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Theory of coevolution of cytoplasmic male-sterility,

nuclear restorer and selfing

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27 **ABSTRACT**

28 Gynodioecy is a sexual polymorphism in angiosperms, where hermaphroditic and female individuals

29 coexist. This is often caused by a cytoplasmic genetic element (CGE) that destroys male functions,

30 which is called cytoplasmic male-sterility (CMS). On the other hand, nuclear genes tend to evolve the

31 ability to restore male function. The coevolutionary process of CMS and the restoration has been studied

32 theoretically. Recently, a theoretical study suggested that these coevolutionary dynamics could be

33 influenced by the rate of selfing within populations, although it assumed that the selfing rate of a

34 population was a fixed parameter. Accordingly, we theoretically study the coevolution of three traits in

35 this paper: CMS, nuclear restorer and selfing rate, in which we hypothesize that selfing evolution can

36 suppress CMS evolution under some conditions. The analysis indicates three significant properties of the

37 system; (1) CMS-restorer evolution can result in bistability under a given selfing rate, (2) the

38 coevolution of three traits can realize intermediate levels of selfing, and (3) the evolution of high levels

39 of selfing is conditionally associated with no CMS and/or no restoration, which may support our

40 hypothesis.

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42 Keywords: gynodioecy, inter-genomic conflict, pollen discounting, pollen limitation, inbreeding

43 depression, cost of restorer

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46　**INTRODUCTION**

47　Gynodioecy is a sexual polymorphism in angiosperms, where hermaphroditic and female individuals

48　coexist within a population. Gynodioecy is often caused by a cytoplasmic genetic element (CGE) which

49　destroys male functions, typically through mitochondrial mutants (see review by Hanson (1991)), which

50　is termed cytoplasmic male-sterility (CMS). Lewis (1941) was a pioneer in the theoretical research of

51　CMS evolution in plants. He showed that the CMS cytoplasm can increase in a population if CMS

52　results in an advantage in female fitness, and that a frequency of CMS reaches a stable equilibrium if the

53　female fitness involves a negative frequency dependence. After this, Lloyd (1974) theoretically studied

54　the dynamics of both nuclear- and cytoplasmic-controlling male-sterility, and investigated the effects of

55　self-fertilization on gynodioecy. Lloyd (1975) then extended his previous study by considering

56　inbreeding depression and showed that male-steriles can be maintained in a population when their

57　obligatorily outcrossed progenies have higher fitness than the inbred progeny (from self-fertilization in

58　hermaphrodites).

59　　　　CGEs are not transmitted to offspring via male gametes, therefore they would evolve male-

60　sterility when CGEs promote the individual female function. On the other hand, the male function can

61　genetically contribute to nuclear transmission via male gametes, which results in the inter-genomic

62　conflict between CGE and nucleus (Burt & Trivers, 2009), by which nuclear restore evolves to recover

63　male function. Incuding this factor, theoretical models were developed to investigate the coevolution

64　between CMS and nuclear restorer allele (Charlesworth & Ganders, 1979). Those early theoretical

65　studies of the coevolution of CMS and nuclear restorer concluded that the final population is fixed for

66　CMS with either restorer fixation (i,e., hermaphrodite) or remains polymorphic for the restorer alleles. In

67　the case with restorer polymorphism, individuals with and without restorer allele develop into

68　hermaphrodite and female, respectively, which corresponds to gynodyoecy in some species, called

69 "nuclear gynodioecy" (NG). In other species, however, both nuclear gene and cytoplasm are responsible

70 for the individual sex determination (Bailey & Delph, 2007b), which is called "nuclear-cytoplasmic

71 gynodioecy" (NCG) (Dornier & Dufay, 2013). In order to explain the role of cytoplasm in determining

72 sexuality, theoretical researchers have included a cost of restoration into the model (Delannay et al.,

73 1981, Charlesworth, 1981, Gouyon et al., 1991, Jacobs & Wade, 2003, McCauley & Bailey, 2009,

74 Bailey & Delph, 2007b, Dufay et al., 2007), which indicated the importance of restoration cost in the

75 stable maintenance of nuclear-cytoplasmic gynodioecy. Theoretical studies have also shown that the

76 restoration cost can contribute to the coexistence of multiple types of resotorer-CMS interactions in a

77 population (Gouyon et al., 1991, Maurice et al., 1993, Bailey et al., 2003). Furthermore, Gouyon et al.

78 (1991) and Dufay et al. (2007) showed that when both restorer and CMS are accompanied by costs, a

79 continuous oscillation with a limit cycle is possible in a gynodioecious population. These theoretical

80 studies of restoration cost have revealed evolutionary dynamics of nuclear-cytoplasmic gynodioecy,

81 although the recent studies tended to focus on self-incompatible species (except for a discussion by

82 Jacobs and Wade (2003)), thereby ignoring effects of selfing that were considered in earlier published

83 studies (Charlesworth, 1981, Delannay et al., 1981).

84 It should be remarked that the theoretical studies of gynodioecy have generally adopted one

85 critical assumption: a negative correlation between female fitness and female frequency in the

86 population, which is typically derived from the process of pollination. This assumption is based on the

87 principle of "pollen limitation" (Knight et al., 2005), by which there is a positive correlation between the

88 fertilization rate of ovules and the density of male organs in the population, so that fertilization is

89 restricted by the frequencies of male-sterile individuals. This limitation results in a negative frequency-

90 dependence in female fitness, which can stabilize gynodioecy (Maurice & Fleming, 1995). Importantly,

91 this process may be enhanced in the presence of self-fertilization in hermaphrodites, where the total

92　exportation of pollen is limited due to individual self-consumption (Charlesworth, 1980). The reduction

93　of pollen exportation by a preceding self-pollination is called "pollen discounting" (Harder & Wilson,

94　1998, Porcher & Lande, 2005a). Through the combination of pollen limitation and discounting, selfing

95　of hermaphrodites can negatively influence the fitness of CMS females by restricting fertilization in

96　outbreeding. It should also be remarked that this also affects the fitness of hermaphrodites with

97　autogamous selfing, because selfing ability without the help of pollinators ensures the fertilization of

98　their own ovules even under severe pollen limitation, which is called "reproductive assurance" (Sicard &

99　Lenhard, 2011). Theoretical studies have found that in a gynodioecious population, autogamous selfing

100　can be an effective assurance for hermaphrodite reproduction (Maurice & Fleming, 1995). Indeed,

101　Lahiani et al. (2015) empirically measured seed production, strength of pollen limitation, and the selfing

102　rates of hermaphroditic individuals in both female-biased and hermaphrodite-biased gynodioecious

103　populations. They clearly indicated that selfing can be a reproductive assurance, by which

104　hermaphrodites are more tolerant to pollen limitation than females especially in female-biased

105　populations.

106　　　　Despite an expectation of a significant effect of selfing, it has rarely been included within

107　theoretical studies of CMS-restorer coevolution. Charlesworth (1981) initially investigated this issue,

108　although the model did not include any cost of restoration. Delannay et al. (1981) and Jacobs and Wade

109　(2003) clarified selection in CMS-restorer coevolution with selfing in the presence of a restoration cost.

110　Dornier and Dufay (2013) modeled coevolutionary dynamics of CMS and restorer alleles with a cost

111　under selfing, based on computer simulations. These studies successfully revealed a relationship

112　between both CMS-restorer coevolution and selfing, although they considered selfing only as a fixed

113　parameter. In reality, however, selfing itself can be an evolutionary trait. Indeed, apart from CMS

114　studies, the evolution of selfing has been investigated both empirically and theoretically. Goodwillie et

115   al. (2005) reviewed the history and background of studies surrounding this issue and summarized the

116   theoretical and empirical achievements to date. More recently, Sicard and Lenhard (2011) reviewed the

117   available empirical research on selfing syndrome, in which they discussed a relationship between the

118   two factors that influence the selfing evolution, i.e., ecological and genetic factors. The ecological

119   factors consist of pollen discounting and pollen limitation, which quantitatively affect individual

120   reproductive success. (As described above, those are also important in the evolution of gynodioecy). On

121   the other hand, genetic factors involve inbreeding depression and a "50% automatic advantage of

122   selfing". When a rare mutant with perfect selfing invades a population with outcrossing only, the

123   individual automatically enjoys a 50% advantage of gene transmission because its pollen not only

124   fertilizes the ovules of the resident individuals, but also monopolizes its own ovules (Fisher, 1941,

125   Porcher & Lande, 2005b). Lloyd (1979) theoretically investigated selfing evolution with both inbreeding

126   depression and this automatic advantage and showed that if the loss of seed success by inbreeding

127   depression is smaller than 0.5, selfing can evolve.

128   Despite the understanding of the importance of selfing evolution in plant reproduction, CMS

129   evolution has not been studied alongside it theoretically. The purpose of the present study is to reveal the

130   effect of the evolution of selfing rate on the coevolution of CMS and nuclear restorer. In this paper, we

131   hypothesize that selfing evolution can suppress the evolution of CMS. For fertilization of ovules, CMS

132   females require hermaphroditic individuals as suppliers of pollen. In the presence of pollen discounting

133   and pollen limitation, selfing reduces the density of exported pollen, by which the evolution of CMS

134   might be suppressed. In order to investigate this process, we theoretically analyze the coevolution of

135   three traits: CMS, nuclear restorer, and selfing rate.

136

137   **MATHEMATICAL MODEL**

138   In our model, we assume a haploid organism with two nuclear loci, which determine a restoration ability

139   of cytoplasmic male sterility and a selfing rate. Theoretical studies of gynodioecy have traditionally

140   focused on diploid model, although studies of pathogen-plant interaction sometime considered haploid

141   plants (Parker, 1994, Agrawal & Lively, 2002) , which has been accepted as an appropriate

142   approximation. In this study, we also assume haploidy for a simplicity of analysis. The restoration is

143   considered to be controlled by a major gene that involves non-restorer and restorer alleles, genotypes of

144   which are denoted by m and M, respectively. On the other hand, the selfing rate is a quantitative trait that

145   is determined by many genes on quantitative loci with additive effects, which can evolve gradually. In

146   order to analyze evolutionary dynamics of this selfing rate, we assume two genotypes, i.e., a resident

147   selfing allele and a mutant selfing allele, between which the sefing rate is slightly different from each

148   other. The resident and mutant genotypes are denoted by s and s', which also represent their selfing rates.

149   In addition to two nuclear genes, the organism includes a cytoplasmic gene that causes male sterility,

150   which is mutant mitochondria. The genotypes of non-CMS and CMS cytoplasm are defined as n and c,

151   respectively. According to the alternative states on 2 nuclear genes and 1 cytoplasmic gene, there are 8

152   possible cyto-genotypes, i.e., ms/n, Ms/n, ms/c, Ms/c, ms'/n, Ms'/n, ms'/c, and Ms'/c, genotypic

153   frequencies of which are denoted by X_{mn}, X_{Mn}, X_{mc}, X_{Mc}, X'_{mn}, X'_{Mn}, X'_{mc} and X'_{Mc}, respectively. Primes on

154   the frequencies represent genotypes of mutant with respect to selfing. We assume that there is a linkage

155   between two nuclear loci with a recombination rate r, although it does not influence equilibria and their

156   stabilities.

157   We assume that male sterility is accompanied by a female advantage, promoting ovule

158   production with a relative advantage k (so that the ovule number becomes $1 + k$), which is achieved only

159   in genotypes ms/c and ms'/c with the CMS and non-restorer allele. It should be noted that our definition

160   of female advantage is different from that of Dornier and Dufay (2013) who analysed an analogous

161   system. In their analysis, female advantage was denoted as a function of selfing rate and inbreeding

162   depression (they expressed it as $k\, w_s/(1 - s\, \delta)$ in a case without the pollen limitation, where w_s

163   represented a negative effect of CMS on ovule production), which represents a relative advantage for

164   inbreeding success. On the contrary to this, we denote female advantage, k, excluding those factors (our

165   $1 + k$ corresponds with $k\, w_s$ in Dornier and Dufay), which we adopt for a convenience in the analysis of

166   selfing evolution.

