The Evolution of Reproductive Phenology in Broadcast Spawners and the Maintenance of Sexually Antagonistic Polymorphism

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Submitted July 19, 2016; Accepted September 29, 2016; Electronically published December 19, 2016

Online enhancements: appendixes, zip file.

abstract: Reproductive phenology is a crucial life-history trait that evolves in response to external environmental conditions and frequency- and density-dependent interactions within species. Broadcast spawners—which represent a large fraction of aquatic biodiversity—evolve phenologies that balance strong density-dependent fertilization success against abiotic environmental conditions that are required for successful reproduction. The overall balance between these processes may be particularly complex in dioecious species, where selection on reproductive timing potentially differs between the sexes. Here, we develop a population genetic model of reproductive phenology in a dioecious broadcast spawning species and show that environmental variability and density-dependent fertilization dynamics naturally give rise to profound sex differences in selection on gamete release strategies. The frequency-dependent nature of sperm competition generates sexually antagonistic selection on reproductive timing and facilitates the maintenance of genetic variation in phenological traits. Selection in females favors monomorphic spawning phenologies that maximize net fertilization success and offspring survival across environmental conditions, whereas selection in males often favors polymorphic phenologies that are primarily shaped by sperm competition. Our model helps explain several well-documented empirical observations in aquatic species, including high intraspecific variance of reproductive phenologies, sex-specific spawning phenologies, and spawning during environmentally suboptimal times.

Keywords: sexual antagonism, sexual conflict, phenology, frequency-dependent selection, sperm competition, external fertilization.

Introduction

Reproductive phenology is a pivotal life-history trait influencing the evolution of plant and animal species. The timing of reproduction determines mating opportunities (Brunet and Charlesworth 1995; Hendry and Day 2005; Elzinga et al. 2007), the environmental conditions experienced by mating adults and their offspring (Cushing 1969, 1990; Brunet and Charlesworth 1995; Elzinga et al. 2007), as well as each individual's energy budget for growth, reproduction, and development (Stearns 1992; Ejsmond et al. 2010). Indeed, many species experience strong stabilizing selection for reproduction to coincide with environmental conditions that are ideal for mating success and offspring survival (Morgan and Christy 1995; Durant et al. 2007; Lowerre-Barbieri et al. 2011). On the other hand, stochastic environmental conditions and frequency- and density-dependent effects that arise during mating can potentially give rise to selection for increased within-individual variance in phenology (Iwasa and Haccou 1994; Devaux and Lande 2010). For example, while positive density dependence in per capita mate encounter, pollinator visitation, and fertilization rates should select for increased reproductive synchrony between members of a population (Allee et al. 1949; Augspurger 1981; Tomaiuolo et al. 2007; Devaux and Lande 2010), negative frequency- and density-dependent mating success arises when individuals are forced to compete for limited mates, pollinators, or fertilizations; these factors can also generate selection for increased variance in phenology (Fisher 1930; Birkhead and Møller 1998; Levitan 2004; Tomaiuolo et al. 2007; Devaux and Lande 2010).

Although frequency- and density-dependent effects are important for a wide range of aquatic and terrestrial taxa, they are likely to be particularly strong and complex in broadcast spawning species (Levitan 1991, 2002, 2004; Rouse and Fitzhugh 1994; Marshall 2002). Most aquatic organisms shed eggs and sperm directly into the water column (Monro and Marshall 2015), and the consequences of reproductive timing are critical. Spawning strategies must take into account both the availability of mates and fertilization opportunities, as well as the favorability of environmental conditions, which vary over time. As we outline below, reproductive phenology strategies that maximize fitness in broadcast spawning males will often differ from those that maximize female fitness. In this context, pleiotropic effects of loci that influence reproductive timing within both sexes may give rise to sexually antagonistic selection (or intralocus sexual conflict), in which the alleles that benefit one sex prove del-

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Am. Nat. 2017. Vol. 189, pp. 000–000.
DOI: 10.1086/690010

eterious when expressed by the other (Kidwell et al. 1977; Rice and Chippindale 2001; Bonduriansky and Chenoweth 2009; Connallon and Clark 2014*b*). Developmental genes underlying phenological traits—such as the age of sexual maturity and the timing of reproduction—may experience both differential expression between the sexes and sexually antagonistic selection (McEuan 1988; Levitan 2004; Lotterhos and Levitan 2011; Barson et al. 2015). The timing of gamete release in broadcast spawners therefore provides a broadly relevant arena for examining selection on sexually antagonistic alleles within an explicit ecological context. Importantly, much research on aquatic systems has focused on the consequences of temporally variable environmental conditions and population density for external fertilization and larval mortality (Denny and Shibata 1989; Marshall 2002; Levitan 2004). This ultimately permits us to link the population genetic processes of sex-secific selection to key ecological drivers of the timing of reproduction.

Although external fertilizers are extraordinarily diverse and taxonomically widespread across the tree of life, we focus on the archetypal external fertilizer—the broadcast spawner—which includes seaweeds, corals, sea stars, and many fish taxa. Broadcast spawning species constitute >50% of marine invertebrates worldwide (Monro and Marshall 2015) and have long served as classic models for the evolution of anisogamy (Parker et al. 1972; Parker 1982). Spawning in broadcasting species has historically been thought to coincide precisely with favorable environmental conditions (e.g., Thorson 1936; Cushing 1969; Harrison et al. 1984; Babcock et al. 1986; Clifton 1997), yet many species exhibit greater variation in reproductive phenology than is expected under this view (McEuan 1988; Marshall 2002; Levitan 2005; Lotterhos and Levitan 2011). Furthermore, many broadcast spawners exhibit protandry, where males release gametes earlier and for longer than females do (McEuan 1988; Levitan 2005; Lotterhos and Levitan 2011). High variance in population phenologies—coupled with sexually dimorphic gamete release strategies—suggests that frequency-dependent, density-dependent, and sex-specific selection may each play critical roles during the evolution of reproductive timing.

For broadcast spawners, individual reproductive success is determined by local gamete concentrations and the kinetics of fertilization. The probability of successful fertilization is governed by the density and reproductive strategies of spawning individuals as well as local hydrodynamic conditions (Denny and Shibata 1989; Levitan 1991, 2002, 2004; Marshall 2002). At low sperm concentrations or rapid advection, the probability of contact between an egg and sperm is low, resulting in sperm-limited fertilization dynamics. At the opposite end of the spectrum, high local sperm concentrations can severely increase egg mortality because of polyspermy, as documented in several aquatic systems (Brawley 1992; Franke et al. 2002; Marshall 2002; Levitan 2004; Levitan et al. 2004). Overall, we expect a bell-shaped relation between population density and the probability of successful fertilization (Millar and Anderson 2003). Sperm-limited contexts should generate sexually concordant selection for increased reproductive synchrony between the sexes, resulting in sperm concentrations that mirror the availability of unfertilized eggs (Tomaiuolo et al. 2007). At high population densities, egg mortality due to polyspermy may lead to strong negative density-dependent selection on spawning time and conflicting selection between the sexes with respect to spawning time (Bode and Marshall 2007; Tomaiuolo et al. 2007; Olito et al. 2015). In synchronously spawning populations, sperm competition may favor the evolution of male gamete release strategies that lead to high rates of polyspermy, suboptimal rates of fertilization, and depressed female fitness (Bode and Marshall 2007; Tomaiuolo et al. 2007; Olito et al. 2015). The evolution of sexually dimorphic reproductive phenologies can potentially resolve this form of sexual conflict. However, such resolutions may involve nontrivial fitness trade-offs between temporally variable sperm concentrations and environmental conditions as well as genetic constraints on the evolution of sexually dimorphic phenotypes (Bode and Marshall 2007; Olito et al. 2015).

In this article, we develop a simple theoretical population genetic model of sex-specific selection on the timing of gamete release phenotypes in a broadcast spawning species. We then explore the robustness of the population genetic model predictions with individual-based quantitative genetic simulations. The models account for environmental variability over time as well as frequency- and density-dependent dynamics of male and female fertilization success. Our models make two important advances on previous theory. First, unlike previous population genetic models of sexually antagonistic selection, we allow selection in both sexes to emerge from underlying processes of frequency- and density-dependent fertilization and spawning phenology. Our models provide a more ecologically grounded approach than classic population genetics models, which typically assign fitness values to genetic variants arbitrarily rather than explicitly modeling them (see Orr 2005). Second, while previous models of the evolution of phenologies generally assume a perfect correlation between male and female phenotypes (Rathcke and Lacey 1985; Tomaiuolo et al. 2007; Devaux and Lande 2008, 2010; but see Iwasa and Haccou 1994; Brunet and Charlesworth 1995), our population genetic models and quantitative genetic simulations explicitly address the consequences of sexually dimorphic gene expression for the maintenance of genetic variation underlying reproductive phenology. Our new models directly explore the influence of frequency-dependent sexually antagonistic selection on the maintenance of polymorphism and the evolution of reproductive phenology, an antagonistically selected life-history trait.

Methods

Our population genetic model follows the evolution of a dioecious population of broadcast spawners. All individuals are haploid, and generations are discreet and nonoverlapping. Our results are nevertheless likely to apply more generally to hermaphrodite populations that are undergoing obligate outcrossing (Jordan and Connallon 2014). We investigate the conditions permitting balanced polymorphism for a pair of alleles at a single locus that influences reproductive phenology. We do not model long-term evolution under recurrent mutations or substitutions. Hence, our models allow us to identify the evolutionary scenarios that are most and least likely to maintain polymorphism, but they do not assume that a polymorphic state is uninvadable or evolutionarily stable. Indeed, recent theory suggests that adaptation may typically involve the successive invasion of alleles subject to transient balancing selection (Sellis et al. 2011). Furthermore, predictions regarding the long-term evolutionary dynamics from our models would require the strong assumption that population densities and environmental conditions remain stable over similar timescales.

