SELECTION FOR POLLEN COMPETITIVE ABILITY IN MIXED MATING SYSTEMS

ABSTRACT

Co-expression of genes in plant sporophytes and gametophytes allows correlated gametic and sporophytic selection. Theory predicts that, under outcrossing, an allele conferring an advantage to pollen competitive ability should rapidly fix within a population unless antagonistic pleiotropy with the sporophyte stage is strong. However, under strong selfing, pollen competitiveness is immaterial because superior and inferior competitors are deposited on opposite stigmas, producing assortative competition. Because many plant species have mixed-mating systems, selfing should be critical in the spread and maintenance of pollen-expressed genes affecting competitiveness. We present two one-locus, two-allele population genetic models for the evolution of a locus controlling pleiotropic antagonism between pollen competitiveness and diploid fitness. Analytical solutions provide minimum and maximum selfing rates allowing invasion of alleles with greater fitness in diploid and haploid stages respectively. Further, polymorphism is only maintained when diploid selection is recessive. Numerically, we show fixation of the allele conferring greater pollen competitiveness may be prevented, even with weak counter-selection in the sporophyte stage, when selfing is sufficiently high. Finally, selfing expands and limits the range of haploid-diploid selection coefficients that allow for polymorphism, depending on dominance and mode of self-pollination.

Key Words: Assortative competition, gametic selection, sporophytic selection, population genetics, mixed mating, genetic correlation

INTRODUCTION

More than 7000 genes are expressed in male gametophytes—pollen grains, pollen tubes and the sperm they transmit—as they compete in the pistil for access to ovules (Page and Grossniklaus 2002; Honys and Twell 2003; Rutley and Twell 2015). As many as 60% of all known plant genes are expressed in both the gametophyte and sporophyte (Borges et al. 2009; Arunkumar et al. 2013). For instance, a gene regulating cell polarity and elongation, *PIP5K4*, is expressed in both pollen tubes and root hairs (Sousa et al. 2008). Over 100 cases of co-expression in pollen tubes and root hairs have been identified (Hadif et al. 2012). This high degree of co-expression presents ample opportunity for selection acting on pollen to affect sporophyte evolution, and *vice versa*. Haldane (1932) was among the first to recognize this potential, writing "Clearly a higher plant species is at the mercy of its pollen grains. A gene which greatly accelerates pollen tube growth will spread through a species even if it causes moderately disadvantageous changes in the adult plant." Put simply, genes conferring a competitive advantage in the gametophyte fix unless there is strong antagonistic pleiotropy of these genes with sporophyte fitness.

Selection should be strong on alleles with concordant impacts on the gametophyte and sporophyte stages. Deleterious recessive mutations, which are masked in the heterozygous sporophyte, are fully exposed to selection in the haploid gametophyte. This facilitates purging, thereby diminishing inbreeding depression (Charlesworth and Charlesworth 1992). Some have suggested that alleles promoting pollen competition will increase offspring quality; large stigmatic pollen loads lead to intense competition in the style, favouring the genetically superior gametophytes, which then contribute their superior genes to the seed (Mulcahy and Mulcahy 1987; Winsor et al. 1987; Winsor et al. 2000; but see Baskin and Baskin 2015; Pélabon et al. 2016). Walsh and Charlesworth (1992) argue that strong selection on alleles with concordant

effects on the gametophyte and sporophyte will soon deplete genetic variation in pollen competitive ability. But, as Haldane suggested, polymorphism may be maintained if an advantage in pollen competitive ability is counterbalanced by fitness costs to the sporophyte.

Immler, Arnqvist and Otto (2012) explored the maintenance of genetic variation under discordant selection between haploid and diploid phases, as well as under sex-specific selection. Considering an autosomal gene, they demonstrated polymorphism is most easily maintained under negative ploidy-by-sex interactions, under which sex-specific selection acts in opposite directions in haploids and diploids, i.e., is ploidally antagonistic. More generally, they conclude that polymorphism can be maintained, even in the absence of sex differences in selection, when selection is opposing between sporophytic and gametophytic stages.

High rates of self-pollination diminish the advantage of pollen competitive ability (Mazer et al. 2010), which may in turn restrict the conditions for stable polymorphism. This is due to assortative competition under self-pollination. Imagine a one locus system, with allele C_g conferring a competitive advantage to pollen (i.e., in the gametophytic phase) and the alternate allele C_s advantages the sporophytic phase. When a C_gC_g homozygote self-pollinates, the C_g allele gets passed to the offspring by necessity and not due to its competitive superiority. On the opposite homozygote, the C_s allele is likewise successful by necessity, despite its competitive inferiority. The competitive advantage of C_g- over C_s-bearing pollen is realized when heterozygotes self-pollinate, but because selfing reduces heterozygote frequency, over time there are fewer and fewer opportunities for C_g to exercise its superiority. Thus, with obligate selfing, the competitively inferior C_s allele gains refuge from competition, and since it confers a fitness benefit in the sporophyte, this allele quickly goes to fixation. Indeed, a comparison between two populations of *Clarkia tembloriensis* (Onagraceae) revealed slower pollen tube growth rates in

69 the highly selfing population than in the highly outcrossing one (Smith-Huerta 1996), consistent
70 with predictions. Additional examples are reviewed by Mazer et al. (2010).
71 While a little over half of plant species are self-incompatible, and thus obligate
72 outcrossers (Igic and Kohn 2006), about 40% of species have mixed mating systems—
73 individuals typically receive mixtures of self and outcross pollen (Schemske and Lande 1985;
74 Goodwillie et al. 2005). Of the 155 studies of mixed mating systems in plants reviewed by
75 Barrett and Eckert (2012), two-thirds report selfing rates in excess of 20%. How do intermediate
76 selfing rates influence the fate of a new mutation increasing pollen competitiveness at the
77 expense of sporophyte?
78 An important paper by Jordan and Connallon (2014) considered the impact of self-
79 fertilization on sexual conflict in hermaphrodites. They found that selfing narrows the conditions
80 for invasion of new alleles conferring male-benefit in the haploid phase if they impose a cost on
81 female function during the diploid phase. Their model considered the particular case in which
82 gamete quality is determined by the genotype of the adult producing it. In this situation a C_g-
83 bearing gamete from a homozygous adult differs from a C_g-bearing gamete produced by a
84 heterozygote, weakening the correspondence of gamete phenotype to haplotype. Gene expression
85 in animal sperm is limited (Parker and Begon 1993), and so it is reasonable to assume genetic
86 paternal effects on sperm quality for these species. In plants, adult diploid condition can affect
87 pollen size and quality (Young and Stanton 1990; Mazer and Gorchov 1996; Galloway 2001;
88 Smith-Huerta et al. 2007). Pollen size, which could well be mediated by genetically influenced
89 resource allocation by the sporophyte, readily responds to artificial selection (Sarkissian and
90 Harder 2001; McCallum and Chang 2016). Here we explore a different and potentially important
91 situation, specifically, when pollen competitive ability is affected by genes expressed directly in

92 the male gametophyte. Here one may expect stronger selection responses because of the stronger
93 correspondence of haplotype to phenotype.

