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# The evolutionary ecology of inbreeding depression in wild plant populations and its impact on plant mating systems

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6

7 **Keywords: Inbreeding Depression, Self-fertilization, Natural Population, Population Genetics,**  
8 **Evolution**

9 **Abstract**

10 Inbreeding depression, the reduced fitness of inbred relative to outbred individuals was described  
11 more than two centuries ago, long before the development of population genetics. Its impact is  
12 central to evolutionary ecology and the evolution of mating systems, in particular self-fertilization in  
13 hermaphrodites. In the first half of the 20<sup>th</sup> century, population genetics revealed a mechanism for  
14 inbreeding depression through homozygosity. Numerous theoretical studies have modeled inbreeding  
15 depression as a function of genetic architecture and analyzed how it varies with population selfing  
16 rates. A major concept in these models is purging, i.e., the purging of recessive deleterious mutations  
17 through inbreeding. Consequently, inbreeding depression is expected to decrease with increasing  
18 population selfing rates. Along with these theoretical studies, many experimental studies, particularly  
19 on plants, have measured inbreeding depression using experimental crosses or directly in the field.  
20 The results of these studies have revealed that the evolutionary ecology of inbreeding depression is  
21 difficult to capture and that empirical data do not exactly match model predictions, specifically  
22 purging efficacy. In addition, the lability of inbreeding depression in natural populations can  
23 qualitatively affect the selective role of inbreeding depression in the evolution of mating systems.  
24 Recently, several studies have demonstrated the role of epigenetics in shedding new light on the  
25 dynamics of inbreeding depression in natural populations. This review provides a general overview  
26 of the studies on inbreeding depression and how various angles can help capture its selective role in  
27 natural populations.

28 **1 Introduction**

29 Nearly a century before Darwin, Thomas Knight (1799) documented that vegetables with inbred  
30 plants were less fit than outbred ones, a phenomenon hereafter referred to as inbreeding depression,  
31 that is not restricted to plants; all diploid and polyploid organisms can exhibit this phenomenon.  
32 Darwin (1876) documented the deleterious effects of inbreeding in 57 species, which was described  
33 before genetics provided a mechanistic explanation. Although Gregor Mendel (1822-1884) was a  
34 contemporary of Darwin, a connection between genetics and natural selection was not established at  
35 this time. A century later, the development of population genetics provided a rationale and  
36 mechanistic model of inbreeding depression (Charlesworth and Charlesworth 2010). Interestingly,

37 Darwin anticipated a number of evolutionary trends, such as the relationship between inbreeding  
38 depression values and mating systems, which was later confirmed by the population genetics theory a  
39 hundred years later.

40 Inbreeding depression in natural populations is of central importance for several reasons. First,  
41 inbreeding depression can have consequences on population demography; this is especially true for  
42 organisms that practice regular inbreeding (e.g., self-fertilization in hermaphroditism) and this may  
43 be particularly harmful in small populations in conservation biology (Saccheri, Kuussaari et al. 1998,  
44 Degottex-Fréy and Cheptou 2023). Second, inbreeding depression is considered a major factor in the  
45 selection of life-history traits in organisms. The most significant trait selected by inbreeding  
46 depression is the mating system. Intuitively, a strong inbreeding depression will select outbreeding  
47 strategies to avoid the deleterious effects of inbreeding. The selective role of inbreeding depression  
48 has been studied as part of the evolution of self-fertilization in hermaphroditic organisms (e.g.,  
49 (Lloyd 1979)), and where organisms evolve their mating strategy through the avoidance of self-  
50 fertilization. An efficient and widespread mechanism to avoid selfing in plants is a self-  
51 incompatibility system (Nettancourt 1977), where pollen cannot germinate on the stigma of the same  
52 plant. When inbreeding depression is not too costly, selfing can be advantageous and self-fertilization  
53 strategies are sometimes adopted by plants (e.g., (Winn and Moriuchi 2009)) or animals ((Ostrowski,  
54 Jarne et al. 2003). Although less investigated, inbreeding depression is expected to cause evolution of  
55 dispersal traits, which is one way to avoid inbreeding depression (Ronce 2007). Third, since the rise  
56 of population genetics, inbreeding depression has also had many connections with population and  
57 quantitative genetics, as it addresses the genetic architecture of traits. Indeed, a trait will be subject to  
58 inbreeding depression depending on the dominance of alleles coding for that trait (see Section 2), and  
59 the frequency of such alleles may change with regular inbreeding. Finally, from an empirical  
60 perspective, inbreeding depression has been estimated several times in population biology, especially  
61 in plants because plants are mostly hermaphroditic, so producing experimentally inbred and outbred  
62 progeny is easy through controlled crosses. This has led to a general formulation of inbreeding  
63 depression in hermaphrodites  $\delta = \frac{W_{out} - W_{self}}{W_{out}}$ , where  $W_{out}$  and  $W_{self}$  capture the performance of  
64 individuals generated from outbreeding and selfing, respectively.

65 A long history of inbreeding depression has been reported in many outbred and inbred organisms in  
66 different environments. Such empirical data represent an unprecedented body of data that allows the  
67 testing or falsification (or at least corroborating or not) of population genetics predictions and  
68 evolutionary ecology models related to inbreeding depression.

69 This provides an overview of inbreeding depression in plants, with specific attention paid to the  
70 interplay between expectations from population genetics theory and empirical data. Far from being a  
71 simple test of theoretical models, the dialectic of theory and data has been fruitful and has enriched  
72 our understanding of the role of inbreeding depression in natural populations.