We assume that the ancestral state of the population is sexually monomorphic with regard to spawning phenology. Conditions affecting density-independent offspring and zygote survival are temporally variable, and the reproductive season consists of two time points: one corresponding to the environmental optimum (t_0) and the other corresponding to a suboptimal time (t_1). All individuals are assumed to have equal resources available for gamete production. Our analysis focuses on the genotypic frequencies ($1 - p$ and p) of wild-type (B_1) and mutant (B_2) alleles at a single locus influencing the proportion of an individuals' gametes that are released at each time point (for a full description of terms, see table 1). Within this framework, polymorphism is proportional to among-individual variance in phenology, while intermediate phenotypes are analogous to within-individual variance. Changes in allele frequencies between generations can be caused by two different sources of selection: (1) selection for fertilization success, which is frequency- and density-dependent, and (2) density-independent viability selection based on offspring survival at each time point.

Table 1: Key terms and parameters

Term	Description
B_1, B_2	Wild-type and mutant alleles
γ, γ^*	Phenotypic values of wild-type and mutant alleles, respectively, for the equal effects and sex-limited models
γ_f, γ_m, γ_f^*, γ_m^*	Phenotypic values of wild-type and mutant alleles for the sex-specific model
$1 - p$, p	Frequency of wild-type and mutant alleles, respectively
n_f, n_m	Effective population density for females and males, respectively
A	Fertilization function shape parameter
d	Difference in environmental quality between time points

Fertilization Dynamics

A variety of fertilization kinetics models have been derived for external fertilizers. Such models predict the probability of mono- and polyspermic fertilizations as a function of sperm concentration and species-specific gametic traits (Millar and Anderson 2003). For generality and analytic tractability, we model fertilization success using a modified Ricker equation that gives the same functional form of density-dependent fertilization probability as fertilization kinetics models (after Bode and Marshall 2007; Olito et al. 2015). The probability of successful fertilization is described by the function

$$F(s_t) = \left(\frac{As_t}{2}\right)^2 e^{2-As_t}, \quad (1)$$

where s_t is the total amount of sperm released at time t_i by all males and A is a positive shape parameter that determines the steepness of the curve and the location of the maximum of the fertilization function. Variation in A changes the fertilizability of eggs, analogous to changes in egg size or the speed of the polyspermy block (Bode and Marshall 2007; Olito et al. 2015). This changes the population density thresholds at which sperm limitation and polyspermy occur but does not qualitatively alter the behavior of the models. Although the traits influencing A certainly evolve jointly with phenologies, we focus on the joint evolution of male and female phenologies, given a fixed value of A, and we present results for only the representative case of $A = 0.1$. As in previous models of fertilization dynamics, we assume that sperm greatly outnumber eggs and, therefore, that fertilization probability is independent of egg availability or female density (Vogel et al. 1982; Millar and Anderson 2003). That sperm concentration dominates the fertilization dynamics in external fertilizers has been experimentally demonstrated by numerous studies in diverse taxa (Rothschild and Swann 1951; Vogel et al. 1982; Levitan 1998, 2004; Marshall et al. 2000; Millar and Anderson 2003). While this assumption may be violated if sex ratios are strongly skewed toward females and sperm concentrations are extremely low, cases where eggs outnumber sperm are unlikely. Moreover, populations where this occurs will make minimal contributions to population production because of low fertilization success. We therefore focus our analyses on the scenario where sperm greatly outnumber eggs.

A Two-Sex, Two-Time-Point Haploid Model of Selection

We begin with a model in which the phenotypic effects of alleles are perfectly correlated between the sexes (hereafter the equal effects model). We subsequently relax this assumption and consider alternative models where phenotypic effects of the alleles differ between the sexes. The phenotypic effects associated with the wild-type (B_1) and mutant (B_2) alleles are γ and γ^*, respectively, which define the proportion of an individual's gametes that are released at the environmentally unfavorable time point (t_1); $1 - \gamma$ and $1 - \gamma^*$ represent the proportion of gametes released at the favorable time point (t_0). Therefore, among the gametes released at each time point (t_0 and t_1, respectively), the frequency of the mutant genotype is

$$t_0 : g_0 = \frac{p(1-\gamma^*)}{(1-p)(1-\gamma) + p(1-\gamma^*)}, \tag{2a}$$

$$t_1 : g_1 = \frac{p\gamma^*}{(1-p)\gamma + p\gamma^*}. \tag{2b}$$

We define the effective density of males and females as follows. Let $n_m = N_m a_m$ represent the effective density of males, which takes into account both the number of males (N_m) and the total amount of sperm released per male (a_m). Likewise, the effective density of females is $n_f = N_f a_f$, where N_f is the number of females and a_f is the total number of eggs released per female. For simplicity, we use the terms "population density," "male density," and "female density" in the results, but in all cases we are referring to effective density, which takes into account the number of individuals as well as the contributions of each to the gamete pool.

Expressions for effective density can be used to quantify the total number of gametes released by females and males at each time point. The total numbers of sperm released at the two time points are $s_0 = n_m[(1-p)(1-\gamma) + p(1-\gamma^*)]$ for t_0 and $s_1 = n_m[(1-p)\gamma + p\gamma^*]$ for t_1. The number of eggs released at each time point follow from similar expressions. Combining with equation (1), the numbers of successfully fertilized eggs at each time point are calculated as

$$t_0 : n_0 = n_f[(1-p)(1-\gamma) + p(1-\gamma^*)] \times F\{n_m[(1-p)(1-\gamma) + p(1-\gamma^*)]\}, \tag{3a}$$

$$t_1 : n_1 = n_f[(1-p)\gamma + p\gamma^*] \times F\{n_m[(1-p)\gamma + p\gamma^*]\}, \tag{3b}$$

where $F(\cdot)$ is the fertilization function described in equation (1). Successfully fertilized eggs at t_1 experience density-independent mortality at a rate $d \in [0, 1]$, resulting from suboptimal environmental conditions. Thus, d defines the steepness of the environmental gradient. At census, the change in frequency of the mutant allele (B_2) due to both sources of selection is

$$\Delta p = g_0 \frac{n_0}{n_0 + n_1(1-d)} + g_1 \frac{n_1(1-d)}{n_0 + n_1(1-d)} - p. \tag{4}$$

The corresponding recursion equation is $p_{t+1} = p(t) + \Delta p$. We determine the evolutionary fate of a mutant allele by evaluating leading eigenvalues associated with the recursion equation,

$$\lambda = \left. \frac{\partial p_{t+1}}{\partial p_t} \right|_{p=\hat{p}},$$

at the boundary equilibria $\hat{p} = 0$ and $\hat{p} = 1$. Eigenvalues in excess of 1 correspond to unstable equilibria. Evaluating the boundary values of λ allows identification of four possible solution sets: stable equilibrium at $\hat{p} = 0$ and unstable at $\hat{p} = 1$; stable equilibrium at $\hat{p} = 1$ and unstable at $\hat{p} = 0$; both boundary equilibria stable; and both boundary equilibria unstable. These boundary eigenvalues roughly correspond to purifying selection against the mutant allele, positive selection for the mutant allele, unstable internal equilibrium, and protected polymorphism (Prout 1968). We explore the prevalence of these different outcomes across male densities ranging between $n_m \in [0, 100]$ and the full spectrum of possible environmental gradients $d \in [0, 1]$.

To further explore the invasibility of wild-type genotypes by mutant alleles as well as the frequency of sexual conflict and protected polymorphism, we evaluated boundary eigenvalues, λ, across $\gamma \times \gamma^*$ parameter space ($0 \le \gamma, \gamma^* \le 1$) at three different male densities ($n_m = 25, 50, 75$) while holding the environmental gradient fixed at an intermediate value of $d = 0.25$. These conditions represent biologically interesting and feasible scenarios spanning a gradient of negative frequency- and density-dependent selection and a moderate environmental gradient. At $n_m = 25$, polyspermy can occur only at the environmentally optimum time, while at $n_m = 50$ and $n_m = 75$, polyspermy can occur at both time points.

In addition to analyzing evolutionary equilibria for the mutant allele frequency, p, we also examined the evolutionarily stable strategy (ESS) for γ under different densities and environmental gradients. For a given set of conditions regarding the environmental gradient and population density, we specifically identified wild-type phenotypes that prohibit invasion of a new mutant genotype (for ESS methods, see app. A; apps. A, B are available online).

Sexual Conflict and Patterns of Gene Expression

We subsequently relaxed the assumption of equal phenotypic effects of mutant alleles in each sex and considered three alternative models of sex-specific gene expression. These alternative models serve two purposes. First, they allow for a more general exploration of the evolutionary consequences of sex-specific mutational effects for repro-

ductive phenology. Second, and as described below, they permit us to identify parameter conditions leading to sexually concordant and sexually antagonistic selection on spawning phenology. The three alternative models refer to the expression pattern of the mutant allele, B_2, corresponding to (1) a female-limited model, (2) a male-limited model, and (3) a model with arbitrary sex-specific expression of the mutant allele. In this way, we explore the cardinal points of possible phenotypic correlations between the sexes (for full derivations of all models, see app. S1; app. S1–S4 are available online).[1] We note that while alternative derivations exist that would fit the verbal definition of sex limited, we have chosen ours for logical consistency with the equal effects model and ease of isolating the response to selection through each sex individually.

In the sex-limited models, mutant individuals of the nonexpressing sex exhibit identical phenotypes to wild-type individuals (γ). Only mutants of the expressing sex exhibit the modified phenotype (γ^*). In this way, phenotypic variation and selection on the mutant phenotype is limited to the sex expressing the mutant phenotype. This formulation is also consistent with the assumption in the equal effects model that the ancestral state has no sexual dimorphism in spawning time. Within our modeling framework, we define sexual conflict as a difference in the predicted stability of the boundary equilibria between the female- and male-limited models under equivalent parameter conditions.

The sex-specific model permits individual mutations to differentially affect the phenotypes of male and female carriers. This model requires an additional variable to account for expression of the wild-type and mutant alleles in each sex. We specify sex-specific phenotypic effects with subscripts m and f (γ_m, γ_f, γ^*_m, and γ^*_f; γ_m and γ_f represent the wild-type phenotypes of males and females, and γ^*_m and γ^*_f represent the phenotypes of individuals that carry a mutant allele). In short, the inclusion of these terms enables both male and female mutants to express modified phenotypes from wild-type individuals of the same sex. We assessed the stability of ESS solutions for this model by evaluating the eigenvalues of the convergence stability and Hessian matrices for $\lambda|_{p=0}$, where $\gamma^*_f = \gamma_f$ and $\gamma^*_m = \gamma_m$ (Otto and Day 2007; app. A). We performed the same analyses described above for all models.

