutant (left side of equation 3). From left to right the value of the mean spawning time of the heterozygote, δ, increases. Open triangles (α = 5). Crosses (α = 7.5). Open circles (α = 10). We look for regions in which the curves are positive. (a) Mutant-to-resident difference small (δ). Invasion is possible only if the spawning event duration is short. (b) As δ increases a longer duration of the spawning event allows for invasion provided that the strength of density dependence is not too weak. (c) As the mutant spawns even further apart invasion is possible only for higher risk of polyspermy and longer duration of the spawning event.

Temporal Isolation

Information about successful invaders does not tell us if and when temporal isolation evolves. An invasion could evolve, for instance, toward a polymorphic equilibrium where the heterozygote frequency is higher than both homozygotes. We thus take it as a criterion for reproductive isolation whenever the frequency of the heterozygote falls below an arbitrary threshold ($p_{12} < 0.01$). We looked at the long-term dynamics for a wide combination of σ and δ for a given strength of negative density dependence ($\alpha = 100$). In all cases temporal isolation evolved when the ratio of δ to σ was roughly between 1 and 2. To understand this, consider first cases in which the ratio is smaller than one; that is, cases in which the standard deviation of the breeding distribution is larger than the mean difference between the mutant heterozygote and the resident. In this situation the mutant spawns close to the rest of the population and its gametes have only a small advantage compared to the resident gametes. Although invasion is still possible (because of negative density dependence), the equilibrium frequency of the heterozygote remains elevated. The equilibrium frequency decreases with increasing values of the ratio δ to σ. When this ratio becomes larger than one then invasion is possible and temporal isolation evolves, because the mutant spawns at the tail of the resident distribution it can still be fertilized by resident sperm, and its eggs avoid polyspermy. If the ratio becomes slightly larger than 2 the heterozygote frequency increases or the system shows periodical oscillations. The goal of this article is, however, to investigate the conditions under which temporal isolation is possible and therefore we do not investigate parameter combinations that result in oscillations. Figure 6 shows the equilibrium frequencies of the heterozygote as a function of the strength of negative density dependence. If the risk of polyspermy is low (middle graph) then temporal isolation is possible for a narrow combination of parameters, namely short spawning event and small mutant to resident difference. If we increase the risk of polyspermy, however, then temporal isolation evolves for a wider parameter range.

Maintenance Of Temporal Reproductive Isolation

We now investigate if two temporally isolated genotypes will resist invasion from yet another mutant that spawn at some intermediate time between the two resident genotypes present in the population. We assume that temporal isolation has already been established between two different alleles and we look at the fate of a third rare mutant whose spawning time is somewhere in between the two residents. Equation (4) shows the criteria for invasion of an intermediate rare allele as derived in Appendix B.

$$\int_{-\infty}^{\infty} \left(e^{-\frac{a(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{a(t-\delta_{22})^2}{2\sigma^2}} \right) e^{-\frac{\alpha}{2\sqrt{2\pi\sigma^2}}\left(e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}}\right)} \times \left[N_{13} e^{-\frac{(t-\delta_{13})^2}{2\sigma^2}} + N_{23} e^{-\frac{(t-\delta_{23})^2}{2\sigma^2}} - \frac{(N_{13}+N_{23})}{2} \left(e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right) \right] dt > 0. \quad (4)$$

The residents' spawning times are δ_{11} and δ_{22}, while the two mutant heterozygotes spawn at times δ_{13} and δ_{23}. Graphical analysis and numerical simulations show that no other allele can invade if temporal isolation has evolved. The resistance to invasion holds because the spawning period between the two resident homozygote phenotypes corresponds to the period during which most of the gametes suffer from polyspermy. This is true regardless of the dominance relations among the alleles. Conditions for temporal reproductive isolation are thus stable as long as the population