## 73 **2 Population genetics of inbreeding depression**

74 Inbreeding depression is defined as a reduction in the fitness for inbred progeny (e.g., selfing)  
75 compared to the fitness of outbred progeny. It is a ubiquitous phenomenon documented in various  
76 organisms such as humans, insects, birds, fish, crustaceans, ferns, and higher plants (Lynch and  
77 Walsh 1997). Inbreeding depression results from increased homozygosity, either through crosses  
78 between related individuals or siblings or selfing, the latter of which represents the ultimate form of  
79 regular inbreeding.

80 **2.1 The genetic basis of inbreeding depression**

81 The population genetics theory of inbreeding depression is synthesized in (Charlesworth and Willis  
 82 2009). Basically, the question is: what are the genetic characteristics required for fitness values to  
 83 decrease because of increased homozygosity in a population? The answer can be determined by  
 84 considering a single locus encoding any trait in a population and analyzing the immediate  
 85 consequences of inbreeding on the fitness of that population. For simplicity, we used hermaphroditic  
 86 organisms that are capable of selfing. In general, we can write a one-locus model with two alleles as:

87		AA	Aa	aa
88	Frequencies	D	H	R
89	Frequencies	D+H/4	H/2	R+H/4
90	(after one generation of selfing)			
91	Fitness values	w1	w2	w3

92

93 Frequencies before and after one generation of selfing are reported and the fitness value of each  
 94 genotype can be calculated. By convention, we assume that  $w1 > w3 > 0$ . The mean population fitness  
 95 without selfing can be easily calculated as  $\bar{w}_o = D w1 + H w2 + R w3$ . This can be compared with  
 96 the mean population fitness after one generation of selfing as  $\bar{w}_s = \left(D + \frac{H}{4}\right) w1 + \frac{H}{2} w2 +$   
 97  $\left(R + \frac{H}{4}\right) w3$ . Inbreeding depression occurs if  $\bar{w}_o > \bar{w}_s$ ; i.e.,  $\bar{w}_o - \bar{w}_s > 0$ . One can easily show that  
 98  $\bar{w}_o - \bar{w}_s = -\frac{H}{4}(w1 - 2 w2 + w3)$ . Thus, the sign of  $\bar{w}_o - \bar{w}_s$  depends on the fitness value of the  
 99 heterozygotes relative to that of the two homozygotes ( $w1$  and  $w2$ ). If alleles  $A$  and  $a$  are strictly  
 100 codominant ( $w2 = \frac{w1+w3}{2}$ ), there is no inbreeding depression. Therefore, strictly codominant genes  
 101 do not contribute to inbreeding depression. For inbreeding depression to occur,  $w2 > \frac{w1+w3}{2}$ ; i.e.,  
 102 the dominance coefficient,  $h$ , of allele  $a$  over  $A$  is below 0.5. Indeed, the condition can be rewritten as  
 103  $w2 = \frac{w1+h.w3}{2}, h < 0.5$ . The one-locus rationale can be extended to multi-locus traits, with two  
 104 genetic hypotheses fulfilling the condition that alleles contribute to inbreeding depression  
 105 (Charlesworth and Willis 2009). The first is the partial dominance hypothesis ( $0 < h < 1/2$ ), where  
 106 partially recessive deleterious alleles arise owing to mutations; and the second is the overdominance  
 107 hypothesis, where heterozygotes are fitter than homozygotes ( $h < 0$ ). The relative contributions of  
 108 over- and partial-dominance were subject to intensive debate in the 1970s (Crow, 1993). However, it  
 109 is now accepted that the partial dominance hypothesis is a major cause of inbreeding depression  
 110 (Charlesworth and Willis 2009, Hedrick and Garcia-Dorado 2016). Empirical studies that measured  
 111 mutation parameters have concluded that the rate of new deleterious mutations lies in the range of 0.1  
 112 to 1.0 per zygote per generation, with the reduction in fitness between 1% and 10% in the  
 113 homozygous state in metazoans (Schoen 2005). The implication of the population genetics theory  
 114 of inbreeding depression is that it is not static and depends on the genetic architecture of the traits  
 115 (e.g., mutation and dominance) and selection, which lowers the frequency of deleterious alleles.  
 116 Deleterious alleles are expected to be maintained at mutation/selection equilibrium in large  
 117 populations, eventually subject to genetic drift when population sizes are small. Experiments  
 118 measuring plant fitness can be used to make inferences about the deleterious effects of mutations but  
 119 do not identify specific loci that generate inbreeding depression. Genetic mapping studies are now

120 possible due to the development of intensive gene markers. Quantitative trait locus (QTL) mapping  
121 can identify the specific loci that cause inbreeding depression and such an approach has been  
122 investigated mostly in crop plants such as maize (Charlesworth and Willis 2009).