In addition to evaluating the boundary values of each λ, we also determined the outcome of the evolutionary invasion analyses by solving for the roots of the difference equation Δp numerically, giving the full solution sets for $0 \leq \hat{p} \leq 1$. Our numerical results were sometimes more complex than implied by the eigenvalue analysis, with the emergence of ≥ 3 distinct internal equilibria for some parameter conditions. However, the numerical results and boundary values for λ were qualitatively similar over most biologically feasible parameter space, and we present only the boundary results here (for representative numerical results, see app. S2). All analyses of the population genetic models were performed using Mathematica 10 (Wolfram Research 2014).

1. Code that appears in the *American Naturalist* is provided as a convenience to the readers. It has not necessarily been tested as part of the peer review.

Testing Model Assumptions

In addition to the haploid models described above, we also derive diploid versions for each model (see app. S1). With codominant phenotypic effects of mutant and wild-type alleles (i.e., $h = 0.5$), the predictions from the diploid and haploid models are equivalent, and we therefore present the results for the haploid case for simplicity. However, in the diploid models, parameter conditions resulting in net overdominance are possible and would be expected to result in increased balancing selection (Fry 2010). Thus, the haploid results probably represent a conservative prediction for the incidence of balancing selection.

The population genetic models make several other simplifying assumptions that could influence the predictions. In particular, (1) developmental traits influencing phenology are unlikely to be controlled by a single locus and (2) two discrete time points may not adequately capture the consequences of more complex patterns of environmental heterogeneity. To evaluate the robustness of our predictions to these assumptions, we performed individual-based quantitative genetic simulations that conceptually parallel the population genetic models. Specifically, we have expanded the model of flowering phenology described by Devaux and Lande (2010) to model individual spawning phenologies as continuous characters that are governed by two polygenic traits: the individual mean (m) and the individual variance (v) of gamete release time. Our simulation model expands on Devaux and Lande (2010) by incorporating dioecy and the possibility of varying degrees of genetic correlation between the sexes while retaining the density-dependent fertilization dynamics that are relevant to broadcast spawners (described in eq. [1]). We simulated the evolution of m and v for populations of broadcast spawners under parameter conditions corresponding to a factorial matrix between two magnitudes of polyspermy (low or high) and two degrees of steepness of the environmental gradient (gentle or steep). We also manipulated the shape of the environmental gradient to be either discrete and symmetrical (with two levels of environmental stress, analogous to $d = 0.25$ or 0.75 in the population genetic models) or continuous and Gaussian (as in Devaux and Lande 2010; for detailed simulation methods, see app. B).

Simulation results were broadly congruent with predictions of the population genetic models. We touch briefly

on these results; for full simulation results, see appendix S3. However, while the simulations show that the population genetic model results are not merely a consequence of their simplifying assumptions, a thorough exploration of the evolution of spawning phenologies mediated by polygenic characters remains an interesting avenue for future research.

Results

Our results address four main theoretical questions, which we explore in detail below. First, what fraction of the parameter space leads to protected polymorphism, and how does sex-specific gene expression impact opportunities for balancing selection? Second, what parameter conditions give rise to sexual conflict over the timing of reproduction, and how often does such conflict lead to balancing selection? Third, when is sexually antagonistic selection expected to drive the evolution of spawning during suboptimal environmental conditions? Finally, how do evolutionarily stable spawning strategies (i.e., genotypes that, when fixed, cannot be displaced by a mutant strategy) vary across different population densities and environmental gradients?

Prevalence of Protected Polymorphism

Our analyses of the models reveal that negative frequency- and density-dependent selection—which arise from sperm competition and polyspermy—may often generate balancing selection to maintain polymorphism in phenologies. However, the opportunity for balancing selection and protected polymorphism hinges on the pattern of gene expression within each sex. In all four models (i.e., the equal effects, female-limited, male-limited, and sex-specific models), the fate of the mutant allele is sensitive to two density thresholds that determine the incidence of polyspermy. At the lower threshold (at $n_m \approx 20$ for the parameter condition $A = 0.1$), eggs begin to suffer polyspermy if all males spawn at the same time (e.g., $\gamma, \gamma^* = 0$). At the higher threshold ($n_m \approx 40$ when $A = 0.1$), eggs begin to suffer polyspermy at both time points if males release equal amounts of sperm at both time points ($\gamma, \gamma^* = 0.5$).

Stable polymorphism is never maintained in the female-limited model, when selection occurs exclusively through females. Rather, the genetic system always evolves to eliminate or fix the mutant allele (figs. 1*A*, 2*A*). At male densities below the lower threshold, female reproduction is sperm limited, and selection favors high reproductive synchrony with males, leading to increased female spawning at the environmental optimum. As male densities increase and elevate the risk of polyspermy, selection favors female spawning away from the environmental optimum. This occurs because the cost of polyspermy at the environmental optimum outweighs the survival costs to offspring that are produced by spawning at the environmentally suboptimal time.

When at least some selection occurs in males (i.e., in all but the female-limited model), opportunities for maintaining polymorphism increase dramatically. In the male-limited model, the likelihood of balanced polymorphism increases with increased polyspermy (i.e., balancing selection becomes more likely after crossing each population density threshold; fig. 1*B*). Between the two density thresholds, the increase in protected polymorphism in the male-limited model appears to come at the cost of positive selection for the mutant allele. This effect is caused by sperm competition; as males increasingly compete for fertilizations, despite increased rates of polyspermy, selection becomes dominated by the frequency-dependent effects of male-male competition, and selection maintains invading mutant alleles at intermediate frequencies rather than fixing them.

In the equal effects model, where spawning phenotypes are perfectly genetically correlated between the sexes, protected polymorphism occurs only above the upper density threshold, when there is no temporal refuge from polyspermy (fig. 1*C*). The fraction of balanced polymorphism is intermediate between the two sex-limited models; selection in males can maintain polymorphic mutant alleles that would otherwise be fixed through positive selection in females, an effect that arises at sufficiently high male densities. In the sex-specific model—where selection occurs through both sexes, but the phenotypic effects of mutations may be sexually dimorphic—protected polymorphism can occur at high and low male densities, with increasing incidence of polymorphism with increasing male densities (fig. 1*D*).

Overall, balancing selection is driven exclusively by selection through males, with selection through females tending to remove polymorphism. Balancing selection is most permissible when selection is male limited. When mutations affect the phenotypes of both sexes, we see intermediate opportunities for balancing selection. Mutant alleles are most likely to be maintained when population densities are high.

Sexual Conflict and Protected Polymorphism

Strong frequency and density dependence of male fertilization success promotes the maintenance of genetic diversity and generates sexual conflict over the timing of gamete release. The extent of discordance in selection between the sexes hinges on population density. At low population densities (and thus lower sperm concentrations), males and females generally experience concordant selection to synchronize reproduction and maximize fertilization success at the environmentally optimal time. As population densities increase, the interests of males and females diverge sharply, with females experiencing strong selection to minimize poly-

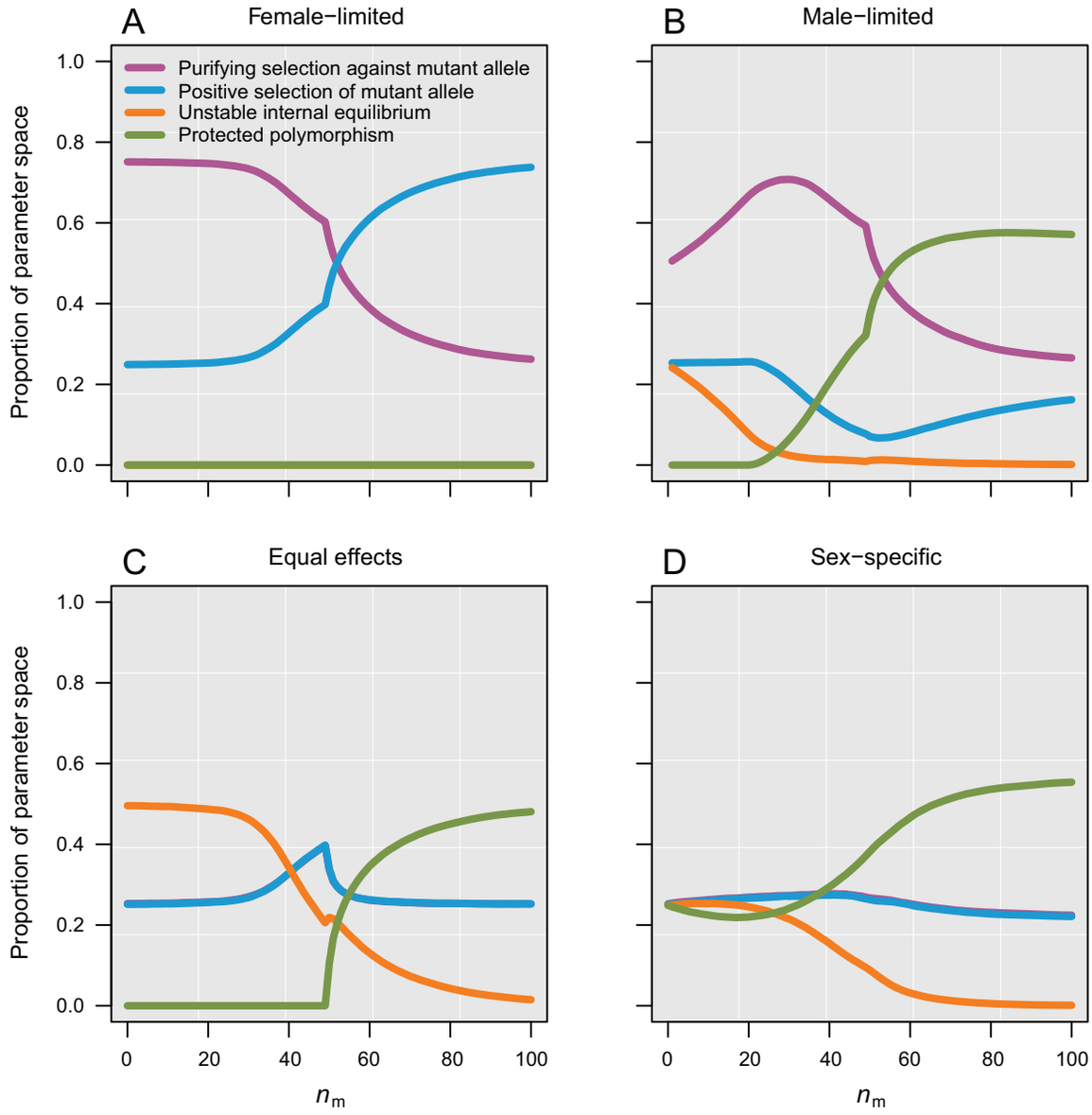

Figure 1: Relative frequency of different evolutionary outcomes for mutant alleles that affect phenology. Results show the evolutionary invasion analysis for each model across $\gamma \times \gamma^*$ parameter space as a function of male density (n_m). Outcomes are based on evaluating the leading eigenvalue, λ, at the boundaries of $p = 0$ and $p = 1$. These results use the parameters $d = 0.25$ and $A = 0.1$. In *C* and *D*, the line for purifying selection is underneath that of positive selection.

spermy, and males experiencing frequency-dependent selection to maximize their relative fertilization success. In all models, the fate of mutant alleles reflects the fitness trade-offs resulting from polyspermy, fertilization success, and the environmental gradient. These complex trade-offs often result in asymmetrical invasion plots (fig. 2) and divergent evolutionary fates for mutations with female-limited versus male-limited phenotypic effects.