167   In this study, we focus on the effects of the selfing rate on coevolution of nuclear restorer and

168   CMS. Selfing influences reproductive success at both the individual and population levels. The first

169   factor is inbreeding depression, which is denoted by a constant δ (i.e., inbreeding success is $1 - \delta$). It

170   should be noted that in reality, inbreeding depression can be a function of selfing rate because selfing

171   tends to sweep out deleterious alleles from the population through strong negative selection on the

172   homozygote of those alleles (Charlesworth et al., 1991, Lande & Schemske, 1985), although we assume

173   a constant inbreeding depression for the simplicity of our analysis. Furthermore, autogamous selfing can

174   reduce the amount of exported pollen if there is a trade-off between pollen number used for self-

175   pollination and outcrossing pollination, i.e., pollen discounting. We denote pollen discounting as a

176   reduction in the number of pollen that an individual releases, which is a function of its selfing rate s as

177   $D_p(s) = 1 - [b + (a - b)s^c]s$, following a formulation by a previous study (Johnston, 1998). In this

178   definition, the negative effect of selfing is expressed as $[b + (a - b)s^c]s$, where a ($0 \le a \le 1$) represents

179   reductions of exported pollen when $s = 1$, b determines the concaveness of the functional form (concave

180   and convex with $a > b$ and $a < b$, respectively), and c (> 0) controls the magnitude of curvature. In

181   addition, $D_p(s)$ is a monotonically decreasing function when $b < a\, (1 + c)/c$. At the population level, the

182   average density of exported pollen can decline with the increase of selfing rate via pollen discounting,

183   and also with the reduction of hermaphrodites as pollen suppliers. The reduction in the density of

184 exported pollen can negatively influence ovule fertilization through pollen limitation. The efficiency of

185 ovule fertilization is defined as an increasing function of the average density of exported pollen P_{EX}, a

186 formulation of which is assumed as $L_p(P_{EX}) = \beta P_{EX} / (\alpha + P_{EX})$. Based on these assumptions, the success

187 of inbreeding and outbreeding are generally weighted by the coefficients $1 - \delta$ and β, respectively. For

188 simplicity, we replace $(1 - \delta) / \beta$ by η. Since $1 - \delta$ is the relative success of inbreeding and β is the

189 maximum fertilization efficiency in outbreeding, η represents the relative advantage of selfing to

190 outbreeding. It should be noted that for outbreeding, the success of exported pollen is determined

191 through pollen competition for ovules, an effect which we incorporate in the model.

192 We consider that the nuclear restorer is accompanied by costs. Various types of expressions are

193 possible for this disadvantage of restoration (Bailey et al., 2003, Dufay et al., 2007). In the present study,

194 we consider two types of negative effect of the restorer allele, in which the restorer allele may reduce

195 either male function (pollen production) or individual performance (relative survivorship of individual)

196 from 1 to $1 - \mu$ ($\mu \leq 1$) in a relative value, which are hereafter referred to as cost types 1 and 2,

197 respectively. Those costs incur in individuals with a restorer allele independently of their cytotypes,

198 which is the so-called "constitutive cost" in the definition by Dufay et al. (2007). Remarkably, it was

199 reported that in *Lobelia siphilitica*, nuclear restore decreased pollen viability even when it did not affect

200 sex determination due to a mismatch of specificity to CMS (Bailey, 2002), which may partly correspond

201 with our cost type 1. We could also consider alternative types of restoration costs that decrease female

202 success only, although dynamic equations are difficult to solve explicitly in this case. Therefore, we

203 exclude this type of cost in the present analysis.

204 Under cost type 1, the male functions of both CMS and non-CMS individuals with the restorer

205 allele are lower than that of an individual without it, and therefore the average density of exported pollen

206 is affected by the restoration cost, a relative value, $P_{EX} = \{X_{mn} + (1 - \mu)(X_{Mn} + X_{Mc})\}D_p(s) +$

207　　$\{X'_{mn} + (1-\mu)(X'_{Mn} + X'_{Mc})\}D_p(s')$. On the other hand, under cost type 2, the frequencies of both

208　　CMS and non-CMS individuals with the restorer allele decline immediately before a measurement of

209　　their genotypic frequencies. In this case, the average density of exported pollen is $P_{EX} =$

210　　$(X_{mn} + X_{Mn} + X_{Mc})D_p(s) + (X'_{mn} + X'_{Mn} + X'_{Mc})D_p(s')$. This expression does not include the

211　　restoration cost μ because the costs have already affected the genotypic frequencies before the census of

212　　frequency. The densities of pollen influence outbreeding success through the fertilization efficiency of

213　　ovules due to pollen limitation, and the competition among pollen for ovules.

214　　　　Based on those assumptions, we formulate the dynamics of genotypic frequencies by

215　　combining the reproductive outcomes of selfing and outbreeding. Here, we show frequency dynamics of

216　　strains with resident selfing rate under cost type 1 in the absence of mutant strains as simple examples.

217　　For simplicity of description, we refer to X_{mn}, X_{Mn}, X_{mc}, and X_{Mc} by w, x, y, z, with which dynamics are

218
$$w_{t+1} = \left[(1-\delta)sw_t + \frac{\beta P_{EX}}{\alpha + P_{EX}}D_p(s)(1-s)\left\{\frac{w_t}{P_{EX}}\left(w_t + \frac{1}{2}x_t\right) + (1-\mu)\frac{x_t+z_t}{P_{EX}}\frac{1}{2}w_t\right\}\right]\frac{1}{W} \ , \qquad (1a)$$

219
$$x_{t+1} = \left[(1-\delta)sx_t + \frac{\beta P_{EX}}{\alpha + P_{EX}}D_p(s)(1-s)\left\{\frac{w_t}{P_{EX}}\frac{1}{2}x_t + (1-\mu)\frac{x_t+z_t}{P_{EX}}\left(\frac{1}{2}w_t + x_t\right)\right\}\right]\frac{1}{W} \ , \qquad (1b)$$

220
$$y_{t+1} = \frac{\beta P_{EX}}{\alpha + P_{EX}}D_p(s)(1-s)\left\{\frac{w_t}{P_{EX}}\left((1+k)y_t + \frac{1}{2}z_t\right) + (1-\mu)\frac{x_t+z_t}{P_{EX}}(1+k)\frac{1}{2}y_t\right\}\frac{1}{W} \ , \qquad (1c)$$

221
$$z_{t+1} = \left[(1-\delta)sz_t + \frac{\beta P_{EX}}{\alpha + P_{EX}}D_p(s)(1-s)\left\{\frac{w_t}{P_{EX}}\frac{1}{2}z_t + (1-\mu)\frac{x_t+z_t}{P_{EX}}\left((1+k)\frac{1}{2}y_t + z_t\right)\right\}\right]\frac{1}{W} \ , \quad (1d)$$

222　　where W represents the average fitness. Variables, parameters and functions that are used in the present

223　　model are summarized in Table 1. Full dynamics of the system are shown in the Appendix A.

224

225　　<u>Equilibrium mating modes under a fixed selfing rate</u>

226　　　　First, we investigate the dynamics in a population with a homogeneous selfing rate without a

227　　mutant selfing rate, i.e., $(X'_{mn}, X'_{Mn}, X'_{mc}, X'_{Mc}) = (0, 0, 0, 0)$. Under both cost types 1 and 2, there are six

228　　types of equilibria, two out of which are equilibrium states with a single genotype only. One equilibrium

229 is $(X_{mn}, X_{Mn}, X_{mc}, X_{Mc}) = (1, 0, 0, 0)$ that comprises a genotype with neither restorer nor CMS, which is

230 always feasible, but stable only if $\{k(1-s) - s\eta\}D_p(s) < s\alpha\eta$. Another solution is $(0, 0, 0, 1)$ with a

231 fixation of both restorer allele and CMS cytotype. It should be remarked that this solution is a subset of

232 an equilibrium with two genotypes $(0, X_{Mn}, 0, X_{Mc})$. The systems also generally have three types of

233 equilibria with two genotypes, and a unique solution with a coexistence of four genotypes. Properties of

234 those equilibria with multiple genotypes depend on cost types.

235 For cost type 1, genotypic frequencies are

236 $$(X_{mn}, X_{Mn}, X_{mc}, X_{Mc}) = (0, X_{Mn}, 0, X_{Mc}), \tag{2a}$$

237 $$(X_{mn}, X_{Mn}, X_{mc}, X_{Mc}) = \left(0, 0, 1 - \frac{2s\alpha\eta + (1+k)(1-s)(1-\mu)D_p(s)}{2\{k(1-s)-s\eta\}(1-\mu)}, \frac{2s\alpha\eta + (1+k)(1-s)(1-\mu)D_p(s)}{2\{k(1-s)-s\eta\}(1-\mu)}\right), \tag{2b}$$

238 $$(X_{mn}, X_{Mn}, X_{mc}, X_{Mc}) = \left(\frac{s\alpha\eta}{\{k(1-s)-s\eta\}D_p(s)}, 0, 1 - \frac{s\alpha\eta}{\{k(1-s)-s\eta\}D_p(s)}, 0\right), \tag{2c}$$

239 $$(X_{mn}, X_{Mn}, X_{mc}, X_{Mc}) =$$

240 $$\left(\frac{1+k}{k} \frac{s\alpha\eta - \{k(1-s)-s\eta\}(1-\mu)D_p(s)}{\{k(1-s)-s\eta\}\mu D_p(s)}, \frac{1}{k\mu}\left((1+k)(1-\mu) - \frac{s\alpha\eta\{1+k(1-\mu)\}}{\{k(1-s)-s\eta\}D_p(s)}\right),\right.$$

241 $$\left.\frac{1}{k}\left(-1 + \frac{s\alpha\eta}{\{k(1-s)-s\eta\}(1-\mu)D_p(s)}\right), \frac{1}{k}\left(1 + k - \frac{s\alpha\eta\{1+k(1-\mu)\}}{\{k(1-s)-s\eta\}(1-\mu)D_p(s)}\right)\right). \tag{2d}$$

242 Equilibrium (2a) is a line rather than a point, which also includes $(0, 0, 0, 1)$. The feasibility and stability

243 conditions of equilibria are summarized in Table 2. The stability condition of the last equilibrium is too

244 complex to be derived analytically, so are examined numerically based on the eigenvalues of the

245 Jacobian matrix of the dynamics. Examples of parameter dependence of those conditions are illustrated

246 in Figure 1 under the parameter set given in Table 1. (We also supplementally check the robustness of

247 this trend with varying parameters (see below)).

248 On the other hand, those for the cost type 2 are

249 $$(X_{mn}, X_{Mn}, X_{mc}, X_{Mc}) = (0, X_{Mn}, 0, X_{Mc}), \tag{3a}$$

250 $\quad$ $(X_{mn}, X_{Mn}, X_{mc}, X_{Mc}) =$

251 $$\left(0,\ 0,\ 1 - \frac{(1-\mu)\{2s\alpha\eta+(1+k)(1-s)D_p(s)\}}{[(1-s)\{k(2-\mu)+\mu\}-2s\eta(1-\mu)]D_p(s)},\ \frac{(1-\mu)\{2s\alpha\eta+(1+k)(1-s)D_p(s)\}}{[(1-s)\{k(2-\mu)+\mu\}-2s\eta(1-\mu)]D_p(s)}\right), \qquad (3b)$$

252 $$(X_{mn}, X_{Mn}, X_{mc}, X_{Mc}) = \left(\frac{s\alpha\eta}{\{k(1-s)-s\eta\}D_p(s)},\ 0,\ 1 - \frac{s\alpha\eta}{\{k(1-s)-s\eta\}D_p(s)},\ 0\right), \qquad (3c)$$

253 $\quad$ $(X_{mn}, X_{Mn}, X_{mc}, X_{Mc}) =$

254 $$\frac{1}{\{2s\eta(1+k\mu)+k(k-1)(1-s)(1-\mu)\}D_p(s)} \times$$

255 $$\left(\frac{[2s\alpha\eta(k+\mu)-(1+k)\{k(1-s)-2s\eta\}(1-\mu)D_p(s)][s\alpha\eta(1+k\mu)+\{s\eta(1+k\mu)-k(1-s)(1-\mu)\}D_p(s)]}{s\eta\mu\{(k-1)\alpha+(1+k)D_p(s)\}},\right.$$

256 $$\frac{-2(s\alpha\eta)^2(1+\mu)(k+\mu)+(1-\mu)\langle s\alpha\eta[k(1-s)\{k(3+\mu)+(1+3\mu)\}-2s\eta\{k(2+\mu)+(1+2\mu)\}]-(1+k)\{k(1-s)-s\eta\}\{k(1-s)-2s\eta\}(1-\mu)D_p(s)\rangle D_p(s)}{s\eta\mu\{(k-1)\alpha+(1+k)D_p(s)\}},$$

257 $$2[s\alpha\eta(1+k\mu)+\{s\eta(1+k\mu)-k(1-s)(1-\mu)\}D_p(s)],$$

258 $$\left.-2(1+k)[s\alpha\eta(1+\mu)-\{k(1-s)-s\eta\}(1-\mu)]D_p(s)\right) . \qquad (3d)$$

259 The equilibrium Eq. (3a) is a line rather than a point, which includes (0, 0, 0, 1). The feasibility and

260 stability conditions of equilibria are summarized in Table 2. Both feasibility and stability conditions of

261 equilibrium (3d) are too complex to be derived analytically, which are examined numerically. Examples

262 of parameter dependence of those conditions are illustrated in Figure 2.

263 $\qquad$ Both Figs. 1 and 2 indicate that the conditions of equilibrium do not overlap each other, except

264 for equilibria (2a) and (3a) which can be bistable with three other equilibria, (1, 0, 0, 0), Eqs. (2c) or (2d)

265 under cost type 1, and (1, 0, 0, 0), Eqs. (3c) or (3d) under cost type 2, respectively (see regions bounded

266 by broken curves). The bistabilities result from a strong positive frequency-dependent selection between

267 non-restorer allele and non-CMS mitochondria. Equilibria (2a) and (3a) associate with a fixation of

268 restorer allele, where CMS mitochondria is neutral due to a suppression of male-sterility. In this case,

269 the frequency of CMS mitochondria changes only by genetic drift, in which the state is relatively stable.