123

## 124 **2.2 Purging inbreeding depression or how inbreeding depression changes with population** 125 **inbreeding**

126 The genetic basis of inbreeding depression implies that it can change with regular inbreeding in  
127 populations. When deleterious recessive mutations cause inbreeding depression, the magnitude of  
128 inbreeding depression ( $\delta$ ) depends on the frequency of the deleterious mutations. While such a  
129 frequency is expected to be  $q = \frac{\mu}{h.s}$  (for  $h \neq 0$ ) in a fully outcrossing populations (i.e., random  
130 mating), where  $\mu$ ,  $h$ , and  $s$  are the mutation rate, the dominance coefficient, and deleterious effect of  
131 the recessive homozygotes, respectively, the frequency is  $\frac{\mu}{s}$  for a fully selfing population and  $\frac{\mu}{s} < \frac{\mu}{h.s}$   
132 for  $h < 1$  (Charlesworth and Charlesworth 2010). Under the partial dominance hypothesis, the purging  
133 process is defined as a reduced frequency of deleterious mutations in inbred populations. While  
134 deleterious mutations can be maintained in the heterozygous state in outcrossing populations because  
135 they have little effect on fitness, inbred populations are expected to eliminate most of their  
136 deleterious mutations because of the higher homozygosity in that population. The concept of purging  
137 has been central to the population genetics of inbreeding depression and predicts that outcrossing  
138 populations should exhibit stronger inbreeding depression than selfing populations. Multilocus  
139 models have been developed to predict the amount of inbreeding depression over the whole genome  
140 as a function of the population selfing rate (Charlesworth, Morgan et al. 1990, Charlesworth and  
141 Willis 2009). Although partial dominance can account for inbreeding depression in wild populations,  
142 it is important to note that in the overdominance hypothesis, inbreeding depression is expected to  
143 increase with population selfing rates.

## 144 **3 Empirical estimates of inbreeding depression in plants**

### 145 **3.1 The phenotypic expression of inbreeding depression in plants**

146 Hermaphroditic plants are ideal candidates for estimating inbreeding depression because  
147 experimental crosses (e.g., hand pollination) easily generates selfed progenies (except in SI species)  
148 and outcrossed progenies from the same mother plant. Measuring progeny fitness from experimental  
149 crosses allows for parameter estimation. Inbreeding depression is expected to develop throughout a  
150 plant's life. In annual plants, it is classically estimated at four stages of the life cycle: seed set,  
151 germination, survival before reproduction, and final biomass or number of flowers as a proxy for  
152 progeny number (Husband and Schemske 1996). Table 1 shows the mean magnitudes of inbreeding  
153 depression in angiosperms, gymnosperms, and gynodioecious species (Data reproduced from Winn  
154 et al., 2011). Using stage values, the inbreeding depression was expressed over the entire plant cycle;  
155 however, a limitation of these datasets is that only annual species were studied. In perennials, an  
156 additional stage must be considered to calculate the survival rate of the next generation. Although  
157 this parameter is often difficult to estimate directly because of the duration of the experiments, there  
158 is evidence that the survival stages may be subject to inbreeding depression (Delmas, Cheptou et al.  
159 2014), in accordance with the fact that perennials are mostly outcrossers (Morgan, Schoen et al.  
160 1997).

### 161 **3.2 Does inbreeding depression decrease with population selfing rates?**

162 In plants, not only can inbreeding depression be experimentally estimated, but the selfing rate from  
163 the studied populations under natural conditions can also be precisely estimated due to neutral co-  
164 dominant markers (e.g., microsatellites, Jarne and Lagoda, 1996). The relationship between  
165 inbreeding depression over the entire life cycle and population selfing rate illustrates the possibility  
166 of purging. Figure 1 shows data for 87 species (angiosperms and gymnosperms; (Winn, Elle et al.  
167 2011)) that indicate a general trend for inbreeding depression that decreases with selfing. The  
168 correlation between inbreeding depression and selfing rate was weak (Spearman rank correlation,  
169  $\rho = -0.18$ , one-tailed  $P = 0.1$ , Winn et al, 2011); that is, selfing explains only a fraction of the  
170 variance in inbreeding depression estimates (Figure 1). This questions the ability of the population  
171 genetics model to predict inbreeding depression values. For instance, the genus *Amsinckia* has low  
172 and nearly similar inbreeding depression values in a set of species despite their contrasting mating  
173 systems (Johnston and Schoen 1996). Such discrepancies between the models and data led (Byers  
174 and Waller 1999) to reduce the importance of purging dynamics in inbreeding depression. More  
175 recently, (Toczydlowski and Waller 2023) analyzed 12 populations of *Impatiens capensis* spanning a  
176 broad range of individual (-0.17 to 0.98) and population ( $F = 0.25 - 0.87$ ) inbreeding. Unexpectedly,  
177 they concluded that inbreeding depression was not systematically lower in inbred populations.  
178 Several arguments have been proposed to account for the differences between model expectations  
179 and experimental data. First, genetic drift can lower the efficacy of selection against deleterious  
180 mutations, especially when the mutation effects are small, which can blur the relationship between  
181 selfing rate and inbreeding depression. (Winn, Elle et al. 2011) suggested that selective interference  
182 could prevent purging and explained the weak relationship between inbreeding depression and  
183 selfing observed in empirical studies. These hypotheses further suggest that the dynamics of  
184 inbreeding depression are not fully captured by the deleterious mutation model. In addition, (Winn,  
185 Elle et al. 2011) reported an intriguing trend for mixed selfers ( $0.2 < s < 0.8$ ) that exhibited higher  
186 inbreeding depression than selfers ( $s > 0.8$ ) and outcrossers ( $s < 0.2$ ). However, this trend must be  
187 interpreted with caution because it could be an artifact of noise within the experimental crosses.  
188 Experimental crosses are often performed in insect-proof greenhouses and outcrossers often produce  
189 a large amount of pollen grains, so there is the possibility that the supposedly “selfing” class of  
190 crosses to be polluted by outcrossing events. Such pollen pollution would result in decreased contrast  
191 between the “outcrossing” and the “selfing” classes of plants, and explain the observed trend.  
192 Because mixed selfers produce far less pollen, this bias was not expected (or at least less expected) in  
193 mixed selfers. Pollen pollution can be controlled by genotyping the progeny, which advocates the use  
194 of genetic markers for accurate inbreeding depression estimates.