94 The paper employs a one-locus model in which allele C_g increases pollen completive
95 ability at the expense of sporophyte fitness. Analytical solutions show there are maximum and
96 minimum selfing rates allowing invasion of C_g and C_s alleles, respectively, and that stable
97 polymorphism is possible only when C_g is recessive. Numerical analyses show that as selfing
98 rate increases, C_g's competitive advantage is negated by increasingly smaller diploid cost.

99 METHODS

100 THE MODELS

101 The departure from Hardy-Weinberg equilibrium caused by selfing necessitates that selection be
102 modelled in terms of genotype frequencies rather than allele frequencies (Crow and Kimura
103 1970). We refer to the frequencies of the C_gC_g, C_gC_s and C_sC_s genotypes as X_0, X_1 and X_2,
104 respectively, where the subscript denotes the number of C_s alleles. Allele frequencies are p and q,
105 for C_g and C_s, respectively.

106 Assume a pleiotropic effect by which the C_g allele increases success in pollen
107 competition but decreases the number of pollen grains and ovules that the diploid plant
108 contributes to the gamete pool (either by reduced survival or lower flower production).
109 Thus, selection favours the C_s allele through both sex functions in the diploid stage, disfavours it
110 in haploid pollen, and has no effect on ovules. Let the fitness of sporophyte C_sC_s be 1, and that of
111 C_gC_g to be 1-s, while the heterozygote C_gC_s is 1-hs, where h is the degree of dominance for
112 fitness. Similarly, define the absolute competitive ability of pollen bearing the C_g allele as 1 and
113 that of pollen bearing the C_s allele as 1-t. Finally, selfing rate, designated by r, varies between 0
114 and 1. All individual plants are expected to have many flowers, and to have the same selfing rate.

Using these parameterisations, we modelled two forms of selfing: fixed selfing and mass-action selfing, defined below.

Fixed selfing

Under fixed selfing, a stigma receives either all self-pollen or all outcross pollen. However, the proportion of flowers on a given plant that receive self-pollen can vary from zero to 1. We can produce recursion equations for genotype frequencies by considering diploid selection and selfing separately. After selection among diploids, genotype frequencies are:

$$x_0 = \frac{(1-s)X_0}{\bar{W}} \tag{1a}$$

$$x_1 = \frac{(1-hs)X_1}{\bar{W}} \tag{1b}$$

$$x_2 = \frac{X_2}{\bar{W}} \tag{1c}$$

where $\bar{W}$ is the mean diploid fitness:

$$\bar{W} = (1-s)X_0 + (1-hs)X_1 + X_2. \tag{2}$$

Next, genotype frequencies following mating are

$$X_0' = r\left[x_0 + \tfrac{1}{2}x_1\left(\tfrac{1}{2-t}\right)\right] + (1-r)\left[\left(x_0 + \tfrac{1}{2}x_1\right)\alpha\right] \tag{3a}$$

$$X_1' = r\left[\tfrac{1}{2}x_1\right] + (1-r)\left[x_0(1-\alpha) + \tfrac{1}{2}x_1 + x_2\alpha\right] \tag{3b}$$

$$X_2' = r\left[x_2 + \tfrac{1}{2}x_1\left(\tfrac{1-t}{2-t}\right)\right] + (1-r)\left[\left(x_2 + \tfrac{1}{2}x_1\right)(1-\alpha)\right] \tag{3c}$$

where α reflects the relative competitiveness of C_g-bearing pollen as a function of the absolute competitiveness conferred by the C_g allele and the frequency of the C_g allele in the pollen pool as a result of genotype frequency following selection:

$$\alpha = \frac{x_0 + \frac{1}{2}x_1}{x_0 + \frac{1}{2}x_1(2-t) + x_2(1-t)}. \quad (4)$$

It follows that the relative competitiveness of C_s-bearing pollen is (1- α).

To understand Equations (3a-c), consider separately the offspring produced via selfing (*r)* and those produced via outcrossing (1-*r*). Under selfing, competition among pollen tubes carrying opposite alleles only occurs within heterozygote stigmas and styles. Assuming heterozygotes produce C_g and C_s pollen in equal numbers, ½ of their offspring will likewise be heterozygous, with the remaining offspring divided between C_gC_g and C_sC_s homozygotes in proportions of $\left(\frac{1}{2-t}\right)$ and $\left(\frac{1-t}{2-t}\right)$ respectively. We assume that each selfing flower produces enough pollen to fertilise its available ovules, such that only female diploid fitness terms are included. Conversely, offspring produced through outcrossing will be a function of female diploid fitness as well as male diploid fitness and the pollen competitive ability conferred by each allele, as specified by α. For sake of simplicity, we make no provision for inbreeding depression; increasing failure rate selfed offspring has the same effect as reducing the basic selfing rate (see Jordan and Connallon 2014).

Mass-action selfing

Under mass-action selfing (Holsinger 1991), all individual stigmas can receive both self- and outcross-pollen, resulting in direct competition for ovules. In such a case, the amount of self-pollen received by a stigma is a function of the following: the proportion of the focal flower's pollen that is dedicated to selfing (i.e., that is deposited on its own stigma), *r*; the quantity of pollen produced by the individual relative to that available through outcrossing; and, the relative competitive ability of the self-pollen.