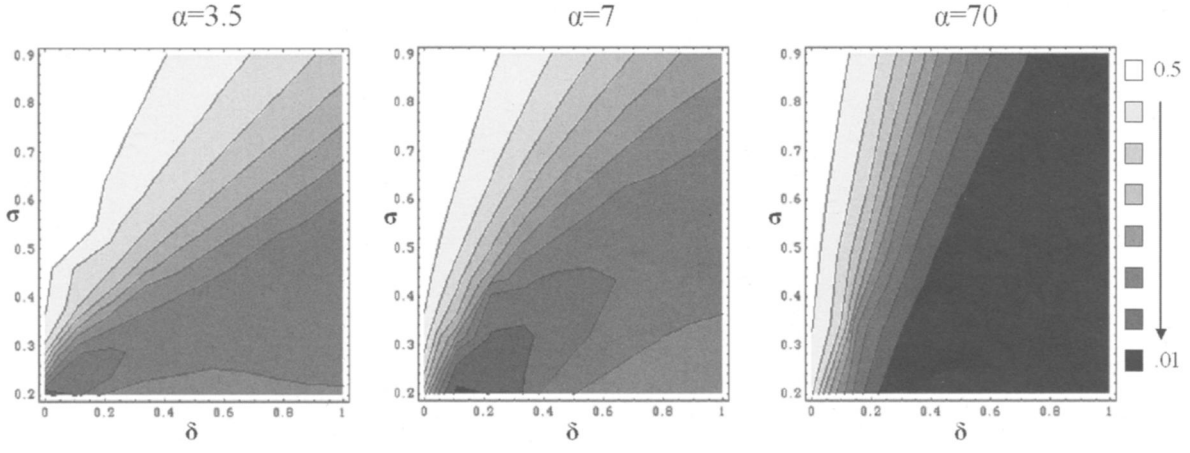

Figure 6. Speciation regions. The x-axis shows the value of the mean spawning time of the heterozygote, δ. The y-axis shows the value of the duration of the spawning event, σ. The value of a cell is the heterozygote equilibrium frequency. From left to right the strength of negative density dependence, α, increases. Dark regions correspond to low heterozygote frequency.

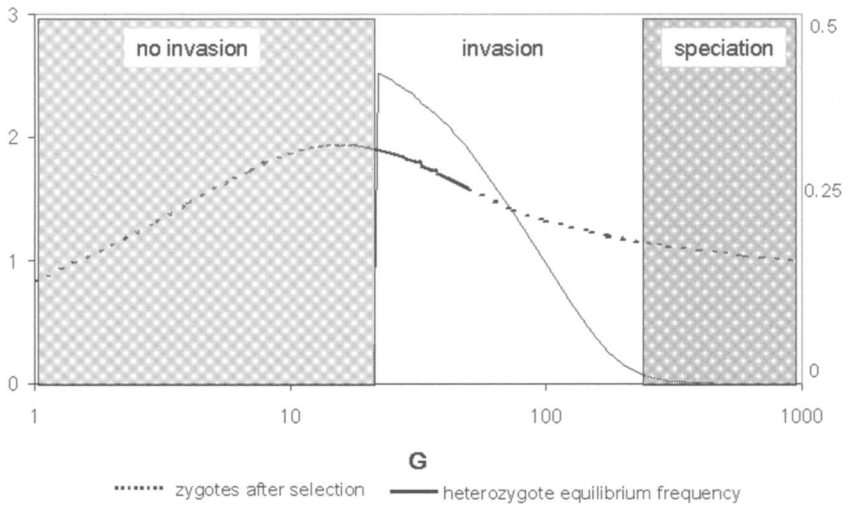

Figure 7. Invasion and speciation as functions of gamete densities. The x-axis is the number of gametes G, on the logarithmic scale (i.e., α from 0.07 to 70). The dotted curve, with scale on the left, represents the fitness of zygotes as a function of density, and was obtained by numerically integrating the fitness function over the spawning period. Heterozygote equilibrium frequency (continuous line) refers to the vertical axis on the right. The mutant-to-resident spawning-time difference and the spawning event duration are kept constant ($\delta = 0.5$, $\sigma = 0.4$).

density does not change. In other words the parameter values that allow temporal reproductive isolation to evolve also prevent invasion from any mutant in between the established residents.

Ecological Robustness

We have shown that temporal isolation may evolve under certain constant ecological conditions. In reality, however, there will be large fluctuations in any of the many factors assumed to be constant in our model. This may include changes in reproductive output, water turbidity, environmental cues, etc. Such changes may undermine our results, for example by changing the probability of polyspermy at a given sperm density, or by allowing gene exchange to take place under certain environmental conditions, but not under others. Here we assess the robustness of our results on the invasion dynamics to environmental fluctuations in density. We used graphical analysis (Fig. 7) to assess the importance of density changes. Invasion of a rare mutant is possible only when density falls in the negative density dependence region of the curve representing the total number of surviving zygotes after selection (i.e., the region of the curve with negative slope). Speciation occurs with higher density. These results suggest that the likelihood of establishing reproductive isolation can be sensitive to fluctuations in spawning densities. In particular knowledge of both qualitative (distribution of fluctuations) and quantitative levels (magnitude of fluctuations) of variation in density is needed to assess the sensitivity of our results.