195 While most of our theoretical knowledge is based on diploids, inbreeding depression has been  
196 estimated in polyploidy species. (Clo and Kolar 2022) reported that phylogenetically young  
197 polyploid lineages have a lower amount of inbreeding depression than their diploid relatives. The  
198 authors suggest the negative effect of polyploidy on the magnitude of inbreeding depression tends to  
199 decrease with time since polyploidization. In crops, (Li and Brummer 2009) suggest that the higher  
200 inbreeding depression observed in some tetraploids could result from the rapid loss of multiple-allelic  
201 interactions within a locus.

### 202 3.3 Field estimates of inbreeding depression and the ecology of inbreeding depression

203 In Darwin’s book The Effect of Cross- and Self-Fertilization in the Vegetable Kingdom, he wrote:

204 *The result was in several cases (but not so invariably as might have been*  
205 *expected) that the crossed plants did not exceed in height the self-fertilized*  
206 *in nearly so great a degree as when grown in pairs in the pots. Thus, with*

207                    *the plants Digitalis, which competed together in pots, the crossed were to*  
208                    *the self-fertilized in height as 100 to 70; whilst those which were grown*  
209                    *separately were only as 100 to 85. Nearly the same result was observed*  
210                    *with Brassica. With Nicotiana the crossed were to the self-fertilized in*  
211                    *height, when grown extremely crowded together in pots, as 100 to 54;*  
212                    *when grown much less crowded in pots as 100 to 66, and when grown in*  
213                    *the open ground, so as to be subjected to but little competition, as 100 to*  
214                    *72 (Darwin, 1876).*

215    If Darwin's hypothesis was that inbreeding depression is likely lower in inbred populations so that  
216    selfing populations pay a low cost of self-fertilization, he also noted that environmental conditions  
217    may affect inbreeding depression values. This hypothesis was not explored until the 1980s, most of  
218    the studies were performed in a single constant environment (greenhouses, common gardens).  
219    Inbreeding has been found to compromise host plant defense gene expression in *Solanum carolinense*  
220    (Campbell, Thaler et al. 2013). In the 2000's, several studies have included the effect of  
221    environmental conditions on inbreeding depression estimates (reviewed in (Crnokrak and Roff 1999,  
222    Armbruster and Reed 2005, Cheptou and Donohue 2011). However, the results show contrasting  
223    trends. (Armbruster and Reed 2005) concluded that stress does not necessarily increase inbreeding  
224    depression, while (Cheptou and Donohue 2011) reported that both higher or lower inbreeding  
225    depression could potentially be found. By modelling inbreeding depression in a quantitative genetic  
226    model, (Ronce, Shaw et al. 2009) found that inbreeding depression would increase under stressful  
227    conditions. This may be due to that fact that stress applied in experiments encompasses a variety of  
228    stress that may act differently on genotypic expression. The question is whether inbreeding  
229    depression measured under standard conditions provides a relevant estimate of inbreeding depression  
230    in natural populations and (Dole and Ritland 1993) made interesting contributions in this regard.  
231    They estimated the inbreeding depression directly in the field in two species of the *Mimulus* genus  
232    over two years. These inferences were based on changes in population inbreeding from seeds to  
233    adults (Table 2). Interestingly, they found that the inbreeding depression estimates were  
234    approximately three times stronger in 1990 than 1989 for both taxa. Because changes in deleterious  
235    gene frequency (i.e., purging) could not account for such inbreeding depression variations in a single  
236    generation, they concluded that inbreeding depression is either affected by environmental conditions  
237    in natural environments or that inbreeding depression likely varies from year to year from the  
238    stochasticity of environmental conditions. The fact that environmental factors are likely to affect  
239    inbreeding depression suggests that recessive mutations are not unconditionally deleterious, but that  
240    their selective effect varies with the environment. It is also possible that certain alleles are beneficial  
241    in certain environments and deleterious in others. For instance, in the species *Silene latifolia*  
242    (Schrieber, Wolf et al. 2019) reported that the magnitude of inbreeding depression in fruit number  
243    was lower in invasive than native populations. While we have compelling evidence of inbreeding x  
244    environment interactions on fitness, the ecology of inbreeding depression remains incomplete and we  
245    are yet to identify the relevant factors affecting the magnitude of inbreeding depression. Some  
246    ecological factors may be inherent to the population, such as density dependence, whereas others  
247    may be caused by external factors like climate and interspecific competition. Regarding density  
248    dependence, (Cheptou and Schoen 2003) estimated inbreeding depression in selfing and outcrossing  
249    *Amsinckia* species ((Johnston and Schoen 1996), which included competition at high density and  
250    between inbred and outbred plants. The rationale was that, in a selfing population, the most  
251    competitive interactions occur within the inbred plants, whereas in an outcrossing population, the  
252    most competitive interactions occur with outbred plants. Interestingly, a larger inbreeding depression  
253    was found in outcrosser taxa than in selfing taxa, which was consistent with the evolutionary models  
254    of selfing.