Assuming diploid selection occurs according to Equations (1a-c), we arrive at the following genotype frequencies after mating:

$$X_0^{'} = x_0\left[\frac{rW_0 + (1-r)\left(x_0 + \frac{1}{2}x_1\right)}{rW_0 + (1-r)\left(x_0 + \frac{1}{2}x_1(2-t) + x_2(1-t)\right)}\right] + \frac{1}{2}x_1\left[\frac{r\frac{1}{2}W_1 + (1-r)\left(x_0 + \frac{1}{2}x_1\right)}{r\frac{1}{2}W_1(2-t) + (1-r)\left(x_0 + \frac{1}{2}x_1(2-t) + x_2(1-t)\right)}\right] \quad (5a)$$

$$X_1^{'} = \frac{1}{2}x_1 + x_0\left[\frac{(1-r)\left(x_2 + \frac{1}{2}x_1\right)(1-t)}{rW_0 + (1-r)\left(x_0 + \frac{1}{2}x_1(2-t) + x_2(1-t)\right)}\right] + x_2\left[\frac{(1-r)\left(x_0 + \frac{1}{2}x_1\right)}{r\frac{1}{2}W_1(1-t) + (1-r)\left(x_0 + \frac{1}{2}x_1(2-t) + x_2(1-t)\right)}\right] \quad (5b)$$

$$X_2^{'} = x_2\left[\frac{rW_2(1-t) + (1-r)\left(x_0 + \frac{1}{2}x_1\right)(1-t)}{rW_2(1-t) + (1-r)\left(x_0 + \frac{1}{2}x_1(2-t) + x_2(1-t)\right)}\right] + \frac{1}{2}x_1\left[\frac{r\frac{1}{2}W_1(1-t) + (1-r)\left(x_2 + \frac{1}{2}x_1\right)(1-t)}{r\frac{1}{2}W_1(2-t) + (1-r)\left(x_0 + \frac{1}{2}x_1(2-t) + x_2(1-t)\right)}\right] \quad (5c)$$

According to Eq. 5a, the two terms represent C_gC_g offspring seed produced by C_gC_g and C_gC_s plants, respectively, with post-selection frequencies of x_0 and x_1. All ovules on the homozygote carry C_g, whereas on heterozygotes only half do. The terms inside the square brackets are the probability that an ovule receives C_g-bearing sperm, which depends on the amount of successful C_g pollen (numerator) divided by the total amount of pollen arriving at the stigma (denominator).

SOFTWARE AND COMPUTATION

Conditions for stable polymorphisms were explored analytically through invasion analyses using Wolfram Mathematica 11.0.1.0. We derived leading eigenvalues for the Jacobian Matrix using the genotype recursions (Eqs. 3a-c, 5a-c). We then determined the conditions that allowed

164 invasion at a boundary by solving for parameters that would produce a leading eigenvalue of an
165 absolute value greater than 1. Invasion analyses were performed for two boundaries: the X_0
166 boundary, at which the C_s allele appears in a population fixed for the C_g allele (i.e., $p = 1$), and
167 the X_2 boundary, at which the C_g allele appears in a population fixed for the C_s allele (i.e., $p = 0$).
168 All numerical results were produced using Visual Basic in Microsoft Excel 2011 Version
169 1610, from which we generated and recorded genotype and allele frequencies over thousands of
170 generations for combinations of selfing, diploid fitness and pollen competitiveness. Long-term
171 allele frequencies were taken from the 20,000th generation and verified by a change in allele
172 frequency, i.e., Δp, of less than 10^{-6}. Hereafter we will refer to these long-term allele frequencies
173 as equilibrium frequencies, as we assume that, with such significantly small changes in allele
174 frequencies, drift will dominate and these long-term frequencies are good approximations of true
175 equilibrium frequencies, where $\Delta p = 0$.

176 RESULTS

177 ANALYTICAL RESULTS

178 We explored the impact of selfing rate on the potential for both C_g and C_s to invade when rare.
179 Invasion analyses at both the X_0 and X_2 boundaries using Equations (3a-c) as well as Equations
180 (5a-c) in their full forms produces extremely complicated leading eigenvalues, limiting their
181 usefulness for predicting population dynamics and drawing clear biological conclusions. To
182 derive more meaningful interpretations, we assumed weak selection, with the diploid phase
183 selection coefficient being considerably smaller than the haploid (i.e., $0 < s << t << 1$).

184 *Fixed selfing*

185 Under fixed selfing, boundary selfing rates permitting invasion of the C_g and C_s alleles,
186 respectively, are as follows:

$$\text{For } C_g\text{: } r < 1 - \frac{s}{(1-2h)s+t} \tag{6a}$$

$$\text{For } C_s\text{: } r > 1 + \frac{s}{(1-2h)s-t} \tag{6b}$$

These analytical solutions are visualised in Figure (1a-c). When mating is random (i.e., $r = 0$), and $s << t$, the C_g allele can always invade, regardless of the degree of dominance h. This is because, without selfing, the C_s allele has no refuge from competition. Conversely, under complete selfing ($r = 1$), each individual sporophyte is a separate mating pool. Heterozygotes are quickly lost by segregation. With a loss of heterozygotes follows decreasing opportunity for competition between C_g- and C_s-bearing pollen. Consequently, the allele conferring higher relative sporophyte fitness in the homozygous state can increase when rare.

The analytical solutions indicate that polymorphism can be maintained in mixed mating systems ($0 < r < 1$), but only if the deleterious effect of the C_g allele in the diploid phase is recessive ($h < 0.5$). When completely recessive ($h = 0$), there is a range of selfing rates in which both alleles can invade when rare, implying protected polymorphism. The C_g allele invades because, when rare, it occurs exclusively in heterozygotes. As such, its negative effect on diploid fitness is entirely masked from selection, and when selfing occurs at moderate levels, there are enough opportunities to outcompete C_s-bearing pollen through greater fertilisation success. Within that same range of selfing rates, the C_s allele can also increase when rare because, 1) heterozygotes, where C_s occurs, have higher relative fitness than the C_gC_g homozygote, and 2) selfing reduces exposure of C_s pollen to competition with the superior C_g pollen.

Additive fitness effects ($h = 0.5$) prohibit polymorphism (Figure 1b). Under this condition, the critical r values for invasion reduce to $1 - \frac{s}{t}$ (Eqn. 6a-b) for both alleles. As h increases, the relative fitness of heterozygotes decreases. It then follows that the initial advantage enjoyed by both alleles when rare also diminishes. To invade, C_g requires low r values to win

208 enough contests to offset the lower fitness of heterozygotes. Conversely, C_s will require higher r
209 values to avoid competitive losses and produce enough high-fitness X_2 homozygotes to offset
210 lower fitness of heterozygotes.