Conflicting selection between the sexes is implied in cases where the evolutionary trajectories of mutant alleles differ between the female- and male-limited models of gene expression. In the female-limited model, mutants releasing more gametes at the environmentally suboptimal time (fig. 2*A*–2*C*, above the diagonal) can invade in two regions of parameter space: (1) when fertilization success at the suboptimal time outweighs the combined fitness costs of

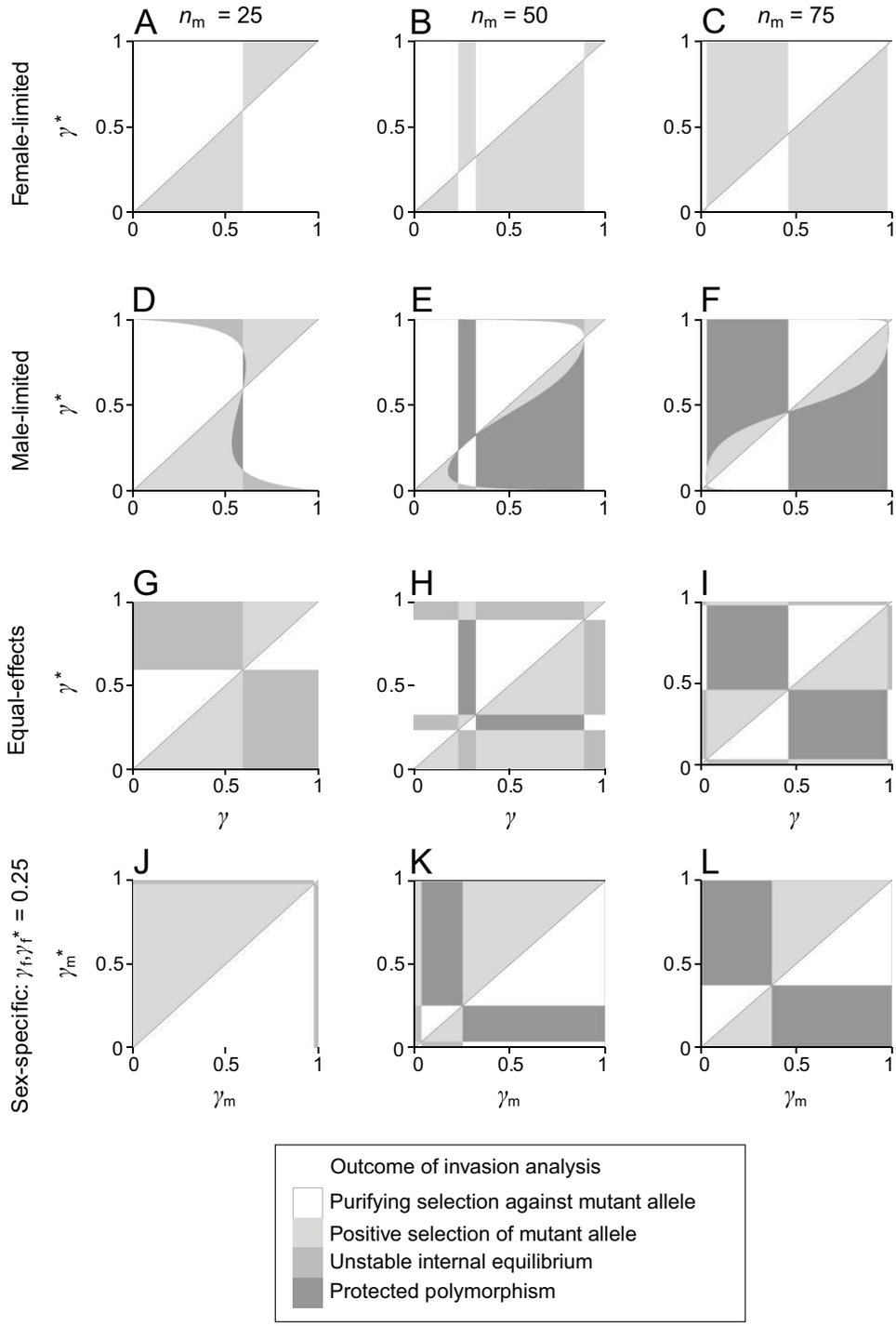

Figure 2: Evolutionary fates of mutant alleles for each model of gene expression. Outcomes of the evolutionary invasion analysis are based on evaluating the leading eigenvalue, λ, at the boundaries of $p = 0$ and $p = 1$ across the full $\gamma \times \gamma^*$ parameter space (or $\gamma_m \times \gamma_m^*$ for the sex-specific model). Mutation effect size is reflected in the magnitude of the vertical deviation away from the diagonal. The sex-specific model results use the parameters $d = 0.25$ and $A = 0.1$ and the parameters $\gamma_f, \gamma_f^* = 0.25$.

polyspermy at the optimal time and poorer environmental conditions at the suboptimal time (e.g., fig. 2*B*, 2*C*, gray regions above the diagonal where $\gamma < 0.5$) and (2) when the fitness cost of sperm limitation at the optimal time exceeds the combined costs of polyspermy and environmental conditions at the suboptimal time (e.g., fig. 2*A*–2*C*, gray regions above the diagonal where $\gamma > 0.5$).

Sperm competition in the male-limited model often drives the evolution of protected polymorphism in regions of parameter space that correspond to positive selection in the female-limited model (fig. 2*D*–2*F*; reflected in fig. 1*A*, 1*B*). The evolutionary outcomes differ between male- and female-limited models because sperm competition leads to frequency-dependent selection in males but not in females. Selection in males can maintain mutant alleles that improve sperm competition against wild-type males but otherwise result in lower net fertilization rates because of polyspermy.

The parameter regions of sexual conflict (and of regions of protected polymorphism in the male-limited model) are determined by the fertilization curve in equation (1). The frequency of sexual conflict increases with mutation size (vertical deviation from the diagonal in fig. 2), but this relation is sensitive to male density (fig. 3*A*). This is because in the regions where sexual conflict occurs, increasing mutation size results in mutants releasing more sperm at the time point where less polyspermy occurs. Mutant alleles can persist at intermediate frequencies when the fitness gains from releasing more sperm at the time point with less polyspermy balance the fitness cost of releasing less sperm at the other time point where sperm competition is strongest. In the limit of arbitrarily large mutation sizes (across all possible values of $\gamma \times \gamma^*$), the overall probability of sexual conflict increases with male density.

In the equal effects model, sexually antagonistic selection constrains the parameter space where protected polymorphism can occur. Conditions for balancing selection are more restrictive when phenotypic effects are tightly correlated between the sexes, relative to the case where selection is male limited. Sexually antagonistic selection does not necessarily result in protected polymorphism, because some mutant alleles that would be maintained by balancing selection in the male-limited model become fixed through directions selection in females. As a result, the equal effects model yields results that are intermediate between the female- and male-limited models (fig. 2*G*–2*I*). When male densities are high enough for polyspermy to occur at the environmental optimum only, this results in a large restriction of the parameter space where polymorphism is predicted to occur (fig. 2*E*, 2*H*; fig. 3*A*, 3*B*, dashed line). When polyspermy occurs at both time points, intermediate frequencies of the mutant allele minimize polyspermy at both time points, increasing female fitness, thereby increasing the parameter space where polymorphism can occur relative to the male-limited model (fig. 2*F*, 2*I*; fig. 3*A*, 3*B*, solid line).

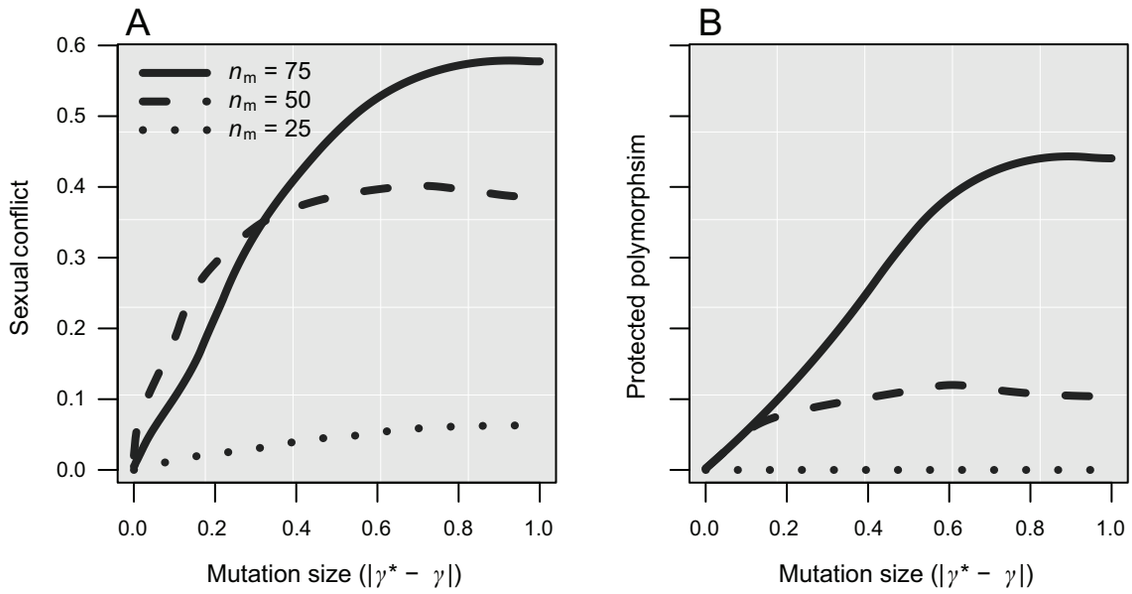

Figure 3: Frequency of sexual conflict (differences in the boundary signatures for λ between the female- and male-limited models; *A*) and protected polymorphism (*B*) in the equal effects model as a function of mutation size ($|\gamma^* - \gamma|$). Results are presented for three different male densities. Note that these results can be recovered directly from the phase space diagrams in figure 2*A*–2*I*. Results use the parameters $d = 0.25$ and $A = 0.1$.