#### 255 4 Demographic consequences of inbreeding depression in natural populations

256 Owing to its effects on fitness, inbreeding depression is expected to affect population demography,  
257 which is a concern in conservation biology (Frankham 1995). (Saccheri, Kuussaari et al. 1998)  
258 provided empirical data that demonstrated that inbreeding can affect population extinction. In the  
259 plant species *Gentiannella campestris*, (Lennartsson 2002) reported on the role of self-fertilization in  
260 population persistence in the field, showing that selfers suffer from a demographic disadvantage  
261 compared with outcrossers and that the time to extinction was greatly reduced in selfers when  
262 compared to that of outcrossers. This was attributed to inbreeding depression over the entire life  
263 cycle of the plant; however, this result was not consistent with population genetics predictions.  
264 Indeed, at equilibrium, the genetic load of a self-fertilizing species is expected to be equal to the  
265 mutation rate  $\mu$ , or half the genetic load of an outcrossing population,  $2\mu$  (Charlesworth and  
266 Charlesworth 2010). The discrepancy between the empirical data and models suggests that other  
267 forces can affect inbreeding depression. For example, it is possible that populations have not reached  
268 equilibrium, especially when endangered species may be out of their evolutionary equilibrium.

#### 269 5 Evolutionary consequences of inbreeding depression in natural populations

270 In wild populations, we classically consider dispersal and mating to be two major traits influenced by  
271 inbreeding depression. Among the different causes of evolution, dispersal has been identified as a  
272 mechanism to avoid the deleterious effects of inbreeding when populations are genetically structured;  
273 i.e., when individuals within the population are genetically related (Bengtsson, 1978; Morgan, 2002).  
274 However, inbreeding avoidance has not been considered a major factor in the evolution of plant  
275 dispersal, rather, inbreeding avoidance is thought to play a role in animals such as mammals,  
276 favoring male-biased dispersal. In contrast, plant mating system evolution has been closely linked to  
277 inbreeding depression since Lloyd's seminal work (Lloyd 1979). In these models, outcrossing avoids  
278 inbreeding depression, while selfing is counter-selected by inbreeding depression, providing a gene  
279 transmission advantage over outcrossing genes (Fisher 1941) and an ecological advantage through  
280 reproductive assurance. Mathematically, the evolution of self-fertilization can be captured by  
281 analyzing the fate of a rare selfing mutant  $s$  in a population with the mean selfing rate  $\bar{s}$ . The fitness  
282 of the rare mutant can be captured by summing the genes transmitted to the next generation through  
283 selfed seeds, outcrossed seeds, and pollen export in the population:

284

$$285 \quad \text{Selfed seeds} \qquad \qquad \text{Outcrossed seeds} \qquad \qquad \text{Pollen export}$$
$$286 \quad w(s, \bar{s}) = f[2. s (1 - \delta) \quad + \quad (1 - s) \quad + \quad (1 - \bar{s})] \quad [1]$$

287 where  $\delta$  is the inbreeding depression parameter and  $f$  is the number of ovules produced by an  
288 individual. It is important to note that the selfed seeds contain two copies of the parental genes,  
289 whereas the outcrossed seeds contained only one copy. From this classical model (Lande and  
290 Schemske 1985), it follows that inbreeding depression is the only parameter that influences the  
291 evolution of self-fertilization. If the inbreeding depression  $\delta > 0.5$ , outcrossing is always favored and  
292 100% outcrossing will evolve in the population. In contrast, if inbreeding depression  $\delta < 0.5$ , selfing  
293 is always favored and 100% selfing will evolve in the population. This simplistic model has been  
294 criticized because it assumes that inbreeding depression is constant; i.e., it does not account for  
295 purging process (see part 1). Population genetic models have been developed to account for the joint  
296 evolution of selfing and inbreeding depressions (Lande and Schemske 1985, Charlesworth, Morgan



297 et al. 1990), in particular the importance of association between inbreeding depression loci and  
 298 selfing modifiers loci (Uyenoyama, Holsinger et al. 1993). Interestingly, the qualitative behavior of  
 299 the model predictions did not change; either 100% selfing or 100% outcrossing evolved in the  
 300 population depending on the initial inbreeding depression value. In these models, purging acts as  
 301 positive feedback on selfing because purging decreases inbreeding depression, favoring the evolution  
 302 of selfing. Many theoretical models have been developed to analyse the evolution of self-fertilization,  
 303 in particular to explain stable mixed mating systems (Goodwillie, Kalisz et al. 2005). Here, I will  
 304 focus on a specific mechanisms maintaining stable mixed selfing rates, based on environment-  
 305 depedant inbreeding depression.

306 In 2001, (Cheptou and Mathias 2001) proposed that, in natural populations, the magnitude of  
 307 inbreeding depression may be subject to fluctuations in time or space due to environmental variations  
 308 (e.g., abiotic or biotic factors). They demonstrated that qualitative changes in the evolution of selfing  
 309 occur because of inbreeding depression variation; in particular, mixed selfing rates are stabilized.  
 310 First, they found that temporal but not spatial variation could maintain mixed selfing. Second, they  
 311 showed that spatiotemporal variation may evolve into polymorphisms in selfing rates. The temporal  
 312 variation case can be illustrated using Lloyd's model. From equation [1], we can rewrite the fitness of  
 313 the rare mutant, assuming that inbreeding depression changes over time:

$$314 \quad wt(s, \bar{s}) = f[2. s (1 - \delta(t)) \quad + \quad (1 - s) \quad + \quad (1 - \bar{s})] \quad [2]$$

315

316 We assume that  $\delta$  varies in time in a stochastic manner where in each generation,  $\delta \in [0,1]$ , is a  
 317 probability distribution. In this context, the relevant fitness measure is the geometric mean over time.

$$W = \lim_{n \rightarrow \infty} \left( \prod_{t=1}^n wt \right)^{1/n}$$

318 Figure 2 depicts the selfing rate evolution, assuming a truncated Gaussian distribution ( $\delta \in [0,1]$ )  
 319 (Cheptou and Schoen 2002). If the temporal variance is higher than zero, there is a possibility that  
 320 mixed selfing rates will evolve.