Discussion

It has been proposed that for sympatric speciation to occur the combined strength of assortative mating and disruptive selection has to exceed a threshold (Gavrilets 2004, p. 342; Bürger et al. 2006). In our model the degree of assortative mating is controlled by the level of gene exchange among genotypes with different spawning-time distributions, and disruptive selection is caused by negative density dependence caused by polyspermy. We have shown that the combination of these mechanisms makes temporal isolation theoretically possible. We have not proven that polyspermy has caused speciation in corals, only that this is a theoretical possibility if the conditions are right. Other biological mechanisms could act in combination with polyspermy that would increase the likelihood of speciation. Predators, for instance, could congregate during the middle of the spawning event to the advantage of the gametes at the tails of the spawning-time distribution.

Eggs possess blocks to polyspermy that can ameliorate the conflict between male and female function (Styan 1998; Franke et al. 2002; Levitan 2004). An efficient polyspermy block would prevent all but one spermatozoan from fusing with the egg and eliminate the cost of developmental failure at high sperm concentrations. However, in spite of these blocks, polyspermy is noted in laboratory and field conditions, and there is variation in the efficiency of these blocks among females and species. At least in the well-studied echinoids, this variation is correlated with ease of fertilization. Eggs that require a low sperm concentration to achieve fertilization are also more susceptible to polyspermy (Levitan et al. 2007). These patterns are correlated among congeneric sea urchins to species differences in crowding. The species found at sperm-limiting, low densities have eggs that are easy to fertilize, but susceptible to polyspermy, whereas the species found at, sperm-competitive, high densities have eggs that require high densities of sperm for fertilization, but are resistant to polyspermy

(Levitan et al. 2007). Even across species with different compatibility proteins, ease of hybridization is related to susceptibility to polyspermy. Divergent recognition proteins increase the threshold sperm concentration for polyspermy, but do not eliminate the possibility (Levitan et al. 2007).

Assuming that the efficiency of polyspermy is a density-dependent trait, the likelihood of polyspermy being a strong selective force driving the evolution of reproductive isolation may be linked to historic sperm availability. As population density increases, any trait that reduces the cost to polyspermy may be selected, be it shifts in spawning time or a more resistant block to polyspermy. However, even the most resistant populations (caused by efficient polyspermy blocks or by polymorphic recognition proteins) will have a threshold spawning density at which eggs succumb to polyspermy, and shifts in spawning behavior are the only escape. It is not yet clear if these thresholds are regularly exceeded in nature. Currently, both worldwide and especially in the Caribbean, coral populations have decreased dramatically for a variety of reasons generally attributed to human influences (e.g., Aronson et al. 2003; Pandolfi et al. 2003). Thus sperm limitation may be more common and polyspermy less common than in the past history of the species. One prediction from our model is that we should see an increase in hybrid fertilization between partially temporally isolated morphs if there is a decrease in local population density. In accordance with this scenario, Vollmer and Palumbi (2002) provide some examples of introgression among decimated coral species with compatible gametes.

If the timing of reproduction is genetically variable, as it often is, then gene exchange should be limited between different genotypes even within a single generation (Hendry and Day 2005). This may represent an intermediate step to temporal isolation, but is neither the beginning nor the end. A selective force must act to initiate the process of temporal lineage split, in our model represented by negative density-dependent selection, and a selective cost must be present to complete or maintain the evolved differences in the timing of reproduction.

The theoretical framework we presented should also apply to the evolution of temporal isolation in flowering plants. Outcrossing plants need to synchronize the release of their gametes to increase the probability of successful pollination. Polyspermy is known to occur in plants (Vigfússon 1970), but is thought to be rare (Scott 2007). Pollinator limitation, however, is widely reported (Burd 1994), and could lead to negative frequency dependence. Consider, for example, a population of flowering plants with individuals that flower at a given time. Such a population should be adapted to the most effective pollinators present in the area during that time. The presence of a limited number of pollinators could then initiate a process of temporal isolation between genotypes that flower or are attractive to pollinators during different days, or at different times during the day. This would occur because individuals flowering during the peak flowering time have on average a lower probability of pollinating (or being pollinated) because of pollinator limitation.