Small-effect mutations generally experience either purifying or positive selection, whereas large-effect mutations are more likely to be maintained as polymorphisms (fig. 3*B*).

We illustrate some features of the sex-specific model's behavior by holding female strategies fixed at the arbitrary value $\gamma_f = \gamma_f^* = 0.25$ and varying the male strategies associated with each genotype (fig. 2*J*–2*L*). In this example, the majority of explored parameter space results in either positive selection for—or purifying selection against—the mutant allele. When polyspermy can occur at only the environmental optimum, selection can maintain polymorphism for mutations with modest to small effect sizes when male and female phenotypes are similar (fig. 2*K*; where $\gamma_m, \gamma_m^* \approx 0.25$). At high population densities, sperm competition and the incidence of polyspermy at the suboptimal time also increase. Consequently, protected polymorphism occurs only when mutant males are able to compensate for their loss of competitive ability against wild-type males with increased fertilization success at the opposite time point (fig. 2*K*, 2*L*, dark gray region above and below the diagonal, respectively).

Environmental Gradients

The steepness of the environmental gradient between spawning time points (d) influences the balance of fitness effects associated with spawning at versus away from the environmental optimum. At high densities, negative frequency- and density-dependent fertilization success drives the evolution of stable polymorphism and spawning at the environmentally suboptimal time, even when the fitness costs of doing so are high. The steepness of the environmental gradient influences the population density at which protected polymorphism becomes common. However, the relation between the frequency of balanced polymorphism and population density remains qualitatively similar across all possible parameter values for d (fig. 4). Mutations causing individuals to release more gametes at the suboptimal time t_1 ($\gamma^* > \gamma$) were favored at low or high population densities (fig. 5). At low densities, fertilization success is sperm limited. In this case, spawning at the suboptimal time is favored only when enough sperm is released that the resulting increase in fertilization success is greater than the survival cost to offspring. At high densities, increased polyspermy at the environmental optimum generates negative density-dependent selection that favors spawning at the suboptimal time, even when the associated reduction in offspring survival is high.

Evolution of Within-Individual Variance

The evolution of within-individual variance (intermediate values of γ) was strongly density dependent (fig. 6; app. S3). At male densities below $n_m \approx 50$, there is no convergence-stable ESS for γ, only a single unstable equilibrium that pushes the evolution of phenologies toward the boundaries of $\gamma = 0$ and $\gamma = 1$, corresponding to no within-individual variance. Thus, low-density populations are predicted to evolve phenologies with no within-individual variance. When male densities are greater than $n_m \approx 50$ (recall that this corresponds to the density threshold where polyspermy can occur at both time points), two additional equilibria appear: one convergence-stable ESS that approaches $\gamma = 0.5$ with increasing n_m and a second unstable equilibrium that approaches $\gamma = 0$ with increasing n_m. Consequently, high within-individual variance is predicted above the upper density threshold at $n_m \approx 50$. The steepness of the environmental gradient modifies the ESS gamete release strategies and unstable equilibrium states, though d does not qualitatively alter their relationships with population density (n_m). Increased within-individual variance is predicted to evolve to minimize the fitness costs of polyspermy arising across different time points or spawning opportunities. These general patterns are robust to the specific form gene expression in each sex (see fig. S5 in app. S2).

The evolution of increased within-individual variance is predicted in both the population and the quantitative genetic models when male densities are sufficiently high to result in polyspermy at multiple time points (see app. S3). Both models for the evolution of within- versus among-individual variance in phenology are similarly influenced by the intensity of polyspermy and changes in environmental conditions between spawning times. However, in contrast to the population genetic models, the quantitative genetic models were sensitive to the gene expression patterns in each sex. In particular, populations tended to have higher within-individual variance in spawning time when phenotypic effects in each sex were uncorrelated (the sex-specific model) than when they were tightly correlated (the equal effects model). Moreover, this result was generally robust to the incidence of polyspermy, or the steepness of the environmental gradient.

Discussion

The Evolution of Phenologies

Spawning in marine organisms is traditionally assumed to be highly synchronized between the sexes and to coincide precisely with optimal environmental conditions (Thorson 1936, 1946, 1950; Cushing 1969, 1990; Harrison et al. 1984; Babcock et al. 1986; Clifton 1997; reviewed in Durant et al. 2007; Lowerre-Barbieri et al. 2011). In contrast to this long-held assumption, several studies have documented conspicuous spawning asynchrony or mismatches between spawning time and ideal environmental conditions. These

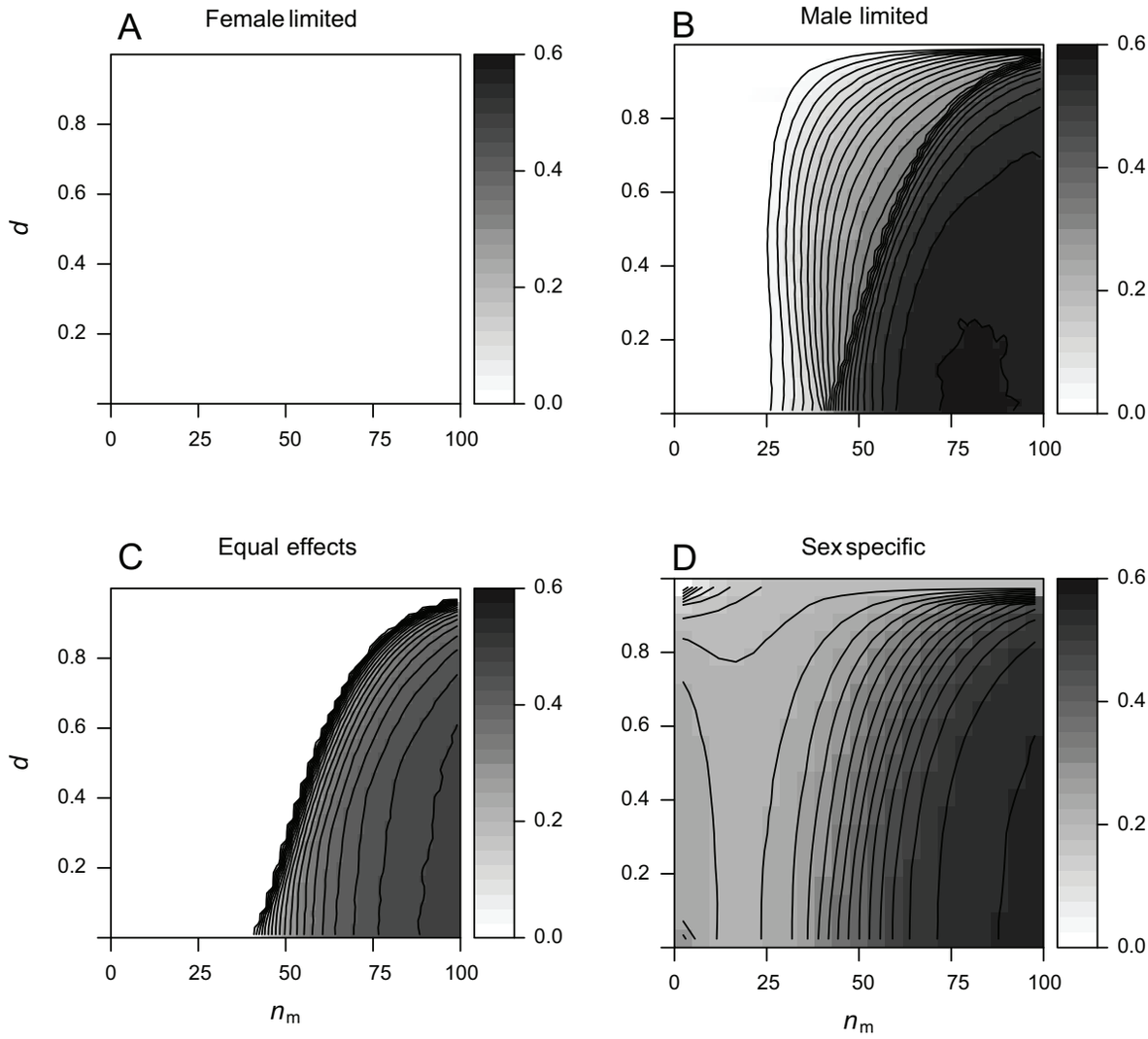

Figure 4: Proportion of $\gamma \times \gamma^*$ parameter space resulting in protected polymorphism for each model as a function of the steepness of the environmental gradient (d), and male density (n_m). Note that the results presented in figure 1 represent a slice through these contours at $d = 0.25$. All plots use a common color scale.

observations come from a diverse range of externally fertilizing species, including corals (Levitan et al. 2004, 2011; Baird et al. 2009), sea urchins (Levitan 2002, 2004; Lotterhos and Levitan 2011), intertidal ascidians (Marshall 2002), and marine polychaetes (Lewis et al. 2002, 2003; Kupriyanova 2006). Many species also exhibit sexually dimorphic spawning behaviors, with males typically spawning earlier and for longer than females (McEuan 1988; Levitan 2005; Lotterhos and Levitan 2011). While environmental stochasticity and bet-hedging strategies may explain some of the observed variance in reproductive phenologies (e.g., Iwasa and Haccou 1994; Devaux and Lande 2010), they do not fully address the possible role of negative frequency- and density-dependent selection in the evolution of spawning strategies, nor do they adequately explain the prevalence of sexually dimorphic phenologies within broadcast spawners (Durant et al. 2007; Olito et al. 2015). Our models help to reconcile these empirical patterns with theory. We show that precise synchrony between spawning times and optimal environmental conditions is predicted only under a narrow set of parameter conditions. We also demonstrate that sexually antagonistic selection over the timing of reproduction can select for sexually dimorphic spawning behaviors and potentially maintain variation in population phenologies.