211 When the disadvantage of the C_g allele in the diploid sporophyte is entirely dominant (h =
212 1), there is a range of selfing rates that prohibit either allele from invading when rare (Figure 1c).
213 For a given value of s, the maximum selfing rate allowing C_g invasion is lowered because its
214 adverse effect on sporophyte fitness is fully expressed by heterozygotes. On the other hand, the
215 critical r for C_s invasion increases when C_g is dominant. When rare, the positive diploid fitness
216 effects of the C_s allele are concealed in heterozygotes. Thus, only very high selfing rates, can
217 provide sufficient refuge from competition to allow C_s to spread.

218 *Mass-action selfing*

219 Considering mass-action selfing, the maximum and minimum r values allowing for invasion of
220 the C_g and C_s alleles can be solved analytically. However, the expressions are are complex
221 difficult to interpret biologically; these are present in Appendix A (online supplemental
222 material). As above, these solutions are visualised in Figure (1d-f).

223 The analytical solutions for mass-action selfing lead to the qualitatively the same
224 outcomes as fixed selfing, i.e., at any given value of dominance (h), the selfing rate (r)
225 prohibiting invasion by C_g decreases as cost increases (Figures 1a-c versus 1d-f).

226 The modest quantitative differences in outcome between the two selfing modes stem
227 from the different impact that diploid cost for competitive ability gets paid through male
228 function. Under mass-action selfing each stigma can receive a mixture of C_g- and C_s- bearing
229 pollen (except when r = 1, i.e., all individuals are entirely selfing). A given selfing rate between
230 0 and 1 thus affords less refuge for C_s from competition with C_g under the mass-action model,

231 compared to fixed selfing. However, this greater opportunity for C_g pollen to outcompete C_s is
232 partly offset through male diploid fitness differences. Under fixed selfing, male diploid fitness
233 (pollen quantity) is moot, since all flowers of each genotype are assumed to produce enough
234 pollen to fertilise all their ovules. Under mass-action selfing, in contrast, the quantity of pollen
235 produced by an individual affects its realized fertilisation success through self-pollen. This
236 limitation is similar to the relative fertilisation success of the C_g allele under fixed selfing, as
237 parameterised by α (Equation 4). The different impacts on male function shift the invasion
238 boundary upward unde mass action selfing. For example, when $h = 0$ (Figures 1a, 1d) and $s =$
239 0.01, the maximum selfing rate allowing the C_g allele to invade increases from $r = 0.5$ to $r = 0.6$.
240 Likewise, the minimum selfing rate allowing C_s to invade increases from $r = 0.75$ to $r = 0.8$.

241 NUMERICAL RESULTS FOR INVASION, FIXATION, AND PROTECTED POLYMORPHISM

242 The analytical results rest on the assumption that, when an allele is rare, its increase can
243 be modelled by considering only the frequencies of heterozygotes and the common homozygote,
244 while ignoring the frequency of the *very* rare homozygote. This assumption is strained at high
245 selfing rates because self-pollination by heterozygotes quickly increases the frequency of the rare
246 homozygote to a level that is no longer negligible, especially if selection during the diploid stage
247 is strong. To arrive at a tractable solution, we assumed that $s << t < 1$. Here we present numerical
248 results for the recursion equations (Equations 3a-c, 5a-c) to probe the potential for polymorphism
249 under broader conditions.

250 We start by running Equations (3a-c) with $t = 0.03$ and $s = 0.01$; these are the values that
251 are illustrated along the top edge of the panes in Figure 1. Figure 2 plots the equilibrium
252 frequency of C_g as a function of selfing rate, r. These numerical results agree with the analytical
253 solution when C_g is recessive ($h = 0$). Figure 2a shows that both alleles are maintained within the

254 range of selfing rates (r = 0.48 to 0.74) predicted in Figure (1a). The numerical results depart
255 slightly from the analytical solution when the two alleles have additive effects (h = 0.5). Rather
256 than an abrupt transition from fixation of C_g to fixation of C_s at a critical selfing rate (Figure 1b),
257 there is a narrow window permitting polymorphism (r = 063 - 0.69; Figure 2b). When the
258 negative impact of C_g on sporophyte fitness is dominant (h = 1), analytical and numerical results
259 agree that high selfing rates prevent invasion by the C_g allele when rare, while low rates prevent
260 C_s invasion. Over an intermediate range of selfing rates, neither allele can increase in frequency
261 when rare (Figure 2c).

262 The numerical results confirm that fixed and mass-action selfing modes have
263 qualitatively the same impact on invasion, fixation and polymorphism (Figure 1, 2). Again, for a
264 given value of h, the minimum and maximum selfing rates allowing for invasion of the C_g and C_s
265 alleles respectively increases under mass-action selfing (Figure 2d-f), as the C_g allele is allotted
266 more opportunities to outcompete the C_s allele. Additionally, the parameter space allowing both
267 polymorphism (Figure 2d) and unstable equilibrium (Figure 2f) contracts under mass action
268 selfing relative to fixed (Figures 2a, c).

269 Returning to Haldane's conjecture that plants are at the mercy of their pollen grains, how
270 strong must sporophyte selection against a pollen-competition allele be to prevent its fixation?
271 The fate of the C_g allele—fixation, elimination or stable polymorphism—depends on the strength
272 of selection during the two life stages, which we can consider as a ratio of diploid cost to haploid
273 benefit, i.e., s/t. At any given s/t ratio, increasing the rate of self-pollination will restrict
274 opportunities for C_g pollen to exercise their competitive superiority, muting the selective
275 advantage. Against this, at any given ratio, Mendelian dominance alters how often the cost of
276 competitive ability gets paid. If C_g is recessive (h = 0) heterozygotes pay no diploid cost. If

277 dominant (h = 1), it masks the C_s benefit and heterozygotes pay full cost; however, since selfing
278 decreases heterozygote frequency, the dominance issue becomes moot. These conditions produce
279 the general outcome that s/t ratios allowing C_g to invade, and to be maintained in stable
280 polymorphism, tend to decrease as both r and s increase (Figure 3).

281 DISCUSSION

282 Classical theory predicts that an allele with an advantage to pollen competitiveness will fix in a
283 population unless a trade-off with the sporophyte stage sufficiently counter-balances this haploid
284 advantage. Our model predicts that even moderate rates of selfing, can slow or prevent the
285 spread of a pollen-expressed allele that confers competitive superiority (Table 1). Self-
286 pollination leads to assortative competition among pollen grains, restricting opportunity for such
287 an allele to exercise its superiority. When homozygotes self, only one haplotype is deposited on
288 the stigma, so by necessity that haplotype cannot be out-competed by another. Alternate
289 haplotypes compete only on heterozygotes. In addition, selfing depresses heterozygote frequency
290 through the generations such that the proportion of the pollen pool engaged in competitive
291 contests falls in proportion to the selfing rate.