270 On the other hand, when the frequency of CMS cytotype is comparatively low, an advantage of

271   restoration is small, in which the restoration allele cannot increase due to its accompanying cost. These

272   reciprocal suppressions cause bistability of the two equilibria.

273   The trends are similar between Figs. 1 and 2, although there are clear differences. In Fig. 1, a

274   hermaphrodite population with a fixation of restorer allele (i.e., $(0, X_{Mn}, 0, X_{Mc})$) tends to occur in

275   relatively wide parameter regions in comparison to Fig. 2. Contrary to this, regions of cytoplasmic

276   gynodioecy without restorer (i.e., $(X_{mn}, 0, X_{mc}, 0)$) become wider in Fig. 2. These differences may result

277   from the influence of a restoration cost on the frequency of the restorer allele. In cost type 1, the

278   restoration cost decreases the restorer alleles in the pollen pool only, but not in the ovule pool, which

279   may mildly suppress restorer evolution. On the other hand, with regards to cost type 2, the restoration

280   cost decreases restoration alleles totally in the entire population, which significantly suppresses the

281   prevalence of the restoration allele. It should be remarked that simple comparisons between two cost

282   cases might be inadequate because the relative effect of pollen limitation is different between those,

283   owing to a difference of census timing of genotypic frequencies (before and after suffering restoration

284   cost in cost types 1 and 2, respectively). However, such comparisons could provide useful information to

285   understand some evolutionary trends.

286   In order to study transient dynamics, we conduct simulations for some example cases, which

287   are illustrated in Figure 3. Figs. 3(a)-(c) represent results in the bistable cases. In the equilibrium (2a) on

288   the X_{Mn}-X_{Mc} axis, CMS mitochondria is neutral owing to restorer fixation, where genetic drift causes the

289   fluctuation of its frequency. Importantly, when the CMS frequency (i.e., X_{Mc}) falls below a threshold, the

290   state becomes unstable, causing an increment in the non-restorer allele. Figure 3(d) illustrates that if the

291   equilibrium (2a) is a unique solution, trajectories starting from an unstable region on the X_{Mn}-X_{Mc} axis

292   finally return to the stable region on the same axis with the disappearance of the non-restorer allele.

293   Accordingly, a combination of genetic drift on mitochondria and the mutational occurrence of non-

294   restorer allele may result in intermittent repeating cycles.

295

296   <u>Selfing evolution from perfect outbreeding and perfect selfing</u>

297   We considered a constant selfing rate in the above analysis, although in reality selfing rate can

298   evolve. This evolution of selfing is analyzed by examining the invasibility of individuals with a mutant

299   selfing rate into a population with a resident selfing rate. We assume that the population always reaches

300   an equilibrium state of restorer and CMS for the resident selfing rate. This assumption may be

301   reasonable because the selfing rate is a quantitative trait which changes more slowly and continuously

302   than allelic dynamics of restorer and CMS. Under bistability of two equilibria, we consider that the

303   system eventually converges to an equilibrium other than Eqs. (2a) and (3a) because those equilibria can

304   be unstable due to genetic drift (see above). In addition to this, when equilibrium (2a) or (3a) is a unique

305   stable solution, we assume that (0, 0, 0, 1) is the realized equilibrium because a fixation of CMS

306   mitochondria can persist for a relatively long time until an emergence of non-CMS mitochondria.

307   First, we analytically investigate the dynamics at the boundary selfing rates, i.e., perfect

308   outbreeding ($s = 0$) and perfect selfing ($s = 1$), by analyzing eigenvalues of the Jacobian matrix of the

309   system with resident and mutant selfing rates. According to Eqs. (2) and (3), at perfect outbreeding with

310   $s = 0$, three types of equilibria occur conditionally in both cost types, i.e., $(0, 0, X_{mx}, X_{Mc})$, $(0, 0, 0, 1)$ and

311   $(1, 0, 0, 0)$ (see also Figs. 1 and 2). In those equilibria, the perfect outbreeding can be either stable or

312   unstable, depending on conditions. For cost type 1, the instability conditions of perfect outbreeding are

313   $\qquad 2\eta\left(1 + \frac{2k\alpha}{(1-\mu)(1+k)}\right) - k(1+b) > 0 \qquad\qquad$ for $\qquad (0, 0, X_{mc}, X_{Mc})\,, \qquad\qquad$ (4a)

314   $\qquad 2\eta\left(1 + \frac{\alpha}{1-\mu}\right) - (1+b) > 0 \qquad\qquad$ for $\qquad (0, 0, 0, 1)\,, \qquad\qquad$ (4b)

315   $\qquad 2\eta(1+\alpha) - (1+b) > 0 \qquad\qquad$ for $\qquad (1, 0, 0, 0)\,. \qquad\qquad$ (4c)

316   On the other hand, those for cost type 2 are

317    $$2\eta\left(1 - \mu + \frac{\alpha\{2k+(1-k)\mu\}}{1+k}\right) - (k + \mu)(1 + b) > 0 \quad \text{for} \quad (0, 0, X_{mc}, X_{Mc}),$$ (5a)

318    $$2\eta(1 + \alpha) - (1 + b) > 0 \qquad \text{for} \qquad (0, 0, 0, 1),$$ (5b)

319    $$2\eta(1 + \alpha) - (1 + b) > 0 \qquad \text{for} \qquad (1, 0, 0, 0).$$ (5c)

320   Under these conditions, selfing can evolve from perfect outbreeding. These conditions tend to be

321   satisfied by larger α and η (= $(1 - \delta) / \beta$), and smaller b, which promotes the evolution of selfing. In

322   addition, from Eqs. (4a) and (5a), we can prove that an increment of k suppresses the evolution under the

323   feasibility and stability conditions of those equilibria. Significantly, an increment of μ promotes selfing

324   evolution in Eqs. (4a) and (4b) but suppresses it in Eq. (5a). This indicates that in equilibria with a

325   fixation of restorer allele, restoration costs conversely affect the selfing evolution from perfect

326   outbreeding depending on cost types. Mechanisms of those parameter dependencies are discussed below

327   (see Discussion).

328   We also analyze selfing evolution from perfect selfing ($s = 1$). It can be analytically shown that

329   with $s = 1$, the original hermaphrodite state, $(1, 0, 0, 0)$, is a unique stable equilibrium in both cost types

330   due to an exclusion of $(0, X_{Mn}, 0, X_{Mc})$ from consideration under bistability (see also Figs. 1 and 2). At

331   equilibrium, perfect selfing can be stable when

332    $$2\eta\left(\frac{\alpha}{1-a} + 1\right) > 1,$$ (6)

333   regardless of cost type. This indicates that under both cost types 1 and 2, perfect selfing tends to persist

334   when α, a or η (= $(1 - \delta) / \beta$) is large (see Discussion). The effects of parameters on instabilities of

335   boundary selfing rates are summerized in Table 3.

336

337   <u>Evolution of intermediate selfing rate</u>

338   Next, we examine the evolution of an intermediate level of selfing rate. The Jacobian matrix of

339   system with $0 < s < 1$ is very complex. In addition to this, equilibrium mating mode discontinuously

340   change with varying selfing rate. These properties mean that analytical derivations of the maximum

341   eigenvalue and its derivative are difficult, therefore, we analyze evolutionary dynamics numerically (see

342   Appendix B). In Figures 1 and 2, solid circles represent selfing rates that are simultaneously

343   convergently and evolutionary stable, whereas open circles indicate such that are convergently unstable.

344   In each panel, the selfing rate evolves along a horizontal direction under a given k value. In the

345   examined case, all convergently stable solutions satisfy evolutionary stability conditions at least under

346   the given conditions. These figures indicate that there can be multiple convergently and evolutionarily

347   stable solutions in the evolution of selfing, and that intermediate selfing rates can be evolutionarily

348   realized depending on parameters. These figures imply that the evolution of selfing rate realizes multiple

349   equilibria, which associate with various types of mating modes.

350   According to the numerical analysis, all solutions that are indicated by solid circles in Figs. 1

351   and 2 are satisfied both convergent and evolutionary stability conditions. In order to examine the

352   evolutionary dynamics of selfing in more detail, we conducted supplemental analysis (see Appendix B),

353   and illustrate a PIP (pairwise invasibility plot) that represent the invasibilities of mutant strategies into

354   populations with resident strategies (Geritz et al., 1998). Supplementary figures 1 and 2 show PIP for $k =$

355   1 cases of Figs. 1 and 2, respectively, by which we can reconfirm evolutionary stabilities of the solid

356   circles graphically.

357   Figures 1 and 2 focus on effects of cost of restorer (μ) and inbreeding depression (δ) on

358   evolution of mating mode and selfing rate. We also examine dependencies of evolution on parameters of

359   pollen limitation (α and β) and pollen discounting (a and b), which are illustrated in Supplementary

360   figures 3-10 (those trends are discussed below). It is remarkable that all results indicate that once selfing

361   rate evolves toward some level, it can prevent CMS mitochondria from fixation. If selfing rate evolves

362   toward $s = 1$, both restorer and CMS are excluded from the population (i.e., $(1, 0, 0, 0)$), which

363   represents a negative correlation between selfing, and CMS and restorer in their coevolution. It should

364   be noted that that with a parameter set of panels (a) in Supplementary Figs. (7)-(10), a pollen

365   discounting function $D_p(s)$ is not a monotonically decreasing function within $0 \leq s \leq 1$, which is locally

366   minimized around $s = 0.69444$. However, those figures suggest that a small deviation from

367   monotonically decreasing manner in pollen discounting does not affect trends significantly.

368

369   **DISCUSSION**

370   <u>Multi-stability in the analyzed system</u>

371   Throughout the analyses, we clarify the effect of selfing on the coevolution of nuclear restorer and CMS

372   cytoplasm. First, we demonstrate the bistability of equilibria under a fixed selfing rate, which was first

373   proposed by Charlesworth (1981) but had not been studied in detail. In the present study, we analytically

374   clarify the conditions for bistability of mating modes in relation to selfing rate. The bistability results

375   from a positive frequency-dependent selection between non-restorer allele and non-CMS mitochondria.

376   The possibility of bistability suggests that if local populations are founded by small groups with various

377   genotypic compositions, steady states might differ among those local populations in a single species.

378   Remarkably, the present analysis shows that one equilibrium in bistability is always the hermaphroditic

379   population with a fixation of restorer allele (i.e., $(0, X_{Mn}, 0, X_{Mc})$). In this case, the frequency of CMS

380   mitochondria is neutral on an X_{Mn}-X_{Mc} axis, which can lead the equilibrium to a region of instability due

381   to genetic drift. Thus, this state ultimately becomes unstable and subsequently converges to an

382   alternative equilibrium. Although the hermaphroditic population with a fixation of restorer could result

383   from founder effects in colonization or stochasticity in dynamics, it may not persist for a long period.

384   Subsequently, evolution of selfing are included in the model, by which we investigate

385   coevolutionary process of three traits, i.e., selfing rate, nuclear restorer and CMS. According to the

386   analysis, multiple selfing rates (including $s = 0$ and 1) can be stable simultaneously under given

387   parameter values and conditions (Figs. 1 and 2). As described above, bistability is possible even with the

388   fixed selfing rate. When we take into account the evolution of selfing, a greater number of diverging

389   states can be possible due to the combination of multi-stabilities of both the CMS-restorer interaction

390   and the realized selfing rate.

391   It is known that gynodioecious species often show extremely wide variation in female

392   frequencies among populations (Lloyd, 1976, Frank, 1989, Webb, 1999). In addition to this, selfing rates

393   are known to vary among populations of gynodioecious species (Dornier & Dufay, 2013). Diversities

394   that are observed in gynodioecious species may be explained by the property of multi-stability of

395   gynodioecy, which is indicated by the analysis presented in this study. Under the multi-stability of

396   mating systems, stochasticities in dynamics and a founder effect in colonization may cause significant

397   fluctuation or variation in system states, which may result in a variation of selfing rates. Interestingly,

398   female frequencies in nuclear gynodioecy tend to be stable among populations in comparison to nuclear-

399   cytoplasmic gynodioecy (Bailey & Delph, 2007a). Figs.1 and 2 show that nuclear gynodioecy is

400   generally monostable without an alternative equilibrium state, which may be a reason for the relative

401   stability of nuclear gynodioecy.

402

403   <u>Driving force for the evolution of selfing from perfect outbreeding</u>

404   The main driving force of selfing evolution may be due to the reproductive assurance of hermaphrodite

405   individuals under pollen limitation. In a CMS-prevailing population, the average density of exported

406   pollen is low because female individuals with CMS don't export pollen at all. In those cases,

407 hermaphroditic individuals evolve higher selfing rates to ensure their ovule fertilization. This selfing

408 evolution further intensifies the lack of exported pollen in the population under pollen discounting,

409 consequently suppressing the evolution of CMS that requires pollen, in other words, the enclosure of

410 pollen by hermaphroditic individuals via selfing subdues CMS cytoplasm.