In our models, sexual conflict over the timing of reproduction was predicted to be common, to be density dependent, and, in many cases, to maintain genetic polymorphism.

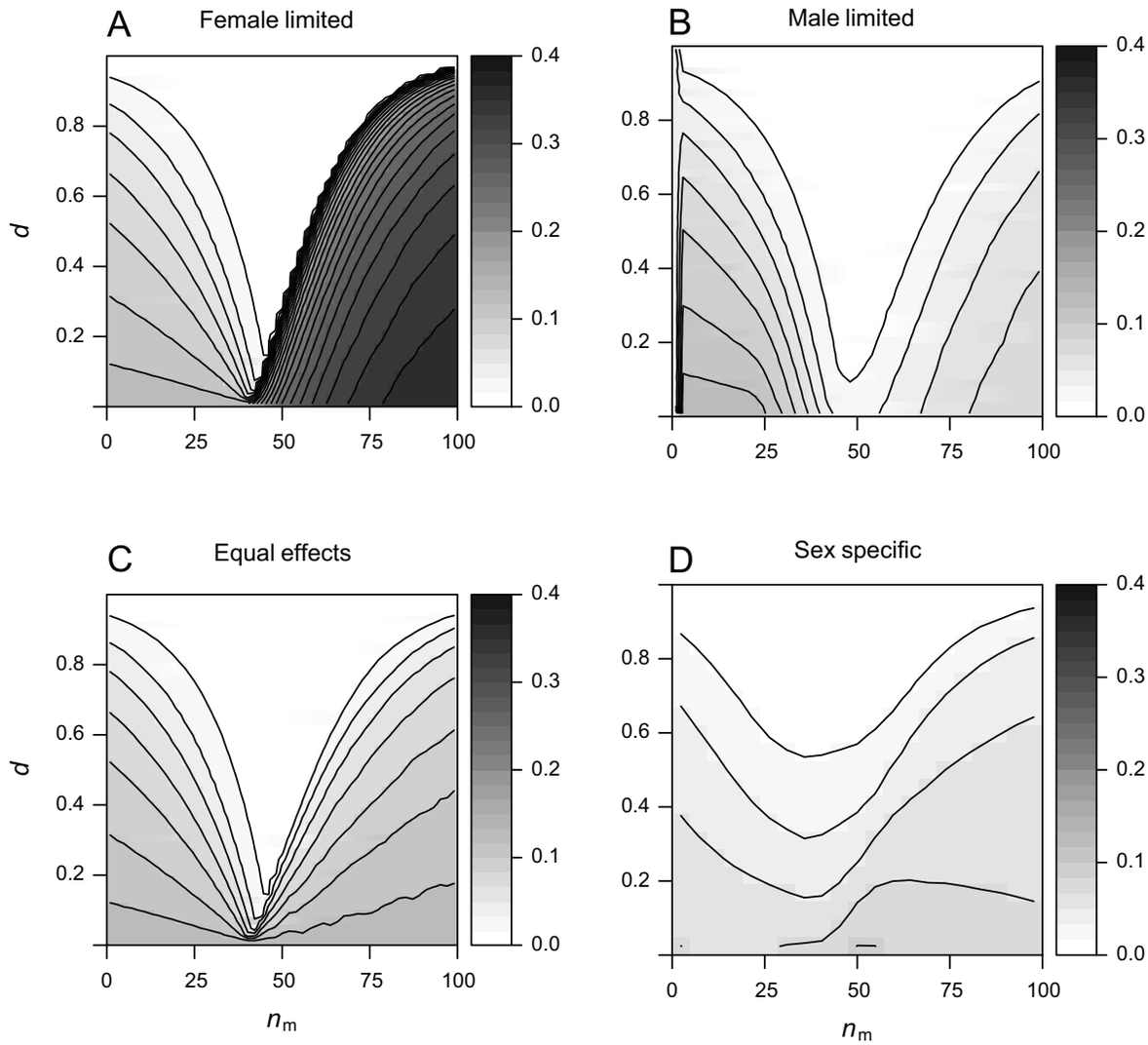

Figure 5: Proportion of $\gamma \times \gamma^*$ parameter space resulting in fixation of mutant alleles with greater phenotypic values than the wild-type ($\gamma^* > \gamma$). This represents the fixation of genotypes that preferentially spawn at the environmentally suboptimal time (t_1). Results are plotted as a function of the environmental gradient (d) and male density (n_m). All use a fixed parameter value for A ($A = 0.1$) and a common color scale.

Selection through females (as in the female-limited gene expression model) favored the evolution of fixed female phenological strategies that reduce the risk of polyspermy, even in cases where fertilization was somewhat sperm limited at suboptimal time points. These results parallel previous predictions regarding frequency-dependent selection on gamete recognition proteins (Levitan and Ferrell 2006). In contrast, protected polymorphism was a common outcome in the male-limited model, which reflects the inherent frequency dependence of sperm competition outcomes (Parker et al. 1972; Parker 1982; Birkhead and Møller 1998). Our results offer an interesting counterpoint to previous studies of sperm competition, which tend to emphasize the evolution of reduced variation around trait values that maximize male competitiveness (e.g., large testes size, ejaculate volume, and competitive sperm morphology; Stockley et al. 1997; reviewed in Wedell et al. 2002). Given its ubiquity, sperm competition offers a potentially common mechanism for generating sexually dimorphic fitness optima and sexually antagonistic selection over traits that affect the fertilization process.

For broadcast spawners, high phenological variances and sexually dimorphic phenologies may be a consequence of sexually antagonistic selection that is driven by sperm com-

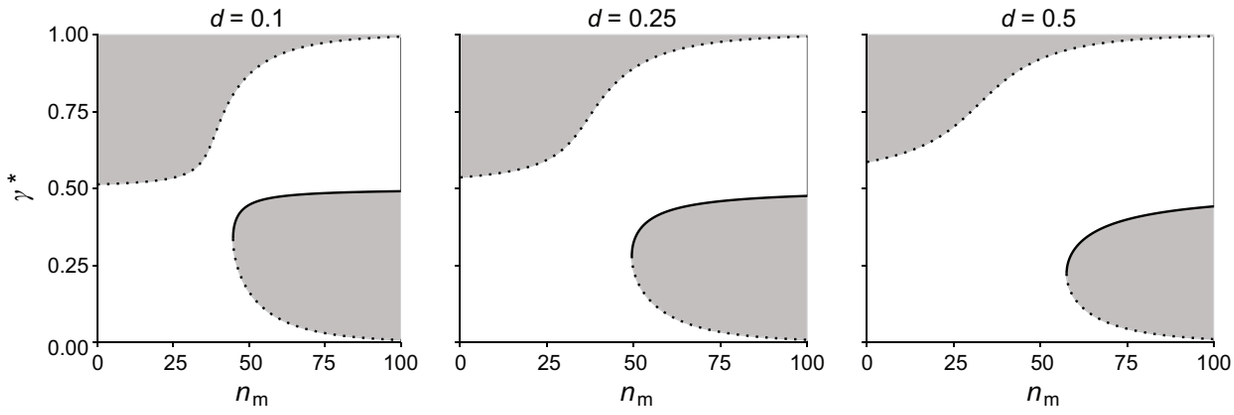

Figure 6: Evolutionarily stable strategy solutions for γ as a function of male density (n_m) and for three different steepnesses of the environmental gradient (d). Solid lines represent convergence-stable equilibrium states, whereas dotted lines represent unstable equilibria. At a given density, populations are expected to evolve away from unstable equilibria and toward nearby stable equilibria along the vertical axis. These results use the parameter $A = 0.1$ and were calculated for the female-limited model. Results for the other three models were nearly identical (see fig. S10, available online).

petition. While acknowledging the difficulties of detecting sexually antagonistic selection at individual loci (e.g., Barson et al. 2015), our model predictions may nevertheless be tested by other means. For example, our models predict that sexual conflict over the timing and duration of spawning should be greatest at high densities. These conditions may, in the long run, give rise to sexually dimorphic phenologies in which males have longer spawning durations and higher among-individual variances than females. Our model predictions could also be tested by estimating the relation between spawning phenotypes (e.g., spawning onset and duration) and the fitness of each sex under sperm-limited versus high-polyspermy conditions. Variances in reproductive success have been shown to differ between males and females across population density gradients (Levitan 2004), and gamete traits and population phenologies are known to experience strong density- and frequency-dependent selection (Levitan 2002; Levitan and Ferrell 2006; Tomaiuolo et al. 2007). However, these patterns have yet to be linked to sex-specific phenological traits or genes expressed in both sexes.

Our results may also help explain the seemingly counterintuitive observation that some species' reproduction occurs during poor environmental conditions. For example, some marine polychaetes conspicuously spawn when temperatures are suboptimal for fertilization success and larval development (Lewis et al. 2002, 2003). Seasonal energy budgets and competition among settling larvae have been proposed to explain these patterns. However, these species are also susceptible to polyspermy and occur at high population densities, conditions that lead to spawning away from the environmental optimum in our models. Conceptually, these predictions are loosely analogous to previous theory on the evolution of polygenic traits in sexually dimorphic species (Lande 1980), where sexual selection and positive genetic covariances between the sexes can cause trait values to evolve away from adaptive peaks that are favored by natural selection. In our models, sexual conflict and polyspermy can drive the evolution of spawning in environmental conditions that are unfavorable for survival. In extreme instances, spawning in suboptimal environments can evolve, even when the mortality cost in the poorer environment reaches 95%.