292 The dominance relationships between the competitive and non-competitive alleles in the
293 diploid stage also influences invasion success. When the fitness costs to the sporophyte of
294 competitive allele is recessive, it is shielded from negative selection during the diploid phase.
295 Full dominance fully exposes it to negative selection. Thus the conditions for invasion narrow
296 with increase in the dominance coefficient (Table 1, Figures 1 and 2).

297 Increased dominance also narrows the condition for stable polymorphism (Table 1).
298 When costs are recessive, heterozygotes gain success as fathers because 50% of their pollen is
299 competitively superior, and they pay no cost. This can lead to overall heterozygote superiority.

300 Under random mating, this over-dominance in total fitness can maintain stable polymorphism
301 (Figures 1-3). But selfing diminished the impact of over-dominance (Figure 3) by eroding
302 heterozygote frequency: heterozygosity lost to segregation is not replenished by matings between
303 opposite homozygotes, and so the competitive allele is eventually carried mostly by the
304 homozygote, who pay full cost. Dominance of the allele precludes stable polymorphism.
305 Although heterozygotes get half the paternal benefit, they but pay full cost (Table 1, Figure 3),
306 making over-dominance for total fitness unachievable.
307 These results are qualitatively similar to the case explored by Jordan and Connallon
308 (2014), where pollen competitive ability is under the control of the paternal genotype. They
309 considered sexually antagonistic selection in a one-locus model, whereby in the diploid, one
310 allele increases the number or quality of sperm produced at the expense of ova, while its
311 alternate promotes ova over sperm. They also found that selfing and dominance restricts the
312 potential for stable polymorphism and ultimately diminish selection through male function.
313 Our results extend these findings to the potentially important case where pollen
314 competitive ability is determined by gene expression in the gametophyte itself, and not by gene
315 products and transcripts carried over during meiosis. With gametophyte gene expression pollen
316 phenotype (competitive ability) is more strongly correlated with haplotype. Specifically,
317 heterozygotes always produce a 50:50 mix of phenotypically and genetically 'strong' and 'weak'
318 pollen competitors. Under paternal control, in contrast, heterozygotes can produce
319 phenotypically all strong ($h = 0$), all weak ($h = 1$), or all intermediate ($h = 0.5$) competitors, even
320 though they always produce a 50:50 genetic mix. In the early stages of invasion, when pollen
321 carrying the competitive allele is produced only by heterozygotes, it will always confer
322 superiority over the alternate allele when expressed by the gametophyte. This clear genetic signal

323 thus expands conditions for a costly allele to invade and spread, and a somewhat stronger selfing
324 rate is required to extinguish the advantage.
325 The models indicate that under outcrossing, where opportunities to express selective superiority
326 are maximized, selection on the gametophyte can overcome greater sporophyte costs than under
327 selfing. Genomic evidence on loci expressed exclusively in pollen, where diploid costs are
328 necessarily zero, bear out relaxed selection under selfing. In the self-incompatible *Capsella*
329 *grandiflora*, Arukumar et al. (2011) found evidence for stronger purifying selection at sites
330 expressed solely in the pollen tubes than those expressed exclusively in seedling. Looking at the
331 highly selfing *Arabidopsis thaliana*, on the other hand, Harrison et al. (2015) detected weaker
332 purifying selection in pollen-exclusive versus sporophyte-exclusive genes, as evidenced by a
333 higher frequency of premature stop codons in the former. Greater scrutiny of loci expressed
334 during both stages are needed to clarify the general importance of negative pleiotropic
335 constraints on the evolution of gametophyte and sporophyte traits.
336

337 **REFERENCES**

338 Arunkumar, R., E. B. Josephs, R. J. Williamson, and S. I. Wright. 2013. Pollen-specific, but not
339 sperm-specific, genes show stronger purifying selection and higher rates of positive
340 selection than sporophytic genes in *Capsella grandiflora*. Mol. Biol. Evol. 30:2475-2486.

341 Barrett, S. C. and C. G. Eckert. 2012. 14 Variation and Evolution of Mating Systems in Seed
342 Plants. Biological approaches and evolutionary trends in plants :229.

343 Barrett, S. C. H. 1998. The evolution of mating strategies in flowering plants. Trends Plant Sci.
344 3:335-341.

345 Baskin, J. M. and C. C. Baskin. 2015. Pollen (microgametophyte) competition: an assessment of
346 its significance in the evolution of flowering plant diversity, with particular reference to
347 seed germination. Seed Sci. Res. 25:1-11.

348 Borg, M., L. Brownfield, and D. Twell. 2009. Male gametophyte development: a molecular
349 perspective. J. Exp. Bot. 60:1465-1478.

350 Campbell, S. A. 2015. Ecological mechanisms for the coevolution of mating systems and
351 defence. New Phytol. 205:1047-1053.

352 Charlesworth, D. and B. Charlesworth. 1992. The effects of selection in the gametophyte stage
353 on mutational load. Evolution 46:703-720.

354 Crow, J. F. and M. Kimura. 1970. Introduction to Population Genetics Theory. Harper & Row,
355 New York.

356 Damgaard, C. and V. Loeschcke. 1994. Genetic-variation for selfing rate the dependence of
357 selfing rate on mating history in *Brassica napus* (rape seed). Heredity 72:570-573.

358 Galloway, L. F. 2001. The effect of maternal and paternal environments on seed characters in the
359 herbaceous plant *Campanula americana* (Campanulaceae). Am. J. Bot. 88:832-840.