321 Overall, the evolutionary models predicted that high inbreeding depression counteracts selfing  
 322 strategies; however, the empirical data do not always follow this approach. (Delmas, Cheptou et al.  
 323 2014) reported interesting results from studying the perennial mass-flowering shrub *Rhododendron*  
 324 *ferrugineum*. They compared the parental inbreeding coefficient with the progeny selfing rate for  
 325 each parent and in absence of inbreeding depression, it was expected that at equilibrium,  $F_{parent} =$   
 326  $\frac{s}{2-s}$ , where  $s$  is the selfing rate in the progeny (Charlesworth and Charlesworth 2010). This study  
 327 revealed that despite the high selfing rate in the progeny,  $F_{parent}$  was close to zero, which requires  
 328 very high inbreeding depression values ( $\delta > 0.9$ ) despite the high selfing rates caused by the absence  
 329 of pollination agents. Such high inbreeding depression values are inconsistent with expectations for  
 330 selfing species. A possible explanation for this outcome is that the population has not reached  
 331 equilibrium because of the recent decline in pollination, and/or inbreeding depression remains strong  
 332 in perennial species and the long generation time does not easily allow for the purging deleterious  
 333 mutations (see also Scofield and Schultz, 2006).

334 We have learned much about the dynamics of inbreeding depression by comparing the model  
335 expectations and empirical estimates of inbreeding depression. However, there are discrepancies  
336 between predictions and data.

## 337 **6 Revisiting inbreeding depression through the lens of epigenetics**

338 Until recently, most of our understanding of inbreeding depression in natural populations has resulted  
339 from the interpretation of inbreeding depression estimates in common environments using population  
340 genetics and, more specifically, the dynamics of deleterious mutations in natural populations.  
341 Recently, several studies have explored the potential role of epigenetics in the magnitude of  
342 inbreeding depression. While the epigenetics of inbreeding depression are still in their infancy, these  
343 results can change our understanding of inbreeding depression in natural populations and are worth  
344 discussing. *Sensu lato* epigenetics can be defined as any biological factor that affects phenotypes  
345 without altering the DNA sequence. In evolutionary ecology, methylation is one of several types of  
346 epigenetic marks that regulate DNA expression. (Stojanova, Maurice et al. 2020) measured  
347 inbreeding depression in the species *Lamium amplexicaule*, which produces cleistogamous and  
348 chasmogamous flowers in various proportions. They produced inbred progeny by hand self-  
349 pollination in chasmogamous and cleistogamous flowers (i.e., obligatory selfed), as well as produced  
350 outbred progeny from hand-outcrossed pollinations. According to the classical inbreeding depression  
351 theory, it was expected that progeny from cleistogamous flowers to behave like self-pollinated  
352 progeny from chasmogamous flowers, having a lower fitness than outbred progeny from  
353 chasmogamous flowers. The striking result of the study was that the “flower effect” on progeny  
354 fitness was statistically significant and had a higher impact on fitness than the “inbred/outbred” status  
355 of progeny, in spite of no major difference in seed sizes. Because *L. amplexicaule* flowers both in  
356 spring and autumn, with a higher cleistogamy rate in autumn, the authors tested the influence of  
357 season on progeny fitness and discovered that cleistogamous progeny performed better in autumn  
358 than in spring, while the reverse was true for both inbred and outbred chasmogamous progeny  
359 (Figure 3). However, this fitness pattern was not consistent with adaptation to environment-  
360 dependent inbreeding depression but was possibly consistent with adaptation to seasonal pollinator  
361 activity. Also, in *Solanum corolinense* (Nihranz, Walker et al. 2020) found transgenerational effects  
362 of herbivory and maternal plant inbreeding. In particular, they found that offspring of damaged plants  
363 flowered earlier and produced more flowers than offspring of undamaged plants.

364

365 This result is puzzling and suggests that epigenetic mechanisms are involved in the magnitude of  
366 inbreeding depression beyond the DNA sequence. (Vergeer, Wagemaker et al. 2012) conducted a  
367 simple and inspiring experiment on the role of methylation in inbreeding depression magnitude. The  
368 authors performed a classical inbreeding depression experiment by comparing selfed and outcrossed  
369 offspring in *Scabiosa columbaria* and concluded that inbreeding depression was high in this species  
370 and that inbred individuals had higher levels of methylation than outbred individuals. Then, they  
371 applied a chemical demethylation treatment (5-azacytidine) to the seedlings and found that the  
372 inbreeding depression was nearly zero in the treated plants (and inbred plants after treatment were  
373 taller than without treatment), clearly demonstrating that methylation is involved in inbreeding  
374 depression (Biemont, 2010; (Han, Wang et al. 2021). However, the way methylation group act in  
375 interaction with inbreeding on fitness is not clearcut. In their review, (Nebert, Gálvez-Peralta et al.  
376 2010) concluded that epigenetics on inbreeding depression can be either beneficial or detrimental.