411   This scenario is supported by an examination of the effects of pollen limitation on selfing

412 evolution. Under pollen limitation, the fertilization of outbred ovules increases with the average density

413 of exported pollen, In the presented model, the function of pollen limitation is determined by two

414 parameters, α and β, which represent an insensitivity of fertilization improvement to pollen density and a

415 maximum level of fertilization respectively, where larger α and smaller β indicate more intensive pollen

416 limitation. Our analysis shows that selfing can evolve from perfect outbreeding under larger α and

417 smaller β (see Eqs. (4) and (5)). This is also supported by supplementary analysis, showing that selfing

418 rate tends to evolve toward higher levels under larger α and smaller β (Supplementary Figs. 3-6).

419 Namely, strong pollen limitation results in higher selfing rates, which is consistent when considering

420 selfing evolution as a reproductive assurance.

421   We also examine the effects of pollen discounting on selfing evolution. Under intensive pollen

422 discounting, an increase in an individual's selfing rate results in a significant reduction in pollen which

423 is exported by the individual. In our model, pollen discounting is determined by two parameters, a and

424 b. In the pollen discounting function, a represents the reduction of exported pollen at perfect selfing ($s =$

425 1), whereas b determines the concaveness of the function, i.e., a large b causes a steep reduction of

426 exported pollen at a small selfing rate. Accordingly, it is expected that both large a and b intensify pollen

427 discounting, resulting in similar effects on selfing evolution. However, this should be considered more

428 carefully because the effects of these two parameters on pollen discounting are not straightforward.

429 Here, we focus on a population with a low selfing rate, where outbreeding is dominant (a case with a

430   higher selfing rate is discussed below, where the results are completely different). Under intense pollen

431   discounting, an individual with selfing exports a small amount of pollen, which spoils an individual's

432   ability in competition among pollen for ovules. This disadvantage is significant in the outbreeding-

433   dominant population, which suppresses selfing evolution at low selfing rates via a negative feedback.

434   Importantly, within a range of low selfing rates, the sensitivity of the pollen discounting function to the

435   selfing rate is mainly governed by the parameter b (but not by a), therefore, smaller b is expected to

436   promote selfing evolution. Indeed, our analysis shows that selfing evolution from perfect outbreeding is

437   promoted by smaller b, but is independent of a (see Eqs. (4) and (5)). In contrast, lager b suppresses

438   selfing evolution via a negative feedback between selfing and pollen discounting. The effect of b at low

439   selfing rates can be also confirmed in Supplementary Figs. 7-10.

440   According to the present analysis, when the advantage of CMS in female is high (larger k),

441   selfing evolution from perfect outbreeding tends to be suppressed in the population of nuclear

442   gynodioecy, $(0, 0, X_{mc}, X_{Mc})$ (see Eqs. (4a) and (5a), and Figs. 1 and 2). When females with CMS

443   produce more ovules, selfing reduces the opportunity to fertilize many ovules through outbreeding (i.e.,

444   higher male success in a female-biased population), a disadvantage by which may suppress selfing

445   evolution. It should be noted that this mechanism is effective only when nuclear genes have some

446   transition pathways from female to hermaphroditic individuals, i.e. the existence of some levels of

447   restorer. (Otherwise, after fertilization with ovules of female individuals, the selfing modifier loses any

448   opportunities to be expressed and to be selected). On the other hand, in a hermaphroditic population ((0,

449   0, 0, 1) and (1, 0, 0, 0)), the advantage of CMS in females does not affect selfing evolution from perfect

450   outbreeding (see Eqs. (4b, c) and (5b, c), and Figs. 1 and 2). Such a population comprises no females

451   regardless of an existence of CMS mitochondria, in which an advantage of CMS in females is not

452   effective to selfing evolution.

453   We find that in nuclear gynodioecy, $(0, 0, X_{mc}, X_{Mc})$ being stable with $k > 0$, the effect of the

454   restoration cost (μ) on selfing evolution from perfect outbreeding depends on the cost type. The effect is

455   promotional in a cost reducing male function (Eq. (4a)), but suppressive in a cost reducing individual

456   performance (Eq. (5a): its left-hand side term is negative and positive at small and large μ, respectively,

457   when its sign changes within $0 \leq \mu \leq 1$). In a population with the cost reducing male function, the larger

458   cost significantly decreases individual pollen production, causing lower average density of exported

459   pollen. In this case, ovules are unlikely to be fertilized by outbreeding under pollen limitation, by which

460   selfing becomes more preferable via intensified reproductive assurance. On the other hand, the cost

461   reducing individual performance does not influence the average density of exported pollen in the

462   population (see above). Instead, the cost totally decreases hermaphroditic individuals that carry a

463   restorer allele, which relatively increases the frequency of CMS females, realizing in a female-biased

464   sex ratio. In this case, exported pollen can achieve a high fertilization success via outbreeding with many

465   females, by which the larger restorer cost suppresses selfing evolution (importantly, those offspring can

466   also be hermaphroditic due to the inheritance of the restorer gene in nuclear gynodioecy). Those

467   mechanisms may result in the opposite dependencies of selfing evolution on the restoration cost between

468   nuclear gynodioecy with different cost types. In the presented analysis, we do not consider alternative

469   types of restoration costs that decrease female success only. In reality, however, it was reported that

470   hermaphrodites carrying nuclear restorer genes had lower seed quantity in *Plantago lanceolata* (de Haan

471   et al., 1997). This type of cost does not influence the average density of exported pollen, but reduce a

472   number of ovules of restored hermaphrodites, which may be also suppressive for selfing evolution.

473

474   <u>Evolution of higher levels of selfing</u>

475   According to the present analysis, intermediate levels of selfing can be achieved evolutionarily in a

476   gynodioecious system. The evolution of intermediate levels of selfing has been a subject of theoretical

477   discussion because it cannot be represented simply (see a review by Goodwillie et al. (2005)). For the

478   stability of intermediate selfing rates, a factor with negative frequency dependence on selfing rate is

479   required. Johnston (1998) indicated that the existence of pollen discounting can result in intermediate

480   levels of selfing that are both convergently and evolutionarily stable, a mechanism which could be

481   effective also in the presented model. Remarkably, the author derived a unique stable solution for an

482   intermediate level of selfing under a given condition, although our model shows the possibility of

483   multiple solutions at intermediate levels (see, for example, Fig. 1(i) with $k = 1.3$). This is because CMS-

484   restorer status can change depending on the selfing rate. In Fig. 1(i), one intermediate level of selfing

485   involves a nuclear gynodioecy with a CMS fixation, whereas another solution is accompanied by a

486   cytoplasmic-nuclear gynodioecy that is potentially bistable with a hermaphrodite. The bistability of

487   intermediate levels of selfing is a characteristic property of the coevolution of CMS, restorer and selfing

488   rate.

489   In the above discussion of selfing evolution from perfect outbreeding, we explain that selfing

490   evolution is suppressed by intense pollen discounting with large b (the concaveness of the functional

491   form), independently of the value of a (reductions of exported pollen when $s = 1$). The trend varies in a

492   population with high selfing rates. In the selfing-dominant population, intense pollen discounting

493   significantly reduces the average density of exported pollen, in which selfing is favored as a

494   reproductive assurance under pollen limitation. In short, under high selfing rates, strong pollen

495   discounting tends to promote selfing evolution. Importantly, at high selfing rates, a parameter a mainly

496   governs the sensitivity of the pollen discounting function to the selfing rate, by which selfing evolution

497   is expected to be promoted by larger a. Such a trend can be confirmed in Supplementary Figs. 7-10 (it

498   should be noted that stable selfing solutions with intermediate values also exist above the plotted range

499   in those figures). Consequently, the effect of pollen discounting on selfing evolution at high selfing rate

500   is opposite to that at low selfing rate, which tends to be controlled by different parameters, *a* and *b*,

501   respectively.

502   It should be noted that intense pollen discounting can result in a positive feedback in selfing

503   evolution in response to CMS prevalence. When CMS evolves and dominates in the population, a

504   scarcity of hermaphrodites that are suppliers of exported pollen intensifies pollen limitation in

505   outbreeding, by which selfing could be favored to ensure reproductive success in hermaphroditic

506   individuals. If the pollen discounting is strong, the increment of selfing rate tends to reduce the average

507   density of exported pollen significantly, which enhances the importance of reproductive assurance.

508   These processes accelerate the evolution of higher selfing rates with a positive feedback between pollen

509   limitation and reproductive assurance through pollen discounting, which gradually suppresses CMS

510   females that require pollen supply from hermaphrodites. The cascade is triggered by CMS evolution and

511   eventually suppress CMS.

512   The presented analysis also shows that perfect selfing ($s = 1$) can be stable under low inbreeding

513   depression (small δ), strong pollen discounting (large *a*), and intensive pollen limitation (large α and

514   small β) (see Eq. (6)). Namely, when both pollen limitation and pollen discounting are strong, perfect

515   selfing tends to be stable (see also Supplementary Figs. 3-10). This can be explained by individuals with

516   perfect selfing ($s = 1$) exporting little pollen in the presence of pollen discounting, by which a lower

517   selfing mutant finds it difficult to outbreed successfully under intense pollen limitation, resulting in a

518   failure of its invasion.

519

520   <u>Influence of selfing evolution on gynodioecy</u>

521   Our analysis shows that the evolution of selfing significantly influences the CMS-restorer interaction. If

522   the selfing rate reaches an intermediate level, nuclear-cytoplasmic gynodioecy and cytoplasmic

523   gynodioecy can be achieved (Figs. 1(e), (f), (i), and 2(a), (e), (f), (i)). Furthermore, if selfing reaches a

524   perfect level ($s = 1$), a hermaphrodite population occurs with neither CMS nor restorer. Namely, when

525   selfing rate evolves toward a substantial level, CMS and restorer are likely to be excluded from the

526   population. This trend is inconsistent with the results of Dornier and Dufay (2013), which illustrated that

527   evolution of nuclear gynodioecy was promoted under higher selfing rates (in Dornier and Dufay, Fig. 3).

528   This may be explained by the absence of pollen limitation in their examined cases.

529   When selfing cannot evolve or stay at very low level ($s \approx 0$), either CMS or restorer allele would

530   be fixed in the population with $k > 0$, which may imply that the failure of selfing evolution intensifies an

531   arms race between CMS and restoration. It should be remarked that with a sufficiently large k, there are

532   also some advantages for non-restore allele. When CMS persists under large k, female individuals with

533   CMS achieve a high reproductive success. In this case, non-restorer allele can increase by hitchhiking on

534   this female advantage, which is a reason that both restorer and non-restorer alleles coexist with a

535   fixation of CMS cytotype under $k > 1$. On the other hand, the evolution of intermediate levels of the

536   selfing rate ($s > 0$) promotes the coexistence of non-CMS and CMS cytotypes, and non-restorer and

537   restorer alleles. If the selfing rate reaches a very high level ($s \approx 1$), both CMS and restorer allele are

538   eliminated from the population, implying that intense selfing suppresses CMS evolution.

539   Lahiani et al. (2015) studied reproductive assurance in nuclear-cytoplasmic gynodioecious

540   species, *Silene nutans* (Caryophyllaceae), which categorized plant habitats into female-biased or

541   hermaphrodite-biased patches. The authors showed that female-biased patches were characterized by

542   more fruit set and higher selfing rate than hermaphrodite-biased patches, which suggested a correlation

543   between female advantage (k) and selfing rate (s). The analysis that is presented here predicts a positive

544   correlation between female advantage (k) and selfing rate (s) in nuclear-cytoplasmic gynodioecy (see

545   Figs.1(e) and (i)). Although a relationship between those values and female ratio is not simple in our

546   analysis (see Eqs. (2d) and (3d)), the observed positive correlation between female advantage and

547   selfing rate is consistent with results of our models, which can be explained by evolution of selfing rate

548   under a given parameter k in the gynodioecious plant.

549

550   <u>Implications of observed gynodioecy</u>

551   Our analysis successfully indicates an evolutionary relationship between gynodioecy and selfing,

552   although it is inconsistent to some observations of selfing in gynodioecy. Dornier and Dufay (2013)

553   reviewed selfing in gynodioecious plants, which indicated that some levels of selfing were observed in

554   nuclear gynodioecy. In our results, nuclear gynodioecy occurs in a region with high female advantage in

555   CMS (large k) and low selfing rate (small s), where stable selfing rates tend to be 0 or small (see Figs. 1

556   and 2, and Supplementary figures). This inconsistency may imply that additional factors are needed to

557   understand the coevolution of gynodioecy and selfing. In the presented study, we focus on dynamics of

558   genotypic frequencies, ignoring population dynamics that may influence mating processes. In addition,

559   we considered pollen discounting is a function of average density of pollen only, although it might be

560   also influenced by an ovule density typically in animal pollination systems via an efficiency of pollinator

561   attraction. Inclusions of such factors may alternate trends of selfing evolution.