360 Goodwillie, C., S. Kalisz, and C. G. Eckert. 2005. The evolutionary enigma of mixed mating
361 systems in plants: occurrence, theoretical explanations, and empirical evidence. Annu. Rev.
362 Ecol. Evol. Syst. 36:47-79.
363 Hafidh, S., K. Breznenová, P. Růžička, J. Feciková, V. Čapková, and D. Honys. 2012.
364 Comprehensive analysis of tobacco pollen transcriptome unveils common pathways in
365 polar cell expansion and underlying heterochronic shift during spermatogenesis. BMC
366 Plant Biol. 12(1): 24.
367 Haldane, J. B. S. 1932. The Causes of Evolution. Reprinted 1990, Princeton University Press.
368 Harrison, M. C., E.B. Mallon, D. Twell, and R.L. Hammond. 2015. Deleterious mutation
369 accumulation in *Arabidopsis thaliana* pollen genes: a role for a recent relaxation of
370 selection. bioRxiv, 016626.
371 Holsinger, K. E. 1991. Mass-action models of plant mating systems: the evolutionary stability of
372 mixed mating systems. Am. Nat. 138:606-622.
373 Igic, B. and J. R. Kohn. 2006. Bias in the studies of outcrossing rate distributions. Evolution 60
374 (5): 1098–1103.
375 Immler, S., G. Arnqvist, and S. P. Otto. 2012. Ploidally antagonistic selection maintains stable
376 genetic polymorphism. Evolution 66:55-65.
377 Jarne, P. and D. Charlesworth. 1993. The evolution of the selfing rate in functionally
378 hermaphrodite plants and animals. Annu. Rev. Ecol. Evol. Syst. 24:441-466.
379 Jordan, C. Y. and T. Connallon. 2014. Sexually antagonistic polymorphism in simultaneous
380 hermaphrodites. Evolution 68:3555-3569.

381 Mazer, S. J., A. A. Hove, B. S. Miller, and M. Barbet-Massin. 2010. The joint evolution of
382 mating system and pollen performance: Predictions regarding male gametophytic evolution
383 in selfers vs. outcrossers. Perspect. Plant Ecol. Evol. Syst. 12:31-41.
384 McCallum, B. and S. M. Chang. 2016. Pollen competition in style: Effects of pollen size on
385 siring success in the hermaphroditic common morning glory, *Ipomoea purpurea*. Am. J.
386 Bot. 103(3):460-470.
387 Mulcahy, D. L. and G. B. Mulcahy. 1987. The effects of pollen competition. Am. Sci. 75:44-50.
388 Parker, G. and M. Begon. 1993. Sperm competition games: sperm size and number under
389 gametic control. Proc. R. Soc. London, Ser. B. 253:255-262.
390 Pélabon, C., L. Hennet, G. H. Bolstad, E. Albertsen, Ø. H. Opedal, R. K. Ekrem, and W. S.
391 Armbruster. 2016. Does stronger pollen competition improve offspring fitness when pollen
392 load does not vary? Am. J. Bot. 103:522-531.
393 Sarkissian, T. S. and L.D. Harder. 2001. Direct and indirect responses to selection on pollen size
394 in *Brassica rapa* L. J. Evol. Biol. 14(3):456-468
395 Schemske, D. W. and R. Lande. 1985. The evolution of self-fertilization and inbreeding
396 depression in plants. II. Empirical observations. Evolution 39:41-52.
397 Smith-Huerta, N. L. 1996. Pollen germination and tube growth in selfing and outcrossing
398 populations of *Clarkia tembloriensis* (Onagraceae). Int. J. Plant Sci. 157:228-233.
399 Sousa, E., B. Kost, and R. Malhó. (2008). *Arabidopsis* phosphatidylinositol-4-monophosphate 5-
400 kinase 4 regulates pollen tube growth and polarity by modulating membrane recycling.
401 Plant Cell, 20(11), 3050-3064.
402 Walsh, N. and D. Charlesworth. 1992. Evolutionary interpretations of differences in pollen tube
403 growth rates. Q. Rev. Biol. 67:19-37.

404 Winsor, J., L. Davis, and A. Stephenson. 1987. The relationship between pollen load and fruit
405 maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo*. Am. Nat.
406 129:643-656.
407 Winsor, J. A., S. Peretz, and A. G. Stephenson. 2000. Pollen competition in a natural population
408 of *Cucurbita foetidissima* (Cucurbitaceae). Am. J. Bot. 87:527-532.
409 Young, H. J. and M. L. Stanton. 1990. Influence of environmental quality on pollen competitive
410 ability in wild radish. Science 248:1631-1633.
411

412 **Tables**

413 TABLE 1. Summary of model outcomes for mutation to an allele that increases pollen (haploid
414 gametophyte) competitive ability, but reduces survivorship or fertility in the (diploid)
415 sporophyte.

	MATING SYSTEM		
EXPRESSION OF C_g IN DIPLOID SPOROPHYTE	**Obligate Outcrossing (r = 0)** *Ample opportunities* to exercise pollen competitive superiority	**Mixed (0 < r <1)** *Fewer opportunities* to exercise pollen competitive superiority	**Obligate Selfing (r = 1)** *No opportunities* to exercise pollen competitive superiority
Recessive Fitness cost paid only by homozygote	C_g goes to fixation if sporophyte cost is less than ½ the pollen competitive benefit, and stable polymorphism unless costs exceed ~10×benefit—*conditions for stable polymorphism broad*	C_g goes to fixation only if sporophyte cost is *substantially* less than ½ the competitive benefit; upper limit for cost to permit stable polymorphism is reduced even more—*conditions for stable polymorphism narrowed*	C_g unable to invade.
Dominant Fitness cost paid *both* by homozygote and heterozygote	C_g goes to fixation if sporophyte cost is less than ½ the pollen competitive benefit, but is eliminated if costs are higher—*no stable polymorphism.*	C_g goes to fixation only if sporophyte cost is *substantially* less than ½ the benefit, but *eliminated* if costs are any higher—*no stable polymorphism.*	C_g unable to invade.

416

417

418 **Figure legends**

419 *Figure 1*

420 Conditions producing fixation, polymorphism and unstable equilibrium (i.e., fixation is
421 dependent on initial frequencies) of two alleles C_g and C_s at an autosomal gene under
422 antagonistic pleiotropy between pollen competitive ability and diploid fitness. All results are for t
423 = 0.03. (a-c) Analytical results for the fixed-selfing model. Potential for polymorphism
424 diminishes as the dominance of the C_g allele increases, eventually being replaced by unstable
425 equilibrium when $h > 0.5$. As selfing rate increases, polymorphism requires greater diploid
426 selection (s) against the C_g allele to offset its greater pollen competitive ability (t). (d-f)
427 Analytical results for the mass-action model. Conditions allowing polymorphism versus unstable
428 equilibrium are similar to those of the fixed-selfing model, though markedly narrower, as
429 individual stigmas never receive entirely self-pollen except when $r = 1$, thus increasing
430 opportunity for competition between C_g- and C_s-bearing pollen.