Temporal reproductive isolation is a theoretical possibility in all biological systems with a limited mating duration. Systems in which gamete release needs to occur synchronously, such as marine broadcast spawners or flowering plants, are, however, the most likely candidates to evolve temporal isolation, not just as a byproduct of allopatric isolation, but also as a consequence of disruptive selection.

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Appendix A. Invasion Dynamics

Following the model notation, invasion dynamics will be characterized by the change of a rare mutant gene frequency (p_2) that is present primarily in the heterozygote form (i.e., $p_2 = N_{12}/2n$). Using some algebra we find that

$$\Delta p_2 = \frac{\int_{-\infty}^{\infty} N_2(t)W(N(t))dt}{\int_{-\infty}^{\infty} N(t)W(N(t))dt} - \frac{N_{12}}{2N}. \qquad \text{(A1)}$$

We can write the numerator as follows

$$\int_{-\infty}^{\infty} N_2(t)W(N(t))dt = \frac{N_{12}G}{2\sqrt{2\pi\sigma^2}}\left(\frac{eb}{a}\right)^a\left(\frac{NG}{\sqrt{2\pi\sigma^2}}\right)^a \times \int_{-\infty}^{\infty} e^{-\frac{a(t)^2}{2\sigma^2}} e^{\frac{-bGNe^{-\frac{(t)^2}{2\sigma^2}}}{\sqrt{2\pi\sigma^2}}} e^{-\frac{(t-\delta)^2}{2\sigma^2}} dt,$$

where $N(t)$ is the number of gametes present in the water at time t and can be well approximated by considering only the gametes carrying the resident allele. The denominator can thus be written as

$$\int_{-\infty}^{\infty} N(t)W(N(t))dt = \frac{NG}{\sqrt{2\pi\sigma^2}}\left(\frac{eb}{a}\right)^a\left(\frac{NG}{\sqrt{2\pi\sigma^2}}\right)^a \times \int_{-\infty}^{\infty} e^{-\frac{a(t)^2}{2\sigma^2}} e^{\frac{-bGNe^{-\frac{(t)^2}{2\sigma^2}}}{\sqrt{2\pi\sigma^2}}} e^{-\frac{t^2}{2\sigma^2}} dt.$$

Canceling out terms we obtain the following criterion for increase of the rare mutant

$$\frac{N_{12}}{2N}\frac{\int_{-\infty}^{\infty} e^{-\frac{a(t)^2}{2\sigma^2}} e^{\frac{-bGNe^{-\frac{(t)^2}{2\sigma^2}}}{\sqrt{2\pi\sigma^2}}} e^{-\frac{(t-\delta)^2}{2\sigma^2}} dt}{\int_{-\infty}^{\infty} e^{-\frac{a(t)^2}{2\sigma^2}} e^{\frac{-bGNe^{-\frac{(t)^2}{2\sigma^2}}}{\sqrt{2\pi\sigma^2}}} e^{-\frac{t^2}{2\sigma^2}} dt} - \frac{N_{12}}{2N} > 0.$$

Multiplying by $2N/N_{12}$ and collecting all terms into a single integral we find

$$\int_{-\infty}^{\infty} e^{-\frac{a(t)^2}{2\sigma^2}} e^{\frac{-\alpha e^{-\frac{t^2}{2\sigma^2}}}{\sqrt{2\pi\sigma^2}}} \left[e^{-\frac{(t-\delta)^2}{2\sigma^2}} - e^{-\frac{(t)^2}{2\sigma^2}} \right] dt > 0, \qquad \text{(A2)}$$

where $\alpha = \text{bGN}$. Equation (A2) is equation (3) in the main text.

Appendix B. Stability of Temporal Reproductive Isolation

In this section we assume that the population consists of two temporally isolated homozygote genotypes present in equal frequency and we investigate the fate of a rare mutant that spawns somewhere in between the two residents. Let us label the three alleles present in the population as 1 and 2 for the residents and 3 for the rare mutant. The population thus consists of two resident genotypes (11 and 22) and two mutant genotypes (13 and 23). Under these assumptions the composition of the gamete pool at time t is approximately

$$N(t) \approx \frac{N}{2}\left[g_{11}(t) + g_{22}(t)\right] \quad \text{and} \quad N_3 = \frac{(N_{13} + N_{23})}{2}.$$