562   There is also another inconsistency in the present model. It is known that cytoplasmic

563   gynodioecy without restoration is very rare in plants (Burt & Trivers, 2009), although it can be achieved

564   in our model. When the cost of restoration is high (large μ), cytoplasmic gynodioecy without restorer

565   (i.e., (X_{mn}, 0, X_{mc}, 0)) is possible within regions of intermediate levels of selfing, which can be an

566   equilibrium of selfing evolution (see Figs. 1(f), and 2(e), (f), (i)). This trend is less significant in the case

567   that restorer cost reduces male function (Fig. 1), especially when the relative advantage of selfing (η) is

568   small (compare between Figs. 1(f) and (i)). In such a case, instead, nuclear-cytoplasmic gynodioecy is

569   possible (Fig. 1(i)), which is widely observed in natural populations (Delph et al., 2007). This may

570   suggest that in plant species the restorer cost tends to affect male function, and that a relative advantage

571   of inbreeding to outbreeding ($\eta = (1 - \delta) / \beta$) may be relatively small. It was reported that hermaphrodites

572   carrying nuclear restorer genes had lower pollen viability in *Lobelia siphilitica* (Bailey, 2002), which

573   may suggested that the restorer cost on male function is not rare.

574

575   In the present analysis, we combine a major gene model (for CSM and restorer) and an adaptive

576   dynamics model (for selfing rate), by which successfully revealed properties of coevolution of

577   gynodioecy and selfing rate. Selfing is generally expected to influence the evolution of cytoplasmic

578   genetic element (CGE) because it affects the inheritance processes of CGEs. Accordingly, the present

579   analysis provides a basis to study evolution of a wide variety of selfish CGEs. For instance, selfing in

580   plants may corresponds with sib mating in animals, therefore it might be also applicable to inter-

581   genomic conflict in animals with sib mating. Our approach involves a potential to contribute on

582   investigations about properties of various intergenomic conflicts.

583

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591

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678

679   **FIGURE CAPTIONS**

680   **Figure 1**. Feasible and stable equilibria and evolutionarily realized selfing rates in the case that restorer

681   cost reduces male function (cost type 1). Parameter μ is the cost of restorer, and $1 - \delta$ is the inbreeding

682   coefficient. Initially, the equilibria are analyzed under a given selfing rate. Depending on parameters, 5

683   types of equilibria are possible. An equilibrium with fixation of nuclear restorer, $(0, X_{Mn}, 0, X_{Mc})$, can be

684   bistable with another equilibrium (regions that are surrounded by a broken curve). In the grayscale chart,

685   characters represent sex determination modes of the population, NG: nuclear gynodioecy, CG:

686   cytoplasmic gynodioecy, and NCG: nuclear-cytoplasmic gynodioecy. H1 and H2 represent

687   hermaphroditic populations without CMS and restorer, and that with a fixation of restorer, respectively.

688   It should be noted that the state H2 can be bistable with other equilibrium states depending on

689   parameters. When H2 is a unique equilibrium, it is indicated by H2'. Subsequently, selfing evolution is

690   examined under equilibrium states. Solid circles represent solutions that are both convergently and

691   evolutionarily stable, whereas open circles are convergently unstable solutions, with given k values. See

692   Table 1 for parameter values.

693   **Figure 2**. Feasible and stable equilibria and evolutionarily realized selfing rates in a case that a restorer

694   cost reduces individual performance (cost type 2). Parameter μ is the cost of restorer, and $1 - \delta$ is the

695   inbreeding coefficient. Solid circles represent selfing rates that are both convergently and evolutionarily

696   stable, whereas open circles are convergently unstable solutions. Parameters and conditions are identical

697   to Fig. 1.

698   **Figure 3**. Simulation of the coevolution of CMS and nuclear restorer under a given selfing rate.

699   Parameter values and conditions correspond to Fig. 1(e) with $k = 0.5$, in which equilibrium restorer-

700   CMS status are bistability of $(0, X_{Mn}, 0, X_{Mc})$ (gray region on X_{Mn}-X_{Mc} axis) and another equilibrium, $(1,$

701   $0, 0, 0)$ in (a), $(X_{mn}, 0, X_{mc}, 0)$ in (b) and $(X_{mn}, X_{Mn}, X_{mc}, X_{Mc})$ in (c). On the other hand, $(0, X_{Mn}, 0, X_{Mc})$ is

702   a unique equilibrium in (d). On the X_{Mn}-X_{Mc} axis, CMS mitochondria are neutral due to a fixation of

703   nuclear restorer, although the equilibrium $(0, X_{Mn}, 0, X_{Mc})$ can be unstable when the CMS frequency goes

704   below a threshold (small X_{Mc}). In (d), Genetic drift and subsequent emergence of non-restorer allele may

705   cause cyclic dynamics.

706

Table 1   Notations of traits and parameters

	Description
Genotypic frequencies	
X_{mn}, X'_{mn}	non-restorer and non-CMS with resident and mutant selfing rate
X_{Mn}, X'_{Mn}	restorer and non-CMS with resident and mutant selfing rate
X_{mc}, X'_{mc}	non-restorer and CMS with resident and mutant selfing rate
X_{Mc}, X'_{Mc}	restorer and CMS with resident and mutant selfing rate
Quantitative genetic trait	
s, s'	resident and mutant selfing rate
Parameters and functions	
$D_p(s)$	pollen discounting: $1 - [b + (a - b)s^c]s$
a	reduction when $s = 1$, $0 \leq a \leq 1$ ($a = 1$ in numerical analysis)
b	concaveness of functional form ($b = 0$ in numerical analysis)
c	magnitude of curvature of functional form, $0 < c$ ($c = 0.5$ in numerical analysis)
$L_p(P_{EX})$	pollen limitation: $\beta P_{EX} / (\alpha + P_{EX})$
P_{EX}	density of exported pollen
α	extent of saturation ($\alpha = 0.1$ in numerical analysis)
β	maximum fertilization efficiency in outbreeding ($\beta = 1$ in numerical analysis)
r	recombination rate between restorer and selfing rate loci ($r = 0.5$ in simulation)
δ	inbreeding depression, $0 \leq \delta \leq 1$
η	$(1 - \delta) / \beta$, relative advantage of inbreeding to outbreeding
k	advantage of CMS in female performance (ovule production)
μ	cost accompanying with restorer allele, $0 \leq \mu \leq 1$

Table 2   Mating system, feasibility and stability conditions of equilibria in Eqs. (2) and (3).

Equilibrium	Mating system	Feasibility and stability conditions
Cost type 1		
(2a)	H	$\{(k-1)(1-s)-2s\eta\}(1-\mu)D_p(s) \leq 2s\alpha\eta$, and $X_{Mn} \leq 1 - \mu$
(2b)	NG	$0 < k(1-s)-s\eta,$ and $\{(k-1)(1-s)-2s\eta\}(1-\mu)D_p(s) > 2s\alpha\eta$
(2c)	CG	$s\alpha\eta \leq \{k(1-s)-s\eta\}D_p(s) \leq s\alpha\eta\frac{1+k(1-\mu)}{(1+k)(1-\mu)}$
(2d)	NCG	$\frac{1+k(1-\mu)}{(1+k)(1-\mu)}s\alpha\eta < \{k(1-s)-s\eta\}D_p(s) < \frac{1}{1-\mu}s\alpha\eta$ (this is a feasibility condition, stability is numerically examined)
Cost type 2		
(3a)	H	$\left\{-\frac{(1-s)(1-2\mu-k)}{2(1-\mu)}-s\eta\right\}D_p(s) \leq s\alpha\eta \leq \left\{\frac{(1-s)(1-2\mu)}{2\mu}-s\eta\right\}D_p(s),$ and $X_{Mn} \leq \frac{-2s\alpha\eta\mu+\{(1-s)(1-2\mu)-2s\eta\mu\}D_p(s)}{(1-s)D_p(s)}$
(3b)	NG	$0 < (1-s)\{k(2-\mu)+\mu\}-2s\eta(1-\mu),$ $s\alpha\eta \leq -\left\{\frac{(1-s)(1-k-2\mu)}{2(1-\mu)}+s\eta\right\}D_p(s),$ and $s\alpha\eta \leq \frac{(1+k)(1-\mu)}{2(k+\mu)}\{k(1-s)-2s\eta\}D_p(s)$
(3c)	CG	$s\alpha\eta \leq \{k(1-s)-s\eta\}D_p(s) \leq s\alpha\eta\frac{1+\mu}{1-\mu}$
(3d)	NCG	(both feasibility and stability are numerically examined)

H: hermaphrodite, NG: nuclear gynodioecy, CG: cytoplasmic gynodioecy, and NCG: nuclear-cytoplasmic gynodioecy

Table 3   Effects of parameters on instabilities of boundary selfing rates (see Eqs. (4)-(6)).

	cost type 1				cost type 2			
	$s = 0$			$s = 1$	$s = 0$			$s = 1$
	NG	H2'	H1	(H1)	NG	H2'	H1	(H1)
a	N/A	N/A	N/A	-	N/A	N/A	N/A	-
b	-	-	-	N/A	-	-	-	N/A
α	+	+	+	-	+	+	+	-
$\eta(= (1-\delta)/\beta)$	+	+	+	-	+	+	+	-
k	-	N/A	N/A	N/A	-	N/A	N/A	N/A
μ	+	+	N/A	N/A	-	N/A	N/A	N/A

NG: nuclear gynodioecy, H2': hermaphrodite with (0, 0, 0, 1), H1: hermaphrodite with (1, 0, 0, 0), "+": promotion of instability, "-": suppression of instability, N/A: no effect

Figure 1

Figure 1

Figure 2

Figure 3

(a) $s = 0.47$

$0 \leq X_{Mn} \leq \mu$

$\mu < X_{Mn} \leq 1$

X_{Mc}

X_{Mn}

X_{mc}

X_{mn}

(b) $s = 0.45$

X_{Mc}

X_{Mn}

X_{mc}

X_{mn}

(c) $s = 0.4$

X_{Mc}

X_{Mn}

X_{mc}

X_{mn}

(d) $s = 0.35$

CMS is neutral due to
fixation of restorer allele

X_{Mc}

X_{Mn}

X_{mc}

X_{mn}

1   **APPENDIX A**

2   We formulate the dynamics of genotypic frequencies by combining the reproductive outcomes of

3   selfing and outbreeding. It should be noted that outbreeding success is affected by the average

4   density of exported pollen through both pollen limitation and pollen competition for ovules. For

5   simplicity of description, we refer to X_{mn}, X_{Mn}, X_{mc}, X_{Mc}, X'_{mn}, X'_{Mn}, X'_{mc} and X'_{Mc} by w, x, y, z, w',

6   x', y' and z' in this section. The offspring genotype in outbreeding between two genotypes is

7   summarized in Supplementary Table 1.

8

9   <u>Dynamic equation with cost type 1</u>

10   First, we consider cost type 1 that the nuclear restorer reduces individual pollen production (in x_t,

11   x'_t, z_t and z'_t), a fraction of which is denoted by μ. A relative amount of pollen that is exported by

12   an individual decreases with its selfing rate, i.e., pollen discounting, which we denote as $D_p(s)$.

13   On the other hand, the relative density of exported pollen in the population can be expressed by

14   the average pollen exportation from individuals, which is formulated as $P_{EX} = \{w_t +$

15   $(1 - \mu)(x_t + z_t)\}D_p(s) + \{w'_t + (1 - \mu)(x'_t + z'_t)\}D_p(s')$. It should be noted that CMS

16   individuals without restorer (i.e., y_t and y'_t) cannot produce pollen at all, but promote ovule

17   production with relative ratio k.