431 *Figure 2*

432 Numerical results of equilibrium frequency of the C_g allele when $s = 0.01$ and $t = 0.03$ across
433 selfing rates $0 < r < 1$. Solid lines indicate equilibrium frequencies starting from the X_0 boundary,
434 i.e., when the C_s allele invades a population fixed for C_g, while dashed lines indicted the opposite
435 boundary. (a-c) Under the fixed-selfing model, the window of selfing rates contracts as
436 dominance increases. (d-f) Under the mass-action model, C_s pollen is never entirely protected
437 from competition with C_g pollen as individual stigmas receive a mixture of both self and outcross
438 pollen for intermediate selfing rates. Thus, maximum and minimum selfing rates allowing
439 fixation or maintenance of C_g and C_s alleles respectively are right-shifted compared to under

fixed selfing, reflecting the greater opportunity for the C_g allele to exercise its advantage in pollen competition with the C_s allele.

Figure *3*

Numerical results for equilibrium frequency of the *Cg* allele across a range of *s/t*, i.e., ratios of diploid and haploid fitness values. Series specify selfing rates *r* ranging between 0 and 1. Under both (a-c) fixed and (d-f) mass-action selfing models, increases in dominance of the *Cg* allele sharply decrease the maintenance of polymorphism, particularly at lower selfing rates. Noticably, for a given combination of selfing rate *r* and dominance *h,* the *Cg* allele persists at higher ratios of *s* and *t* under mass-action selfing relative to fixed-selfing , reflecting the greater opportunity for *Cg*-bearing pollen to compete *Cs*-bearing pollen under mass-action selfing.

Appendix A

Conditions for stable polymorphisms were explored analytically through invasion analyses. We derived leading eigenvalues for the Jacobian Matrix using the genotype recursions (Eqs. 3a-c, 5a-c) in the main text. We then determined the conditions that allowed invasion at a boundary by solving for parameters that would produce a leading eigenvalue of an absolute value greater than 1. Invasion analyses were performed for two boundaries: the X_0 boundary, at which the C_s allele appears in a population fixed for the C_g allele (i.e., $p = 1$), and the X_2 boundary, at which the C_g allele appears in a population fixed for the C_s allele (i.e., $p = 0$).

The analytical solutions specifying the selfing rates required for invasion C_g and C_s alleles are provided below:

For C_g: $r <$ (A1)

$$-\frac{-3\,s+4\,h\,s+t}{3\,s-6\,h\,s}+(2^{1/3}\,((9-30\,h+28\,h^2)\,s^2+(-9+14\,h)\,s\,t+t^2))/(3\,(-1+2\,h)\,s\,((54-270\,h+396\,h^2-160\,h^3)\,s^3-3\,(-9+12\,h+4\,h^2)\,s^2\,t+3\,(-9+14\,h)\,s\,t^2+2\,t^3+3\,\sqrt{3}\,\sqrt{-(s-2\,h\,s)^2\,(576\,h^4\,s^4-32\,h^3\,s^3\,(27\,s-37\,t)+12\,h\,s\,t\,(135\,s^2-100\,s\,t+17\,t^2)+4\,h^2\,s^2\,(81\,s^2-558\,s\,t+205\,t^2)+t\,(-432\,s^3+441\,s^2\,t-134\,s\,t^2+9\,t^3))}\,)^{1/3})+\frac{1}{3\times 2^{1/3}\,(-1+2\,h)\,s}((54-270\,h+396\,h^2-160\,h^3)\,s^3-3\,(-9+12\,h+4\,h^2)\,s^2\,t+3\,(-9+14\,h)\,s\,t^2+2\,t^3+3\,\sqrt{3}\,\sqrt{-(s-2\,h\,s)^2\,(576\,h^4\,s^4-32\,h^3\,s^3\,(27\,s-37\,t)+12\,h\,s\,t\,(135\,s^2-100\,s\,t+17\,t^2)+4\,h^2\,s^2\,(81\,s^2-558\,s\,t+205\,t^2)+t\,(-432\,s^3+441\,s^2\,t-134\,s\,t^2+9\,t^3))}\,)^{1/3}$$

For C_s: $r >$ (A2)

$$\frac{1}{6}\,(\frac{6\,(s-4\,h\,s+t)}{3\,s-6\,h\,s}+(2\,2^{1/3}\,((7-26\,h+28\,h^2)\,s^2+(5-14\,h)\,s\,t+t^2))/((-1+2\,h)\,s\,((-20+42\,h+84\,h^2-160\,h^3)\,s^3+3\,(7-20\,h+4\,h^2)\,s^2\,t+3\,(-5+14\,h)\,s\,t^2-2\,t^3+3\,\sqrt{3}\,\sqrt{-(s-2\,h\,s)^2\,(36\,(1-5\,h+4\,h^2)^2\,s^4-4\,(-35+177\,h-330\,h^2+296\,h^3)\,s^3\,t+(61-440\,h+820\,h^2)\,s^2\,t^2+2\,(35-102\,h)\,s\,t^3+9\,t^4)}\,)^{1/3})+\frac{1}{(-1+2\,h)\,s}2^{2/3}\,((-20+42\,h+84\,h^2-160\,h^3)\,s^3+3\,(7-20\,h+4\,h^2)\,s^2\,t+3\,(-5+14\,h)\,s\,t^2-2\,t^3+3\,\sqrt{3}\,\sqrt{-(s-2\,h\,s)^2\,(36\,(1-5\,h+4\,h^2)^2\,s^4-4\,(-35+177\,h-330\,h^2+296\,h^3)\,s^3\,t+(61-440\,h+820\,h^2)\,s^2\,t^2+2\,(35-102\,h)\,s\,t^3+9\,t^4)}\,)^{1/3})$$