377 Several lessons were learned from these experiments. First, they provide mechanisms for  
378 environment-dependent inbreeding depression. Epigenetic marks can be generated by environmental  
379 conditions (e.g., stress), which can affect the magnitude of inbreeding depression. Second, as noted

380 by (Han, Wang et al. 2021), the magnitude of inbreeding depression cannot be fully captured by the  
381 dynamics of “unconditional” deleterious mutations in populations. The experiments discussed  
382 suggest that inbreeding depression may be evolutionarily labile and that it includes epigenetic  
383 regulation. This opens a new perspective in inbreeding depression studies, in which ecological  
384 factors must be included to understand the dynamics of inbreeding depression in natural populations  
385 and its evolutionary consequences on life history traits. In the future, analyzing patterns of  
386 epigenetics (e.g. methylation) in relation to ecological factors should help to clarify how epigenetics  
387 modulate inbreeding depression and if such epigenetic patterns are adaptive with regards to natural  
388 selection in the wild.

389

## 390 **7 Conclusion**

391 More than two centuries after its discovery, inbreeding depression has remained an active area of  
392 research. Inbreeding depression in plants has been closely linked to the evolution of plant mating  
393 systems for two reasons. First, inbreeding depression values are expected to counter-select selfing  
394 genes, and evolutionary models have characterized inbreeding depression values that prevent selfing  
395 genes from evolving in the population (see the  $\delta$ -threshold, part 4, Lloyd, 1979). Secondly,  
396 population genetics has established that the extent of inbreeding depression is linked to selfing rates  
397 (i.e., the purging process). However, these two approaches are distinct and even after 40 years of  
398 research, the link between inbreeding depression and selfing remains unclear (Byers and Waller,  
399 1999).

400 Although much progress has been made in population genetics to capture the genomic architecture of  
401 inbreeding depression, more recent studies have revealed that the magnitude of inbreeding depression  
402 cannot rule out the importance of environmental conditions. This is especially important when  
403 analyzing the selective role of inbreeding depression in mating systems. Indeed, the value of  
404 inbreeding depression in favoring selfing genes is meaningful. The epigenetic approach *sensu lato*  
405 appears to be promising for investigating inbreeding depression in natural populations (Han et al,  
406 2021) and is likely to enrich our understanding of the genetics underlying inbreeding depression.

## 407 **8 Conflict of Interest**

408 All financial, commercial or other relationships that might be perceived by the academic community  
409 as representing a potential conflict of interest must be disclosed. If no such relationship exists,  
410 authors will be asked to confirm the following statement:

411 *The authors declare that the research was conducted in the absence of any commercial or financial*  
412 *relationships that could be construed as a potential conflict of interest.*

## 413 **9 Author Contributions**

414 P.-O. Cheptou wrote the entire manuscript.

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509

510 **Figure legends**

511 **Figure 1** : Relationships between inbreeding depression and population selfing rates illustrated with  
512 progeny array analysis using microsatellites in 87 plant populations (from Winn et al, 2011).

513 **Fig 2** : The evolution of self-fertilization based on equation [2] (see main text) under temporal  
514 variation of inbreeding depression. The different evolutionary stable strategies (e.g., complete  
515 selfing, mixed selfing, and complete outcrossing) are depicted as a function of the mean ( $\mu$ ) and the  
516 temporal variance ( $\sigma^2$ ) of the inbreeding depression distribution using a truncated Gaussian  
517 distribution ( $0 < \delta < 1$ ). Boundaries among complete selfing and mixed selfing and complete  
518 outcrossing are depicted by the black lines (based on Cheptou and Schoen, 2002).

519 **Fig 3**: Mean and standard errors for the number of flowers in *Lamium amplexicaule* experimental  
520 crosses: outcrossed progeny (solid black line), Chasmogamous selfed progeny (dotted black lines)  
521 and Cleistogamous selfed progeny (dotted gray lines). The experiment was performed in an  
522 experimental garden both in the spring and in autumn, which are the two flowering seasons for *L.*  
523 *amplexicaule* natural populations.

524

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526

527 Table 1: Means ( $\pm$ SE) inbreeding depression expressed at the four successive life- cycle stages: seed  
528 set, germination, survival before reproduction, growth/reproduction for all taxa, angiosperms only,  
529 gymnosperms only, and gynodioecious taxa only.

530

	<i>N</i>	<i>Seed set</i>	<i>Germination</i>	<i>Survival to flowering</i>	<i>Growth/reproduction</i>
All taxa	68	0.206 (0.032)	0.116 (0.018)	0.134 (0.032)	0.220 (0.019)
Angiosperms	58	0.143 (0.026)	0.127 (0.019)	0.119 (0.035)	0.226 (0.024)
Gymnosperms	10	0.571 (0.098)	0.053 (0.032)	0.211 (0.089)	0.187 (0.033)
Gynodioecious taxa	10	0.287 (0.073)	0.239 (0.051)	0.234 (0.149)	0.247 (0.065)

531 Data taken from Winn et al (2011).

532

533



534 **Table 2** : Field estimates of inbreeding depression for two consecutive years in the genus *Mimulus*.