18   Frequencies dynamics of strains with a resident selfing rate s are

19   $w_{t+1} = \left[\!\!\left[(1 - \delta)sw_t + \frac{\beta P_{EX}}{\alpha + P_{EX}} \left[D_p(s) \left\langle (1 - s)\left\{ \frac{w_t}{P_{EX}}\left(w_t + \frac{1}{2}x_t\right) + (1 - \mu)\frac{x_t + z_t}{P_{EX}}\frac{1}{2}w_t \right\} + \right.\right.\right.$

20   $\left. (1 - s')\left\{ \frac{w_t}{P_{EX}}\left(\frac{1}{2}w'_t + \frac{1}{2}(1 - r)x'_t\right) + (1 - \mu)\frac{x_t + z_t}{P_{EX}}\frac{1}{2}rw'_t \right\} \right\rangle +$

21   $\left.\left. D_p(s')(1 - s)\left\{ \frac{w'_t}{P_{EX}}\left(\frac{1}{2}w_t + \frac{1}{2}rx_t\right) + (1 - \mu)\frac{x'_t + z'_t}{P_{EX}}\frac{1}{2}rw_t \right\} \right]\right]\!\!\right]\frac{1}{W} \ ,$

22   (A1a)

23 $\quad x_{t+1} = \Big[\!\!\Big[(1-\delta)sx_t + \frac{\beta P_{EX}}{\alpha + P_{EX}}\Big[D_p(s)\Big\langle(1-s)\Big\{\frac{w_t}{P_{EX}}\frac{1}{2}x_t + (1-\mu)\frac{x_t+z_t}{P_{EX}}\Big(\frac{1}{2}w_t + x_t\Big)\Big\} +$

24 $\quad\quad\quad\quad (1-s')\Big\{\frac{w_t}{P_{EX}}\frac{1}{2}rx'_t + (1-\mu)\frac{x_t+z_t}{P_{EX}}\Big(\frac{1}{2}(1-r)w'_t + \frac{1}{2}x'_t\Big)\Big\}\Big\rangle +$

25 $\quad\quad\quad\quad D_p(s')(1-s)\Big\{\frac{w'_t}{P_{EX}}\frac{1}{2}(1-r)x_t + (1-\mu)\frac{x'_t+z'_t}{P_{EX}}\Big(\frac{1}{2}rw_t + \frac{1}{2}x_t\Big)\Big\}\Big]\Big]\!\!\Big]\frac{1}{w}$,

26 $\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad$ (A1b)

27 $\quad y_{t+1} = \frac{\beta P_{EX}}{\alpha + P_{EX}}\Big[D_p(s)\Big\langle(1-s)\Big\{\frac{w_t}{P_{EX}}\Big((1+k)y_t + \frac{1}{2}z_t\Big) + (1-\mu)\frac{x_t+z_t}{P_{EX}}(1+k)\frac{1}{2}y_t\Big\} +$

28 $\quad\quad\quad\quad (1-s')\Big\{\frac{w_t}{P_{EX}}\Big((1+k)\frac{1}{2}y'_t + \frac{1}{2}(1-r)z'_t\Big) + (1-\mu)\frac{x_t+z_t}{P_{EX}}(1+k)\frac{1}{2}rw'_t\Big\} +$

29 $\quad\quad\quad\quad D_p(s')(1-s)\Big\{\frac{w'_t}{P_{EX}}\Big((1+k)\frac{1}{2}y_t + \frac{1}{2}rz_t\Big) + (1-\mu)\frac{x'_t+z'_t}{P_{EX}}(1+k)\frac{1}{2}ry_t\Big\}\Big]\frac{1}{w}$,

30 $\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad$ (A1c)

31 $\quad z_{t+1} = \Big[\!\!\Big[(1-\delta)sz_t +$

32 $\quad\quad\quad\quad \frac{\beta P_{EX}}{\alpha + P_{EX}}\Big[D_p(s)\Big\langle(1-s)\Big\{\frac{w_t}{P_{EX}}\frac{1}{2}z_t + (1-\mu)\frac{x_t+z_t}{P_{EX}}\Big((1+k)\frac{1}{2}y_t + z_t\Big)\Big\} +$

33 $\quad\quad\quad\quad (1-s')\Big\{\frac{w_t}{P_{EX}}\frac{1}{2}rz'_t + (1-\mu)\frac{x_t+z_t}{P_{EX}}\Big((1+k)\frac{1}{2}(1-r)y'_t + \frac{1}{2}z'_t\Big)\Big\}\Big\rangle +$

34 $\quad\quad\quad\quad D_p(s')(1-s)\Big\{\frac{w'_t}{P_{EX}}\frac{1}{2}(1-r)z_t + (1-\mu)\frac{x'_t+z'_t}{P_{EX}}\Big((1+k)\frac{1}{2}ry_t + \frac{1}{2}z_t\Big)\Big\}\Big]\Big]\!\!\Big]\frac{1}{w}$,

35 $\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad$ (A1d)

36 $\quad$ whereas those with a mutant selfing rate s' are

37 $\quad w'_{t+1} = \Big[\!\!\Big[(1-\delta)s'w'_t +$

38 $\quad\quad\quad\quad \frac{\beta P_{EX}}{\alpha + P_{EX}}\Big[D_p(s)(1-s')\Big\{\frac{w_t}{P_{EX}}\Big(\frac{1}{2}w'_t + \frac{1}{2}rx'_t\Big) + (1-\mu)\frac{x_t+z_t}{P_{EX}}\frac{1}{2}(1-r)w'_t\Big\} +$

39 $\quad\quad\quad\quad\quad\quad D_p(s')\Big\langle(1-s)\Big\{\frac{w'_t}{P_{EX}}\Big(\frac{1}{2}w_t + \frac{1}{2}(1-r)x_t\Big) + (1-\mu)\frac{x'_t+z'_t}{P_{EX}}\frac{1}{2}rw_t\Big\} +$

40 $\quad\quad\quad\quad\quad\quad (1-s')\Big\{\frac{w'_t}{P_{EX}}\Big(w'_t + \frac{1}{2}x'_t\Big) + (1-\mu)\frac{x'_t+z'_t}{P_{EX}}\frac{1}{2}w'_t\Big\}\Big\rangle\Big]\Big]\!\!\Big]\frac{1}{w}$,

41 $\qquad$ (A1e)

42 $\quad x'_{t+1} = \llbracket (1-\delta)s'x'_t +$

43 $\qquad \frac{\beta P_{EX}}{\alpha + P_{EX}} \Big[D_p(s)(1-s') \Big\{ \frac{w_t}{P_{EX}} \frac{1}{2}(1-r)x'_t + (1-\mu)\frac{x_t+z_t}{P_{EX}} \Big(\frac{1}{2}rw'_t + \frac{1}{2}x'_t \Big) \Big\} +$

44 $\qquad D_p(s') \Big\langle (1-s) \Big\{ \frac{w'_t}{P_{EX}} \frac{1}{2}rx_t + (1-\mu)\frac{x'_t+z'_t}{P_{EX}} \Big(\frac{1}{2}(1-r)w_t + \frac{1}{2}x_t \Big) \Big\} +$

45 $\qquad (1-s') \Big\{ \frac{w'_t}{P_{EX}} \frac{1}{2}x'_t + (1-\mu)\frac{x'_t+z'_t}{P_{EX}} \Big(\frac{1}{2}w'_t + x'_t \Big) \Big\} \Big\rangle \Big] \rrbracket \frac{1}{W}$,

46 $\qquad$ (A1f)

47 $\quad y'_{t+1} =$

48 $\qquad \frac{\beta P_{EX}}{\alpha + P_{EX}} \Big[D_p(s)(1-s') \Big\{ \frac{w_t}{P_{EX}} \Big((1+k)\frac{1}{2}y'_t + \frac{1}{2}rz'_t \Big) + (1-\mu)\frac{x_t+z_t}{P_{EX}}(1+k)\frac{1}{2}(1-r)y'_t \Big\} +$

49 $\qquad D_p(s') \Big\langle (1-s) \Big\{ \frac{w'_t}{P_{EX}} \Big((1+k)\frac{1}{2}y_t + \frac{1}{2}(1-r)z_t \Big) + (1-\mu)\frac{x'_t+z'_t}{P_{EX}}(1+k)\frac{1}{2}ry_t \Big\} +$

50 $\qquad (1-s') \Big\{ \frac{w'_t}{P_{EX}} \Big((1+k)y'_t + \frac{1}{2}z'_t \Big) + (1-\mu)\frac{x'_t+z'_t}{P_{EX}}(1+k)\frac{1}{2}y'_t \Big\} \Big\rangle \Big] \frac{1}{W}$,

51 $\qquad$ (A1g)

52 $\quad z'_{t+1} = \llbracket (1-\delta)s'z'_t +$

53 $\qquad \frac{\beta P_{EX}}{\alpha + P_{EX}} \Big[D_p(s)(1-s') \Big\{ \frac{w_t}{P_{EX}} \frac{1}{2}(1-r)z'_t + (1-\mu)\frac{x_t+z_t}{P_{EX}} \Big((1+k)\frac{1}{2}ry'_t + \frac{1}{2}z'_t \Big) \Big\} +$

54 $\qquad D_p(s') \Big\langle (1-s) \Big\{ \frac{w'_t}{P_{EX}} \frac{1}{2}rz_t + (1-\mu)\frac{x'_t+z'_t}{P_{EX}} \Big((1+k)\frac{1}{2}(1-r)y_t + \frac{1}{2}z_t \Big) \Big\} +$

55 $\qquad (1-s') \Big\{ \frac{w'_t}{P_{EX}} \frac{1}{2}z'_t + (1-\mu)\frac{x'_t+z'_t}{P_{EX}} \Big((1+k)\frac{1}{2}y'_t + z'_t \Big) \Big\} \Big\rangle \Big] \rrbracket \frac{1}{W}$.

56 $\qquad$ (A1h)

57 In these equations, *W* represents the average fitness.

58

59 <u>Dynamic equation with cost type 2</u>

60 Next, we consider cost type 2 that the nuclear restorer reduces individual performance (in x_t, x'_t, z_t

61   and z'_t). This cost is assumed to reduce individual survivorship immediately before the census

62   timing of genotypic frequencies, a fraction of which is denoted by μ. The relative density of

63   exported pollen in the population can be expressed by the average pollen exportation from

64   individuals, which can be formulated as $P_{EX} = \{w_t + (x_t + z_t)\}D_p(s) + \{w'_t +$

65    $(x'_t + z'_t)\}D_p(s')$.

66   Similarly to the previous case, frequencies of genotypes with a resident selfing rate s

67   are expressed as

$$w_{t+1} = \Bigg[\!\Bigg[(1-\delta)sw_t + \frac{\beta P_{EX}}{\alpha + P_{EX}}\Bigg[D_p(s)\Bigg\langle(1-s)\left\{\frac{w_t}{P_{EX}}\left(w_t + \frac{1}{2}x_t\right) + \frac{x_t+z_t}{P_{EX}}\frac{1}{2}w_t\right\} + $$

$$(1-s')\left\{\frac{w_t}{P_{EX}}\left(\frac{1}{2}w'_t + \frac{1}{2}(1-r)x'_t\right) + \frac{x_t+z_t}{P_{EX}}\frac{1}{2}rw'_t\right\}\Bigg\rangle + $$

$$D_p(s')(1-s)\left\{\frac{w'_t}{P_{EX}}\left(\frac{1}{2}w_t + \frac{1}{2}rx_t\right) + \frac{x'_t+z'_t}{P_{EX}}\frac{1}{2}rw_t\right\}\Bigg]\!\Bigg]\!\Bigg]\frac{1}{\overline{W}}\ ,$$

                                                                                      (A2a)

$$x_{t+1} = (1-\mu)\Bigg[\!\Bigg[(1-\delta)sx_t + \frac{\beta P_{EX}}{\alpha + P_{EX}}\Bigg[D_p(s)\Bigg\langle(1-s)\left\{\frac{w_t}{P_{EX}}\frac{1}{2}x_t + \frac{x_t+z_t}{P_{EX}}\left(\frac{1}{2}w_t + x_t\right)\right\} + $$

$$(1-s')\left\{\frac{w_t}{P_{EX}}\frac{1}{2}rx'_t + \frac{x_t+z_t}{P_{EX}}\left(\frac{1}{2}(1-r)w'_t + \frac{1}{2}x'_t\right)\right\}\Bigg\rangle + $$

$$D_p(s')(1-s)\left\{\frac{w'_t}{P_{EX}}\frac{1}{2}(1-r)x_t + \frac{x'_t+z'_t}{P_{EX}}\left(\frac{1}{2}rw_t + \frac{1}{2}x_t\right)\right\}\Bigg]\Bigg]\!\Bigg]\frac{1}{\overline{W}}\ ,$$

                                                                                      (A2b)

$$y_{t+1} = \frac{\beta P_{EX}}{\alpha + P_{EX}}\frac{1}{P_{EX}}\Bigg[D_p(s)\Bigg\langle(1-s)\left\{\frac{w_t}{P_{EX}}\left((1+k)y_t + \frac{1}{2}z_t\right) + \frac{x_t+z_t}{P_{EX}}(1+k)\frac{1}{2}y_t\right\} + $$

$$(1-s')\left\{\frac{w_t}{P_{EX}}\left((1+k)\frac{1}{2}y'_t + \frac{1}{2}(1-r)z'_t\right) + \frac{x_t+z_t}{P_{EX}}(1+k)\frac{1}{2}rw'_t\right\}\Bigg\rangle + $$

$$D_p(s')(1-s)\left\{\frac{w'_t}{P_{EX}}\left((1+k)\frac{1}{2}y_t + \frac{1}{2}rz_t\right) + \frac{x'_t+z'_t}{P_{EX}}(1+k)\frac{1}{2}ry_t\right\}\Bigg]\frac{1}{\overline{W}}\ ,$$

                                                                                      (A2c)

80 $\quad z_{t+1} = (1-\mu)[\![(1-\delta)sz_t +$

81 $\quad \frac{\beta P_{EX}}{\alpha+P_{EX}}\Big[D_p(s)\Big\langle(1-s)\Big\{\frac{w_t}{P_{EX}}\frac{1}{2}z_t + \frac{x_t+z_t}{P_{EX}}\Big((1+k)\frac{1}{2}y_t + z_t\Big)\Big\} +$