The fitness consequences of a particular phenological strategy should depend on aspects of each species' life history. For example, larger eggs present a bigger target for sperm-egg collisions and lower the effective densities at which polyspermy occurs (Millar and Anderson 2003; Bode and Marshall 2007; Olito et al. 2015; app. B). Egg size also covaries strongly with other life-history traits, such as the mode of larval development (Marshall and Keough 2007). Species with nonfeeding (lecithotrophic) larvae have larger eggs (on average) than species with feeding (planktotrophic larvae) and require favorable physicochemical conditions for development (Strathmann 1985; Marshall and Keough 2007). Species with planktotrophic larvae—which have the same physicochemical requirements and additionally rely on planktonic food resources—are more sensitive to environmental conditions during larval development (Vance 1973; Strathmann 1985; Marshall and Keough 2007). The elevated environmental sensitivities of these species should, according to our models, lead to the evolution of relatively low among-individual variances in phenology. In contrast, lecithotrophs should experience selection for increased var-

iance in phenology because of their larger eggs and presumably lower population density thresholds for polyspermy.

Although our models are tailored for broadcast spawners, the results may hold for the evolution of phenologies in other organisms, particularly dioecious and wind-pollinated flowering plants. Positive and negative frequency- and density-dependent pollen transport is a pervasive feature of reproduction in flowering plants resulting from patch attractiveness, competition for pollinator visitation, the dynamics of wind dispersal of pollen, and polyspermy (Allee et al. 1949; Augspurger 1981; Brunet and Charlesworth 1995; Spielman and Scott 2008; Friedman and Barrett 2009; Devaux and Lande 2010). If the consequences of frequency-dependent pollen transport for reproductive success fundamentally differ between sexes or sex roles, there is potential for sexually antagonistic selection to expand opportunities for polymorphism in floral and phenological traits that are expressed in both sexes (Jordan and Connallon 2014). Indeed, similar to the empirical patterns observed in broadcast spawners, males of many dioecious plant species flower earlier and for longer and generally produce larger flowering displays than female plants (Charlesworth et al. 1987; Brunet and Charlesworth 1995; Kudo 2006). In wind-pollinated species, sex allocation is size dependent and strikingly male biased but is often protogynous (females flower before males; Friedman and Barrett 2009). However, applications of our model predictions to angiosperms must be made with caution because fertilization within this group is internal. While among-male competition during pollen transport superficially resembles that of broadcast spawners, males also compete during the gametophytic stage, with competitive outcomes mediated by interactions with female stylar tissue. These interactions can result in complex fertilization dynamics, including but not limited to negative density dependence (Spielman and Scott 2008; Friedman and Barrett 2009; Harder et al. 2016). Moreover, since the majority of angiosperms are hermaphroditic, floral and phenological traits may be influenced by additional factors, including self-fertilization and selection arising within the context of selfing (Harder and Barrett 1995; Kudo 2006; Friedman and Barrett 2009). Evolutionary responses to negative frequency dependence can also drive adaptive allocation to the less common sex role at any point in the flowering season (Brunet and Charlesworth 1995; Brookes and Jesson 2010; Ishii and Harder 2012).

Sexual Conflict and Protected Polymorphism

Population density, gene expression, and mutation effect size each influenced the likelihood of sexually antagonistic selection maintaining polymorphism. At high population densities, mutations with modest to large phenotypic effect sizes were often subject to sexually antagonistic selection (i.e., the female-limited model predicted fixation, and the male-limited model predicted protected polymorphism). In cases where the mutation was expressed by both sexes, polymorphism was maintained in only a subset of the parameter space of sexual conflict. Large-effect mutations were relatively likely to be maintained as protected polymorphisms.

These results complement recent theoretical predictions about mutation size, sexual antagonism, and balancing selection from simple fitness landscape models of sex-specific adaptation (e.g., Fisher's geometric model; Connallon and Clark 2014*a*, 2014*b*). Sexually antagonistic selection is a common outcome of adaptation in these models, with mutations of intermediate size most likely to experience balancing selection. Our models expand these predictions by providing an explicit ecological mechanism for fitness trade-offs over female and male spawning strategies. In the current context, sexually antagonistic fitness trade-offs increase with population density because polyspermy decreases the net fitness cost to males of spawning away from the environmental optimum. Large-effect mutations are relatively likely to result in balancing selection because they result in higher sperm concentrations (and relatively high male fertilization success) at the suboptimal time, which helps to offset the cost of lower environmental quality. In contrast, females have no frequency-dependent incentive for incurring environmental fitness costs by spawning at the suboptimal time. While polyspermy may also drive females to spawn away from the environmental optimum, conditions leading them to do so often differ from those that incentivize males.

Evolution of Within-Individual Variance

While increased polymorphism leads to increased among-individual variance in our models, the evolution of intermediate gamete release phenotypes (i.e., where individuals spawn across both time points) corresponds to increased within-individual variance. For flowering plants, density-dependent pollination success (e.g., due to patch attractiveness and competition for pollinators) has been predicted to influence the evolution of population phenologies through evolutionary changes in the individual mean and variance of flowering display times (Elzinga et al. 2007; Devaux and Lande 2008, 2010). Among- and within-individual variance in flowering phenology is predicted to respond differently to positive and negative density-dependent pollinator visitation, with disruptive selection driving increased among-individual variance and stabilizing selection decreasing within-individual variance (Devaux and Lande 2010). Our models partly corroborate these findings for dioecious species. Our models also reveal the effects of genetic correlations between the sexes on the evolution of within- and among-individual variances in population phenology, is-

sues that were not considered by previous theory regarding flowering phenologies in simultaneously hermaphroditic plants. In our models, negative density-dependent fertilization success resulted in the same pattern of increased within-individual variance, independent of among-individual variance in spawning time or the pattern of gene expression in each sex. However, our quantitative genetic simulations gave somewhat different results. The prevalence of sexually antagonistic selection and the evolution of within- and among-individual variance in population phenology depended on disruptive selection because of polyspermy, the severity of the environmental gradient, and the genetic correlation of mutant phenotypic effects between the sexes (app. S3). Overall, our results suggest that alleles affecting among-individual variance are often under sexually antagonistic selection, that those affecting within-individual variance are under sexually antagonistic selection under some conditions, and that within- and among-individual variance components may respond differently to net stabilizing and disruptive selection, depending on the correlation of phenotypic effects between sexes. A thorough exploration of the joint effects of these processes on the evolution of reproductive phenologies remains an open area for future study.

Acknowledgments

We thank C. Venables, the editors, and five anonymous reviewers for their helpful comments. We are particularly indebted to S. Proulx for a careful review that greatly improved the models and manuscript. This work was funded by a Monash University Dean's International Postgraduate Student Scholarship to C.O. and Australian Research Council grants to D.J.M. and T.C.

Literature Cited

Allee, W. C., A. E. Emerson, O. Park, T. Park, and K. P. Schmidt. 1949. Principles of animal ecology. Saunders, Philadelphia.

Augspurger, C. K. 1981. Reproductive synchrony of a tropical shrub: experimental studies on effects of pollinators and seed predators on *Hybanthus prunifolius* (Violaceae). Ecology 62:775–788.

Babcock, R. C., G. D. Bull, P. L. Harrison, A. J. Heyward, J. K. Oliver, C. C. Wallace, and B. L. Willis. 1986. Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. Marine Biology 90:379–394.

Baird, A. H., J. R. Guest, and B. L. Willis. 2009. Patterns in reproductive biology of scleractinian corals. Annual Review of Ecology, Evolution, and Systematics 40:551–571.

Barson, N. J., T. Aykanat, K. Hindar, M. Baranski, G. H. Bolstad, P. Fiske, C. Jacq, et al. 2015. Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. Nature 528:405–408.

Birkhead, T. R., and A. P. Møller. 1998. Sperm competition and sexual selection. Academic Press, London.

Bode, M., and D. J Marshall. 2007. The quick and the dead? sperm competition and sexual conflict in the sea. Evolution 61:2693–2700.

Bonduriansky, R., and S. F. Chenoweth. 2009. Intralocus sexual conflict. Trends in Ecology and Evolution 24:280–288.

Brawley, S. H. 1992. Fertilization in natural populations of the dioecious brown alga *Fucus ceranoides* and the importance of the polyspermy block. Marine Biology 113:145–157.

Brookes, R. H., and L. K. Jesson. 2010. Do pollen and ovule number match the mating environment? an examination of temporal change in a population of *Stylidium armeria*. International Journal of Plant Sciences 171:818–827.

Brunet, J., and D. Charlesworth. 1995. Floral sex allocation in sequentially blooming plants. Evolution 49:70–79.

Charlesworth, D., D. W. Schemske, and V. L. Sork. 1987. The evolution of plant reproductive characters: sexual versus natural selection. Pages 317–336 *in* S. C. Stearns, ed. The evolution of sex and its consequences. Birkhauser, Boston.

Clifton, K. E. 1997. Mass spawning by green algae on coral reefs. Science 275:1116–1118.

Connallon, T., and A. G. Clark. 2014*a*. Balancing selection in species with separate sexes: insights from Fisher's geometric model. Genetics 197:991–1006.

———. 2014*b*. Evolutionary inevitability of sexual antagonism. Proceedings of the Royal Society B 281:20132123.

Cushing, D. H. 1969. The regularity of the spawning season of some fishes. ICES Journal of Marine Science 33:81–92.

———. 1990. Plankton production and year-class strength in fish populations: and update of the match/mismatch hypothesis. Advances in Marine Biology 26:249–293.

Denny, M. W., and M. F. Shibata. 1989. Consequences of surf-zone turbulence for settlement and external fertilization. American Naturalist 134:859–889.

Devaux, C., and R. Lande. 2008. Incipient allochronic speciation due to non-selective assortative mating by flowering time, mutation, and genetic drift. Proceedings of the Royal Society B 275: 2723–2732.

———. 2010. Selection on variance in flowering time within and among individuals. Evolution 64:1311–1320.

Durant, J. M., D. Ø. Hjermann, G. Ottersen, and N. C. Stenseth. 2007. Climate and the match or mismatch between predator requirements and resource availability. Climate Research 33:271–283.

Ejsmond, M. J., M. Czarnołeski, F. Kapustka, and J. Kozłowski. 2010. How to time growth and reproduction during the vegetative season: an evolutionary choice for indeterminate growers in seasonal environments. American Naturalist 175:551–563.