---

	<b>Relative fitness of selfed progeny</b>	
	<i>Mimulus guttatus</i>	<i>Mimulus platycalix</i>
1989	0.09	0.17
1990	0.28	0.49

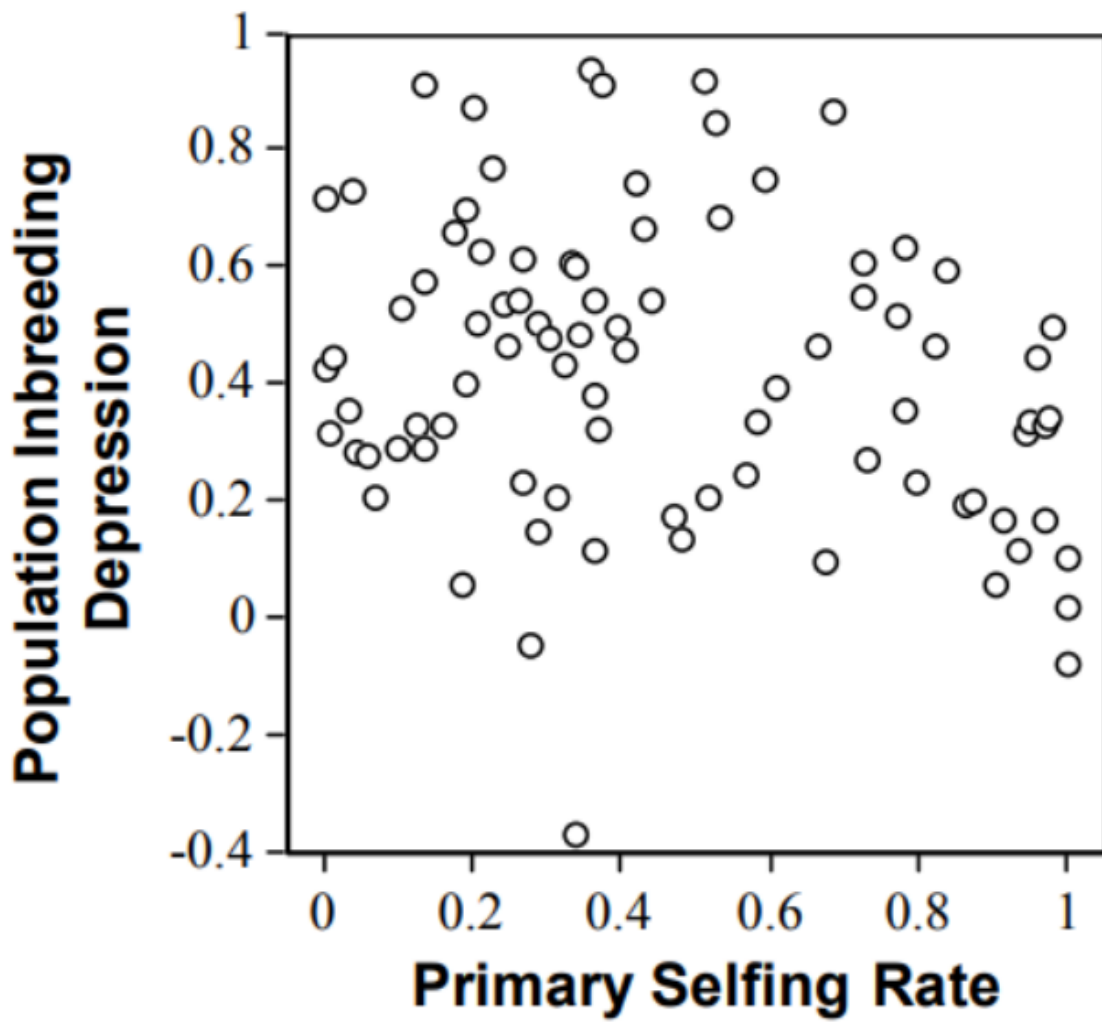
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535 Data from (Dole and Ritland 1993).

536

537 Figure1

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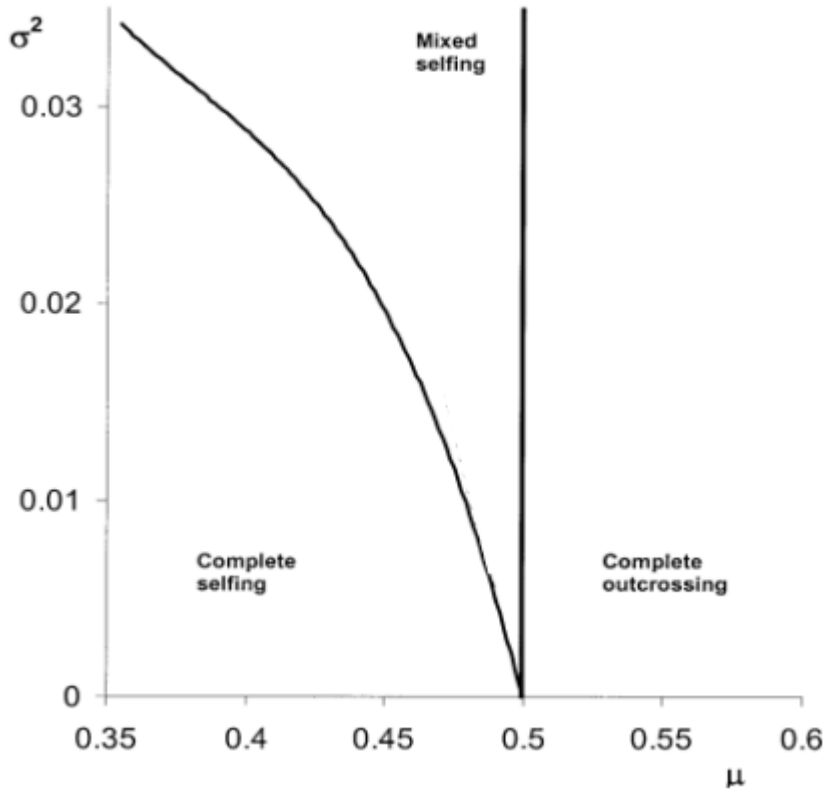


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540 Figure 2

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554 Figure 3

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