Using some algebra we find that

$$\Delta p_3 = \frac{\int_{-\infty}^{\infty} N_3(t) W(N(t))\, dt}{\int_{-\infty}^{\infty} N(t) W(N(t))\, dt} - \frac{N_3}{N}. \qquad \text{(B1)}$$

As usual for invasion we need $\Delta p > 0$

$$\frac{\int_{-\infty}^{\infty} W(N(t)) \left[\frac{1}{2} N_{13} g_{13}(t) + \frac{1}{2} N_{23} g_{23}(t)\right] dt}{\int_{-\infty}^{\infty} \left(\frac{N}{2} g_{11}(t) + \frac{N}{2} g_{22}(t)\right) W(N(t))\, dt} - \frac{N_3}{N} > 0.$$

We can write the numerator of the integral fraction as follows

$$\begin{aligned}\int_{-\infty}^{\infty} N_3(t) W(N(t))\, dt = {} & \frac{G}{2\sqrt{2\pi\sigma^2}} \left(\frac{eb}{a}\right)^a \left(\frac{NG}{\sqrt{2\pi\sigma^2}}\right)^a \\ & \times \int_{-\infty}^{\infty} \left[N_{13} e^{-\frac{(t-\delta_{13})^2}{2\sigma^2}} + N_{23} e^{-\frac{(t-\delta_{23})^2}{2\sigma^2}} \right] \\ & \times \left[e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right]^a \\ & \times e^{\frac{-bGN}{2\sqrt{2\pi\sigma^2}} \left(e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right)} dt.\end{aligned}$$

The denominator of the integral fraction can be written as

$$\begin{aligned}\int_{-\infty}^{\infty} N(t) W(N(t))\, dt = {} & \frac{NG}{2\sqrt{2\pi\sigma^2}} \left(\frac{eb}{a}\right)^a \left(\frac{NG}{2\sqrt{2\pi\sigma^2}}\right)^a \\ & \times \int_{-\infty}^{\infty} \left[e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right] \\ & \times \left[e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right]^a \\ & \times e^{\frac{-bGN}{2\sqrt{2\pi\sigma^2}} \left(e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right)} dt\end{aligned}$$

Canceling out terms we obtain

$$\frac{\int_{-\infty}^{\infty} \left[N_{13} e^{-\frac{(t-\delta_{13})^2}{2\sigma^2}} + N_{23} e^{-\frac{(t-\delta_{23})^2}{2\sigma^2}} \right] \left[e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right]^a e^{\frac{-bGN}{2\sqrt{2\pi\sigma^2}} \left(e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right)} dt}{N \int_{-\infty}^{\infty} \left[e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right] \left[e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right]^a e^{\frac{-bGN}{2\sqrt{2\pi\sigma^2}} \left(e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right)} dt} - \frac{N_3}{N} > 0; \qquad \text{(B2)}$$

Multiplying by N, collecting all terms into a single integral, and defining $\alpha = \text{bGN}$, we find

$$\begin{aligned}& \int_{-\infty}^{\infty} \left(e^{-\frac{a(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{a(t-\delta_{22})^2}{2\sigma^2}} \right) e^{-\frac{\alpha}{2\sqrt{2\pi\sigma^2}} \left(e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right)} \\ & \quad \times \left[N_{13} \left(e^{-\frac{(t-\delta_{13})^2}{2\sigma^2}} - \frac{1}{2} \left(e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right) \right) \right] dt \\ & + \int_{-\infty}^{\infty} \left(e^{-\frac{a(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{a(t-\delta_{22})^2}{2\sigma^2}} \right) e^{-\frac{\alpha}{2\sqrt{2\pi\sigma^2}} \left(e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right)} \\ & \quad \times \left[N_{23} \left(e^{-\frac{(t-\delta_{23})^2}{2\sigma^2}} - \frac{1}{2} \left(e^{-\frac{(t-\delta_{11})^2}{2\sigma^2}} + e^{-\frac{(t-\delta_{22})^2}{2\sigma^2}} \right) \right) \right] dt > 0. \qquad \text{(B3)}\end{aligned}$$

Rearranging (B3) we obtain equation (4) in the text. If (B3) is positive the mutant is able to invade. We only need to sample

the region of the parameter space for which temporal reproductive isolation evolves. For this section we set α to 70 and numerically investigate if invasion is possible for values of δ and σ that result in temporal isolation (black zone in the right panel of Fig. 6). Results from numerical simulations (not shown) indicate that the value of (B4) is always negative and thus invasion is not possible.