82 $\quad (1-s')\Big\{\frac{w_t}{P_{EX}}\frac{1}{2}rz'_t + \frac{x_t+z_t}{P_{EX}}\Big((1+k)\frac{1}{2}(1-r)y'_t + \frac{1}{2}z'_t\Big)\Big\}\Big\rangle +$

83 $\quad D_p(s')(1-s)\Big\{\frac{w'_t}{P_{EX}}\frac{1}{2}(1-r)z_t + \frac{x'_t+z'_t}{P_{EX}}\Big((1+k)\frac{1}{2}ry_t + \frac{1}{2}z_t\Big)\Big\}\Big]\!]\!]\frac{1}{W}$,

84 $\quad$ (A2d)

85 $\quad$ whereas those with a mutant selfing rate s' are

86 $\quad w'_{t+1} = [\![(1-\delta)s'w'_t +$

87 $\quad \frac{\beta P_{EX}}{\alpha+P_{EX}}\Big[D_p(s)(1-s')\Big\{\frac{w_t}{P_{EX}}\Big(\frac{1}{2}w'_t + \frac{1}{2}rx'_t\Big) + \frac{x_t+z_t}{P_{EX}}\frac{1}{2}(1-r)w'_t\Big\} +$

88 $\quad D_p(s')\Big\langle(1-s)\Big\{\frac{w'_t}{P_{EX}}\Big(\frac{1}{2}w_t + \frac{1}{2}(1-r)x_t\Big) + \frac{x'_t+z'_t}{P_{EX}}\frac{1}{2}rw_t\Big\} +$

89 $\quad (1-s')\Big\{\frac{w'_t}{P_{EX}}\Big(w'_t + \frac{1}{2}x'_t\Big) + \frac{x'_t+z'_t}{P_{EX}}\frac{1}{2}w'_t\Big\}\Big\rangle\Big]\!]\!]\frac{1}{W}$,

90 $\quad$ (A2e)

91 $\quad x'_{t+1} = (1-\mu)[\![(1-\delta)s'w'_t +$

92 $\quad \frac{\beta P_{EX}}{\alpha+P_{EX}}\Big[D_p(s)(1-s')\Big\{\frac{w_t}{P_{EX}}\frac{1}{2}(1-r)x'_t + \frac{x_t+z_t}{P_{EX}}\Big(\frac{1}{2}rw'_t + \frac{1}{2}x'_t\Big)\Big\} +$

93 $\quad D_p(s')\Big\langle(1-s)\Big\{\frac{w'_t}{P_{EX}}\frac{1}{2}rx_t + \frac{x'_t+z'_t}{P_{EX}}\Big(\frac{1}{2}(1-r)w_t + \frac{1}{2}x_t\Big)\Big\} +$

94 $\quad (1-s')\Big\{\frac{w'_t}{P_{EX}}\frac{1}{2}x'_t + \frac{x'_t+z'_t}{P_{EX}}\Big(\frac{1}{2}w'_t + x'_t\Big)\Big\}\Big\rangle\Big]\!]\!]\frac{1}{W}$,

95 $\quad$ (A2f)

96 $\quad y'_{t+1} = \frac{\beta P_{EX}}{\alpha+P_{EX}}\Big[D_p(s)(1-s')\Big\{\frac{w_t}{P_{EX}}\Big((1+k)\frac{1}{2}y'_t + \frac{1}{2}rz'_t\Big) + \frac{x_t+z_t}{P_{EX}}(1+k)\frac{1}{2}(1-r)y'_t\Big\} +$

97 $\quad D_p(s')\Big\langle(1-s)\Big\{\frac{w'_t}{P_{EX}}\Big((1+k)\frac{1}{2}y_t + \frac{1}{2}(1-r)z_t\Big) + \frac{x'_t+z'_t}{P_{EX}}(1+k)\frac{1}{2}ry_t\Big\} +$

98 $\quad (1-s')\Big\{\frac{w'_t}{P_{EX}}\Big((1+k)y'_t + \frac{1}{2}z'_t\Big) + \frac{x'_t+z'_t}{P_{EX}}(1+k)\frac{1}{2}y'_t\Big\}\Big\rangle\Big]\frac{1}{W}$,

99 $\qquad$ (A2g)

100 $\quad z'_{t+1} = (1-\mu)[\![(1-\delta)s'w'_t +$

101 $\quad \frac{\beta P_{EX}}{\alpha+P_{EX}}\Big[D_p(s)(1-s')\Big\{\frac{w_t}{P_{EX}}\frac{1}{2}(1-r)z'_t + \frac{x_t+z_t}{P_{EX}}\Big((1+k)\frac{1}{2}ry'_t + \frac{1}{2}z'_t\Big)\Big\} +$

102 $\quad D_p(s')\Big\langle(1-s)\Big\{\frac{w'_t}{P_{EX}}\frac{1}{2}rz_t + \frac{x'_t+z'_t}{P_{EX}}\Big((1+k)\frac{1}{2}(1-r)y_t + \frac{1}{2}z_t\Big)\Big\} +$

103 $\quad (1-s')\Big\{\frac{w'_t}{P_{EX}}\frac{1}{2}z'_t + \frac{x'_t+z'_t}{P_{EX}}\Big((1+k)\frac{1}{2}y'_t + z'_t\Big)\Big\}\Big\rangle\Big]\!]\!]\frac{1}{W}\ .$

104 $\qquad$ (A2h)

105 In these equations, *W* represents the average fitness.

106

107 **APPENDIX B**

108 <u>Invasibility of mutant selfing rate</u>

109 We consider invasions of $s - 10^{-8}$ and $s + 10^{-8}$ mutant selfing rates into a resident mutation rate *s*,

110 dominant eigenvalues of which are denoted by λ_- and λ_+, respectively. The mutant can increase in

111 the population when a norm of the dominant eigenvalue $\|\lambda\|$ is greater than 1. Therefore, the trait

112 value evolves towards a larger value when $\|\lambda_-\| \leq 1$ and $\|\lambda_+\| > 1$ (i.e., $\|\lambda_-\| \leq 1 < \|\lambda_+\|$),

113 and towards a lower value when $\|\lambda_-\| > 1$ and $\|\lambda_+\| \leq 1$ (i.e., $\|\lambda_-\| > 1 \geq \|\lambda_+\|$). Since the

114 norm of the dominant eigenvalue of a resident selfing rate *s* itself is always 1, these conditions

115 can be simplified as $\|\lambda_-\| < \|\lambda_+\|$ and $\|\lambda_-\| > \|\lambda_+\|$, respectively, during the transient process

116 towards an evolutionary singular point. Accordingly, we search for interior convergently stable

117 selfing rates by looking for changing points of sign $\|\lambda_+\| - \|\lambda_-\|$ from positive to negative by

118 using a bisection method for a value of *s*. We also examine the evolutionary stability of those

119 solutions, in which a resident selfing rate can be stable if both $\|\lambda_+\| \leq 1$ and $\|\lambda_-\| \leq 1$ hold

120 simultaneously. On the other hand, boundaries *s* = 0 and *s* = 1 can be convergently and

121 evolutionarily stable solutions when neighborhoods of those boundaries satisfy $\|\lambda_+\| - \|\lambda_-\| <$

122 0 and $\|\lambda_+\| - \|\lambda_-\| > 0$, respectively.

123

124 <u>PIP of selfing evolution</u>

125 We plot PIP with examinations of mutant invasibility by using the same procedure as above,

126 although the interval of selfing is set to 0.005 rather than 10^{-8} to reduce the calculation load.

127 Supplementary figures 1 and 2 represent PIP for $k = 1$ cases of Figs. 1 and 2, respectively. A

128 grayscale chart above each panel indicates a mating mode under the given resident selfing rate,

129 which corresponds to grayscale in Figs. 1 and 2. In those figures, the gray regions indicate a

130 successful invasion of a rare mutant strategy (a coordinate of the vertical axis) into the

131 population of resident strategy (a coordinate of the horizontal axis), while the white regions

132 represent the failure of mutant invasion. On the diagonal line, the mutant strategy coincides with

133 the resident strategy. When the plot is white both above and below a point on the diagonal line, it

134 indicates that mutants with slightly larger and smaller values of strategies than the resident

135 strategy cannot increase in the resident population, implying evolutionary stability of the resident

136 strategy. In those figures, solid circles represent convergently and evolutionarily stable states,

137 whereas open circles are convergently unstable states (as Figs. 1 and 2). According to the figures,

138 we can graphically reconfirm evolutionary stabilities of equilibria that are indicated by the solid

139 circles.

140   Interestingly, in Supplementary figure 1(i), a range of selfing rates can be continuously

141 evolutionarily stable, which is indicated by the region with a black arrow. This seems

142 inconsistent to Fig. 1(i), although it may result from the difference of mutation interval between

143 the two figures. In the PIP, we set an interval of mutation at 0.005, which is smaller than that of

144   the above numerical analysis, 10^{-8}. The larger interval in PIP results in a range of selfing rates

145   that are continuously evolutionarily stable. Indeed, an examination with a smaller interval of

146   selfing rate (i.e., 10^{-8}) suggests that the boundary of invasibility adjoins the diagonal line,

147   implying that the evolutionarily stable selfing rate is a point rather than a region.

148

149

150 **CAPTIONS OF SUPPLEMENTARY FIGURES**

151 **Supplementary Figure 1**. Pairwise invasibility plot (PIP) of selfing rate. Each panel indicates a

152 PIP under the same condition as a corresponding panel of Fig. 1 with $k = 1$. Solid circles

153 represent selfing rates that are both convergently and evolutionarily stable, whereas open circles

154 are convergently unstable solutions. A grayscale chart above each panel indicates a mating mode

155 under the given resident selfing rate, which correspond to grayscale in Fig. 1. In panel (i), an

156 arrow indicates a region where a certain range of selfing rates are continuously evolutionary

157 stable (see Appendix B).

158 **Supplementary Figure 2**. Pairwise invasibility plot (PIP) of selfing rate. Each panel indicates a

159 PIP under the same condition as a corresponding panel of Fig. 2 with $k = 1$. Solid circles

160 represent selfing rates that are both convergently and evolutionarily stable, whereas open circles

161 are convergently unstable solutions. A grayscale chart above each panel indicates a mating mode

162 under the given resident selfing rate, which correspond to grayscale in Fig. 2.

163 **Supplementary Figure 3**. Feasible and stable equilibria and evolutionarily realized selfing rates

164 in a case that a restorer cost reduces male function (cost type 1), with varying parameters of

165 pollen limitation function, α and β. Solid circles represent selfing rates that are both convergently

166 and evolutionarily stable, whereas open circles are convergently unstable solutions. In this figure,

167 $\mu = 0.75$ and $1 - \delta = 0.75$, other parameters are shown in Table 1. The panels (b), (e), and (h) of

168 this figure represent similar results to Figs. 1(h), (e) and (b), respectively, because of

169 correspondences between μ, α and η ($= (1 - \delta)/\beta$) values, i.e., $\eta = 0.25$, 0.5 and 1.0.

170 **Supplementary Figure 4**. Feasible and stable equilibria and evolutionarily realized selfing rates

171 in a case that a restorer cost reduces individual performance (cost type 2), with varying

172 parameters of pollen limitation function, α and β. Parameters and conditions are identical to

173   Supplementary Fig. 3. The panels (b), (e), and (h) of this figure represent similar results with

174   Figs. 2(h), (e) and (b), respectively, because of correspondences between μ, α and η (= $(1 - \delta)/\beta$)

175   values, i.e., η = 0.25, 0.5 and 1.0.

176   **Supplementary Figure 5**. Pairwise invasibility plot (PIP) of selfing rate. Each panel indicates a

177   PIP under the same condition as a corresponding panel of Supplementary Fig. 3 with k = 1. Solid

178   circles represent selfing rates that are both convergently and evolutionarily stable, whereas open

179   circles are convergently unstable solutions. A grayscale chart above each panel indicates a

180   mating mode under the given resident selfing rate, which corresponds to grayscale in

181   Supplementary Fig. 3.

182   **Supplementary Figure 6**. Pairwise invasibility plot (PIP) of selfing rate. Each panel indicates a

183   PIP under the same condition as a corresponding panel of Supplementary Fig. 4 with k = 1.

184   Parameters and conditions are identical to Supplementary Fig. 4.

185   **Supplementary Figure 7**. Feasible and stable equilibria and evolutionarily realized selfing rates

186   in a case that a restorer cost reduces male function (cost type 1), with varying parameters of

187   pollen discounting function, a and b. Solid circles represent selfing rates that are both

188   convergently and evolutionarily stable, whereas open circles are convergently unstable solutions.

189   In this figure, μ = 0.75 and $1 - \delta$ = 0.5, other parameters are shown in Table 1. Panel (i) of this

190   figure corresponds to Fig. 1(e) due to identical parameters.