Elzinga, J. A., A. Atlan, A. Biere, L. Gigord, A. E. Weis, and G. Bernasconi. 2007. Time after time: flowering phenology and biotic interactions. Trends in Ecology and Evolution 22:432–439.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon, Oxford.

Franke, E. S., R. C. Babcock, and C. A. Styan. 2002. Sexual conflict and polyspermy under sperm-limited conditions: in situ evidence from field simulations with the free-spawning marine echinoid *Evechinus chloroticus*. American Naturalist 160:485–496.

Friedman, J., and S. C. H. Barrett. 2009. Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. Annals of Botany 103:1515–1527.

Fry, J. D. 2010. The genomic location of sexually antagonistic variation: some cautionary comments. Evolution 64:1510–1516.

Harder, L. D., M. A. Aizen, and S. A. Richards. 2016. The population ecology of male gametophytes: the link between pollination and seed production. Ecology Letters 19:497–509.

Harder, L. D., and S. C. H. Barrett. 1995. Mating cost of large floral displays in hermaphrodite plants. Nature 373:512–515.

Harrison, P. L., R. C. Babcock, G. D. Bull, J. K. Oliver, C. C. Wallace, and B. L. Wallace. 1984. Mass spawning in tropical reef corals. Science 223:1186–1189.

Hendry, A. P., and T. Day. 2005. Population structure attributable to reproductive time: isolation by time and adaptation by time. Molecular Ecology 14:901–916.

Ishii, H. S., and L. D. Harder. 2012. Phenological associations of within- and among-plant variation in gender with floral morphology and integration in protandrous *Delphinium glaucum*. Journal of Ecology 100:1029–1038.

Iwasa, Y., and P. Haccou. 1994. ESS emergence pattern of male butterflies in stochastic environments. Evolutionary Ecology 8:503–523.

Jordan, C. Y., and T. Connallon. 2014. Sexually antagonistic polymorphism in simultaneous hermaphrodites. Evolution 68:3555–3569.

Kidwell, J. F., M. T. Clegg, F. M. Stewart, and T. Prout. 1977. Regions of stable equilibria for models of differential selection in the two sexes under random mating. Genetics 85:171–183.

Kudo, G. 2006. Flowering phenologies of animal-pollinated plants: reproductive strategies and agents of selection. Pages 139–158 *in* L. D. Harder and S. C. H. Barrett, eds. Ecology and evolution of flowers. Oxford University Press, Oxford.

Kupriyanova, E. K. 2006. Fertilization success in *Galeolaria caespitosa* (Polychaeta: Serpulidae): gamete characteristics, role of sperm dilution, gamete age, and contact time. Scientia Marina 70S3:309–317.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–305.

Levitan, D. 1991. Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. Biological Bulletin 181:261–268.

———. 1998. Does Bateman's principle apply to broadcast-spawning organisms? egg traits influence in situ fertilization rates among congeneric sea urchins. Evolution 52:1043–1056.

———. 2002. Density-dependent selection on gamete traits in three congeneric sea urchins. Ecology 83:464–479.

———. 2004. Density-dependent sexual selection in external fertilizers: variances in male and female fertilization success along the continuum from sperm limitation to sexual conflict in the sea urchin *Strongylocentrotus franciscanus*. American Naturalist 164: 298–309.

———. 2005. Sex-specific spawning behaviour and its consequences in an external fertilizer. American Naturalist 165:682–694.

Levitan, D., and D. L. Ferrell. 2006. Selection on gamete recognition proteins depends on sex, density, and genotype frequency. Science 312:267–269.

Levitan, D., N. D. Fogarty, J. Jara, K. E. Lotterhos, and N. Knowlton. 2011. Genetic, spatial, and temporal components of precise spawning synchrony in reef building corals of the *Montastraea annularis* species complex. Evolution 65:1254–1270.

Levitan, D., H. Fukami, J. Jara, D. Kline, T. M. McGovern, K. E. McGhee, C. A. Swanson, and N. Knowlton. 2004. Mechanisms of reproductive isolation among sympatric broadcast-spawning corals of the *Montastraea annularis* species complex. Evolution 58:308–323.

Lewis, C., P. J. W. Olive, and M. G. Bentley. 2003. Pre-emptive competition as a selective pressure for early reproduction in the polychaete *Nereis virens*. Marine Ecology Progress Series 254:199–211.

Lewis, C., P. J. W. Olive, M. G. Bentley, and G. Watson. 2002. Does seasonal reproduction occur at the optimal time for fertilization in the polychaetes *Arenicola marina* L. and *Nereis virens* Sars? Invertebrate Reproduction and Development 41:61–71.

Lotterhos, K. E., and D. Levitan. 2011. Gamete release and spawning behaviour in broadcast spawning marine invertebrates. Pages 99–120 *in* J. L. Leonard and A. Córdoba-Aguilar, eds. The evolution of primary sexual characters in animals. Oxford University Press, Oxford.

Lowerre-Barbieri, S. K., K. Ganias, F. Saborido-Rey, H. Murua, and J. R. Hunter. 2011. Reproductive timing in marine fishes: variability, temporal scales, and methods. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 3:71–91.

Marshall, D. J. 2002. In situ measures of spawning synchrony and fertilization success in an intertidal, free-spawning invertebrate. Marine Ecology Progress Series 236:113–119.

Marshall, D. J., and M. K. Keough. 2007. The evolutionary ecology of offspring size in marine invertebrates. Advances in Marine Biology 53:1–60.

Marshall, D. J., C. A. Styan, and M. J. Keough. 2000. Intraspecific covariation between egg and body size affects fertilization kinetics of free-spawning marine invertebrates. Marine Ecology Progress Series 195:305–309.

McEuan, F. S. 1988. Spawning behaviors of northeast Pacific sea cucumbers (Holothuroidea: Ecinodermata). Marine Biology 98:565–585.

Millar, R. B., and M. J. Anderson. 2003. The kinetics of monospermic and polyspermic fertilization in free-spawning marine invertebrates. Journal of Theoretical Biology 224:79–85.

Monro, K., and D. J. Marshall. 2015. The biogeography of fertilisation mode in the sea. Global Ecology and Biogeography 24:1499–1509.

Morgan, S. J., and J. H. Christy. 1995. The adaptive significance of the timing of larval release by crabs. American Naturalist 145:457–479.

Olito, C., M. Bode, and D. J. Marshall. 2015. Evolutionary consequences of fertilization mode for reproductive phenology and asynchrony. Marine Ecology Progress Series 537:23–38.

Orr, H. A. 2005. The genetic theory of adaptation: a brief history. Nature Reviews Genetics 6:119–127.

Otto, S. P., and T. Day. 2007. A biologist's guide to mathematical modeling in ecology and evolution. Princeton University Press, Princeton, NJ.

Parker, G. A. 1982. Why are there so many tiny sperm? sperm competition and the maintenance of two sexes. Journal of Theoretical Biology 96:281–294.

Parker, G. A., R. R. Baker, and V. G. F. Smith. 1972. The origin and evolution of gamete dimorphism and the male-female phenomenon. Journal of Theoretical Biology 36:529–553.

Prout, T. 1968. Sufficient conditions for multiple niche polymorphism. American Naturalist 102:493–496.

Rathcke, B., and E. P. Lacey. 1985. Phenological patterns of terrestrial plants. Annual Review of Ecology and Systematics 16:179–214.

R Development Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.

Rice, W. R., and A. K. Chippindale. 2001. Intersexual ontogenetic conflict. Journal of Evolutionary Biology 14:685–693.
Rothschild, L., and M. M. Swann. 1951. The fertilization reaction in the sea urchin: the probability of a successful sperm-egg collision. Journal of Experimental Biology 28:403–416.
Rouse, G., and K. Fitzhugh. 1994. Broadcasting fables: is external fertilization really primitive? sex, size, and larvae in sabellid polychaetes. Zoologica Scripta 23:271–312.
Sellis, D., B. J. Callahan, D. A. Petrov, and P. W. Messer. 2011. Heterozygote advantage as a natural consequence of adaptation in diploids. Proceedings of the National Academy of Sciences of the USA 108: 20666–20671.
Spielman, M., and R. J. Scott. 2008. Polyspermy barriers in plants: from preventing to promoting fertilization. Plant Reproduction 21:53–65.
Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford.
Stockley, P., M. J. G. Gage, G. A. Parker, and A. P. Møller. 1997. Sperm competition in fishes: the evolution of testis size and ejaculate characteristics. American Naturalist 249:933–954.
Strathmann, R. R. 1985. Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. Annual Reviews in Ecology and Systematics 16:339–361.
Thorson, G. 1936. The larval development, growth, and metabolism of Arctic marine bottom invertebrates, etc. Meddelelser om Grønland 100:1–155.
———. 1946. Reproduction and larval development of Danish marine bottom invertebrates, with special reference to planktonic larvae in the Sound (Øresund). Reitzel, Copenhagen.
———. 1950. Reproductive and larval ecology of marine bottom invertebrates. Biological Reviews 25:1–45.
Tomaiuolo, M., T. F. Hansen, and D. Levitan. 2007. A theoretical investigation of sympatric evolution of temporal reproductive isolation as illustrated by marine broadcast spawners. Evolution 61: 2584–2595.
Vance, R. R. 1973. More on reproductive strategies in marine benthic invertebrates. American Naturalist 107:353–361.
Vogel, H., G. Czihak, P. Chang, and W. Wolf. 1982. Fertilization kinetics of sea urchin eggs. Mathematical Biosciences 58:189–216.
Wedell, N., M. J. G. Gage, and G. A. Parker. 2002. Sperm competition, male prudence, and sperm-limited females. Trends in Ecology and Evolution 17:313–320.
Wolfram Research. 2014. Mathematica. Version 10.1. Wolfram Research, Champaign, IL.
Wright, S. 1931. Evolution in Mendelian populations. Genetics 16: 97–159.

Associate Editor: Stephen R. Proulx
Editor: Yannis Michalakis

Galeolaria geminoa is a broadcast spawning marine tube worm common on the east coast of Australia and a particularly tractable experimental species for studies of reproduction in broadcast spawners. This female has recently spawned in a laboratory setting; her eggs can be seen in the background, attached to her setae. Photo credit: Laura McLeod/Marshall lab.