Figures

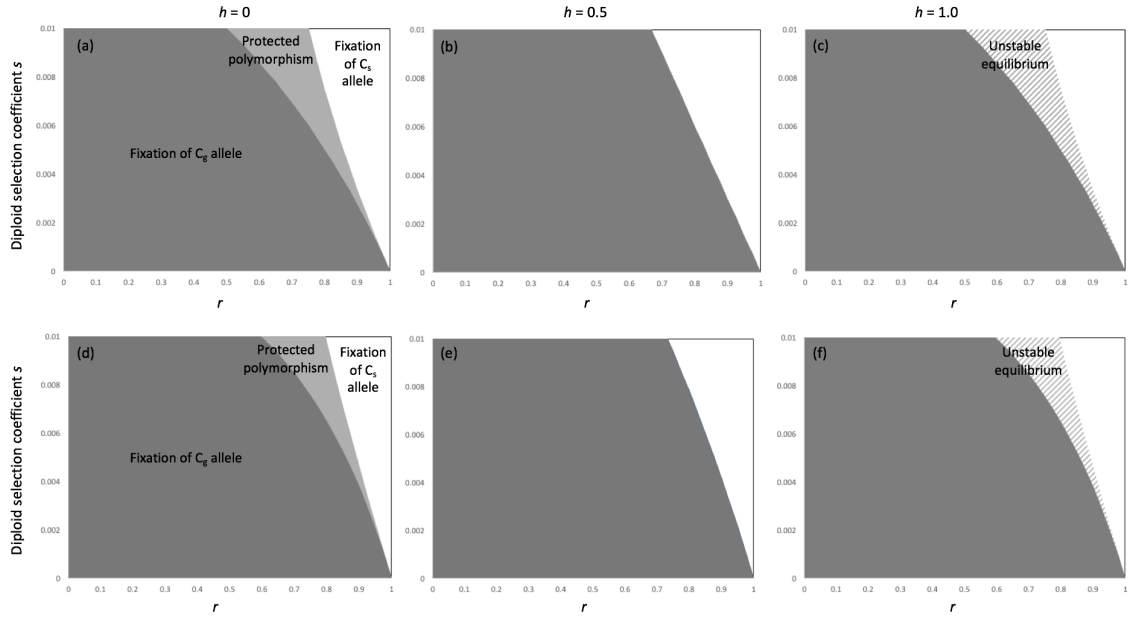

Figure 1

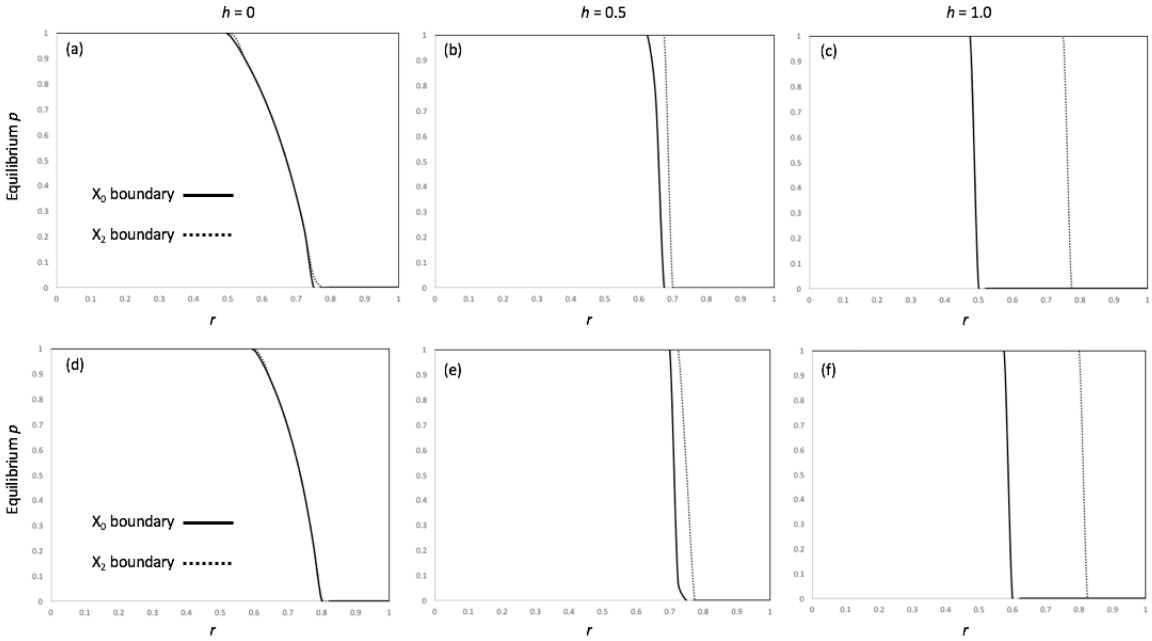

Figure 2

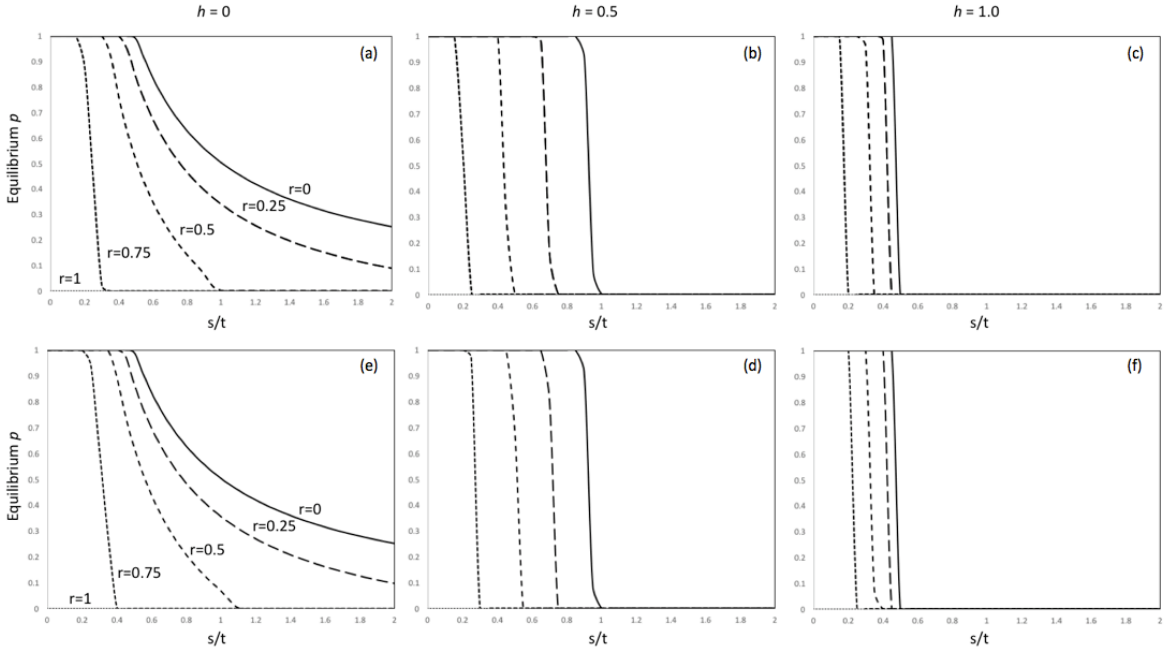

Figure 3