191   **Supplementary Figure 8**. Feasible and stable equilibria and evolutionarily realized selfing rates

192   in a case that a restorer cost reduces individual performance (cost type 2), with varying

193   parameters of pollen discounting function, a and b. Parameters and conditions are identical to

194   Supplementary Fig. 7. The panel (i) of this figure corresponds to Fig. 2(e) due to identical

195   parameters.

196 **Supplementary Figure 9**. Pairwise invasibility plot (PIP) of selfing rate. Each panel indicates a

197 PIP under the same condition as a corresponding panel of Supplementary Fig. 7 with $k = 1$. Solid

198 circles represent selfing rates that are both convergently and evolutionarily stable, whereas open

199 circles are convergently unstable solutions. A grayscale chart above each panel indicates a

200 mating mode under the given resident selfing rate, which corresponds to grayscale in

201 Supplementary Fig. 7.

202 **Supplementary Figure 10**. Pairwise invasibility plot (PIP) of selfing rate. Each panel indicates a

203 PIP under the same condition as a corresponding panel of Supplementary Fig. 8 with $k = 1$.

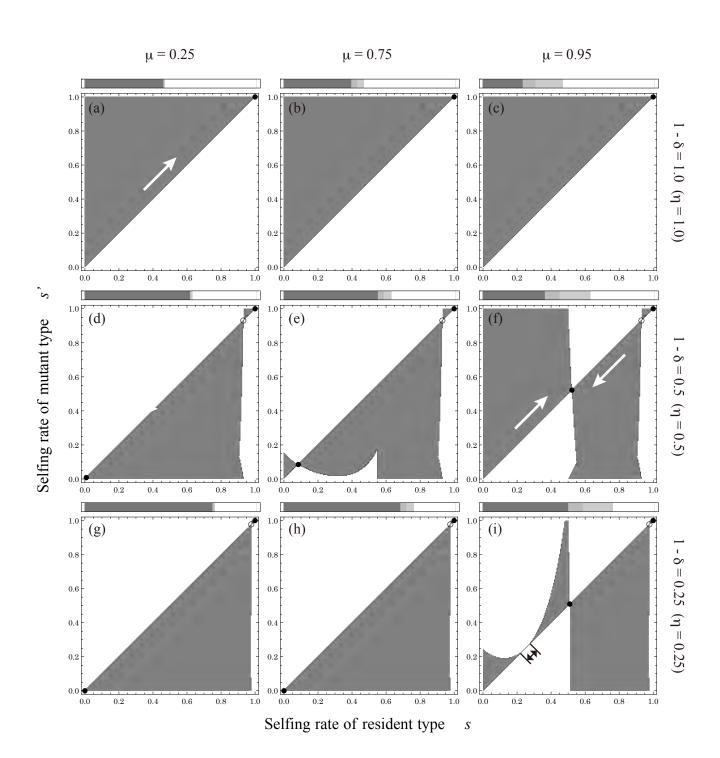204 Parameters and conditions are identical to Supplementary Fig. 8.

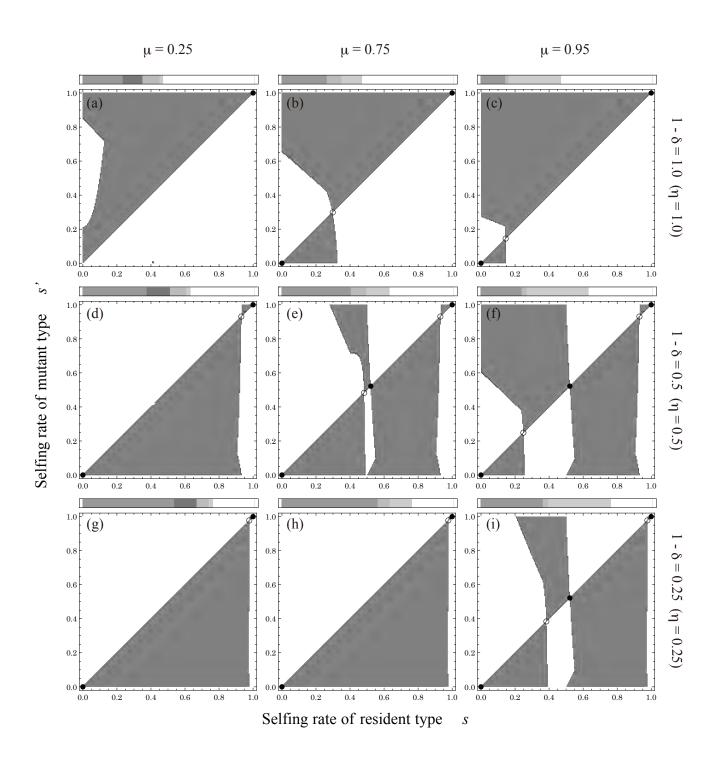205 **Supplementary Table 1** Mating table of outbreeding

206

| | | | normal mitochondria | | | | CMS mitochndria | | | |
| | | | wildtype selfing | | mutant selfing | | wildtype selfing | | mutant selfing | |
	pollen\ovule		w ms/n	x Ms/n	w' ms'/n	x' Ms'/n	y (female) ms/c	z Ms/c	y' (female) ms'/c	z' Ms'/c
normal mitochondria / wildtype selfing Dp(s)	w	ms	w:1	w:1/2 x:1/2	w:1/2 w':1/2	w:1/2(1−r) w':1/2r x':1/2(1−r) x:1/2r	y:1	y:1/2 z:1/2	y:1/2 y':1/2	y:1/2(1−r) y':1/2r z':1/2(1−r) z:1/2r
	x	Ms	w:1/2 x:1/2	x:1	w':1/2(1−r) w:1/2r x:1/2(1−r) x':1/2r	x:1/2 x':1/2	y:1/2 z:1/2	z:1	y':1/2(1−r) y:1/2r z:1/2(1−r) z':1/2r	z:1/2 z':1/2
normal mitochondria / mutant selfing Dp(s')	w'	ms'	w:1/2 w':1/2	w':1/2(1−r) w:1/2r x:1/2(1−r) x':1/2r	w':1	w':1/2 x':1/2	y:1/2 y':1/2	y':1/2(1−r) y:1/2r z:1/2(1−r) z':1/2r	y':1	y':1/2 z':1/2
	x'	Ms'	w:1/2(1−r) w':1/2r x':1/2(1−r) x:1/2r	x:1/2 x':1/2	w':1/2 x':1/2	x':1	y:1/2(1−r) y':1/2r z':1/2(1−r) z:1/2r	z:1/2 z':1/2	y':1/2 z':1/2	z':1
CMS mitochndria / wildtype selfing Dp(s)	y									
	z	ms	w:1/2 x:1/2	x:1	w':1/2(1−r) w:1/2r x:1/2(1−r) x':1/2r	x1:1/2 x':1/2	y:1/2 z:1/2	z:1	y':1/2(1−r) y:1/2r z:1/2(1−r) z':1/2r	z:1/2 z':1/2
CMS mitochndria / mutant selfing Dp(s')	y'									
	z'	Ms'	w:1/2(1−r) w':1/2r x':1/2(1−r) x:1/2r	x:1/2 x':1/2	w':1/2 x':1/2	x':1	y:1/2(1−r) y':1/2r z':1/2(1−r) z:1/2r	z:1/2 z':1/2	y':1/2 z':1/2	z':1

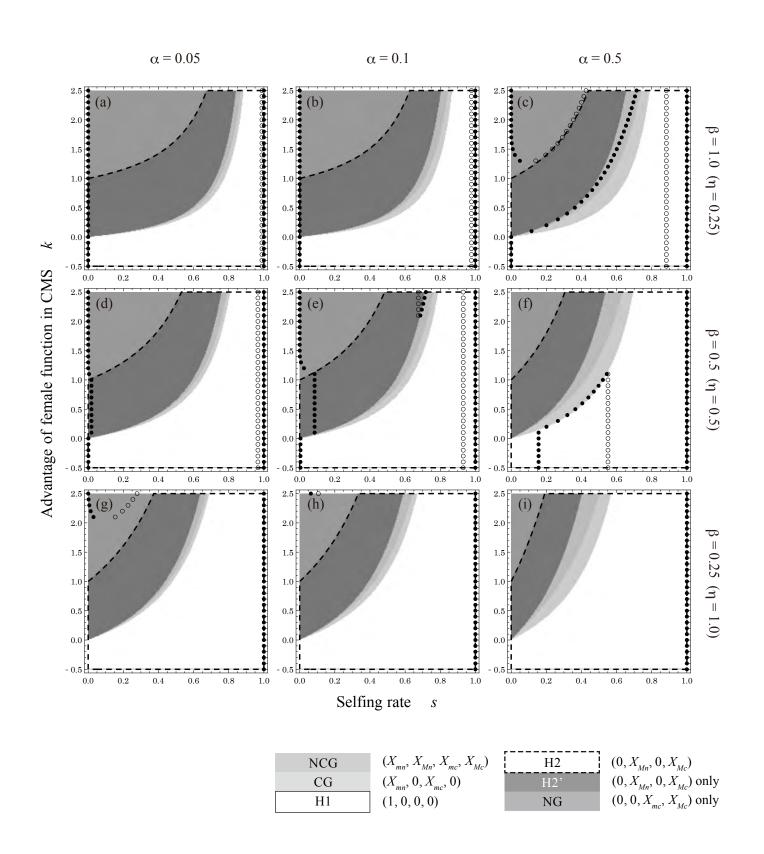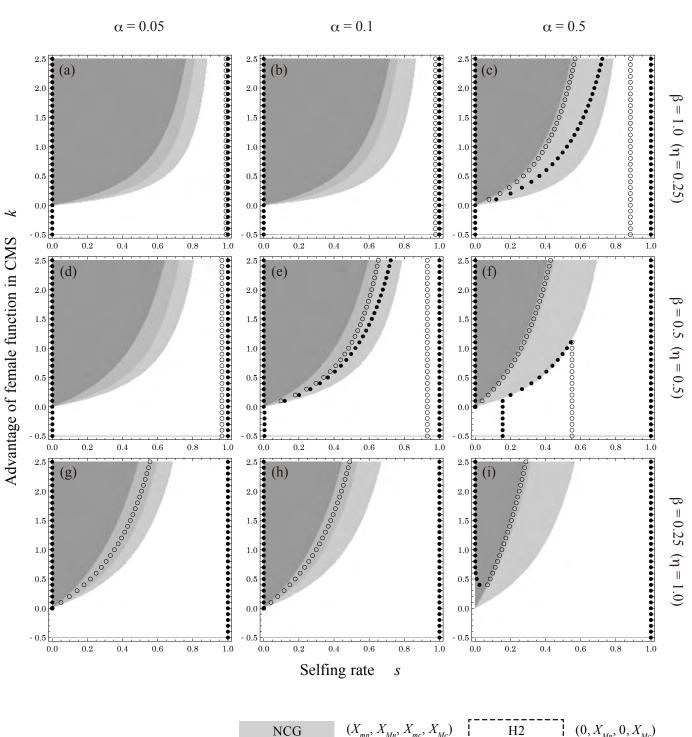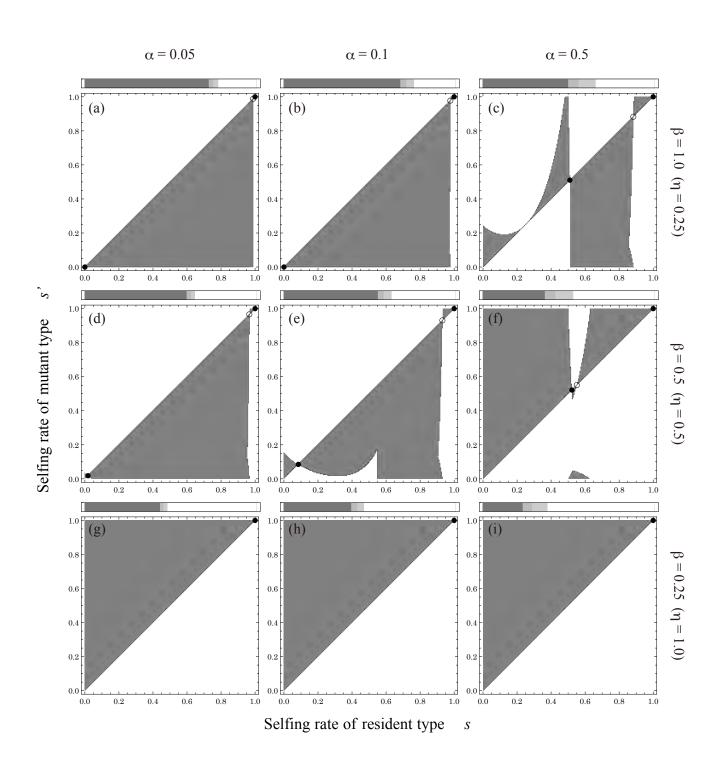† Rows with the same color represet the sama mating consequence

207

208

209

210

$a = 0.2$ $a = 0.6$ $a = 1.0$

$b = 1.0$

$b = 0.5$

$b = 0.0$

Selfing rate of mutant type s'

Selfing rate of resident type s