

# Evolution of exalbuminous seeds as a result of competition between maternally derived and paternally derived genes

Satoki Sakai

*Department of Ecology and Evolutionary Biology,  
Graduate School of Life Sciences,  
Tohoku University, Aoba, Japan*

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## ABSTRACT

**Background:** The endosperm of angiosperm seeds absorbs resources from the female parent to support the embryo. The endosperm has three nuclei; two are maternally derived (madumnal) and one is paternally derived (padumnal). Some species have albuminous seeds; that is, at seed maturity, the endosperm contains most of the seed's stored resources and the embryo is small. Other species have exalbuminous seeds; that is, before seed maturity, the cotyledons absorb most or all the endosperm and the embryo is relatively large. There are two sorts of exalbuminous seeds: (1) The cotyledons of some species absorb most of the endosperm towards the end of maturation. Their genes regulate the absorption process but the embryo does not directly absorb resources from the female parent. (2) The cotyledons of other species completely absorb the endosperm very early in seed maturation, and then absorb resources from the female parent directly. In such species, genes expressed in cotyledons communicate with parental tissues regarding demand for resources.

**Question:** How might natural selection account for the differences in exalbuminous seed development?

**Method:** Analysis of evolutionarily stable strategy (ESS) using a game-theoretic model of seed production.

**Key assumptions:** Madumnal and padumnal genes regulate resource absorption via the endosperm and embryos, their relative contributions being proportional to their genome ratios in those organs. Excessively high absorption rates result in abortion of seeds but a large embryo (relative to endosperm) is advantageous for germination.

**Predictions:** Resource absorption directly from the parent by the embryo is favoured if the abortion cost associated with that strategy is relatively low compared with the abortion cost associated with resource absorption by the embryo from the endosperm. Alternatively, the process whereby exalbuminous seeds that mature without direct absorption by the embryo is favoured if the abortion cost associated with resource absorption by the embryo from the endosperm is relatively low and a large embryo is advantageous. Also, it would appear that the difference in the genome ratio between the endosperm (2m : 1p) and the embryo (1m : 1p) affects

the ESS: the ESS is not simply the strategy by which a high survival rate of the fertilized ovules during development is realized.

*Keywords:* albuminous seed, embryo, endosperm, exalbuminous seed, genomic imprinting, intragenomic conflict, resource absorption.

## INTRODUCTION

The endosperm, whose primary function is to absorb resources from the female parent for nutritive support of the embryo, has several different destinies during seed maturation among the angiosperm taxa. In monocotyledons, the endosperm develops well and contains the bulk of the stored resources for seeds (Costa *et al.*, 2004). The embryo in a mature seed is small and begins to absorb resources from the endosperm at germination. In many dicotyledons, on the other hand, most resources are held in cotyledons and to a lesser extent in the endosperm (Costa *et al.*, 2004). That is, the endosperm is absorbed by the embryo before the end of seed maturation, with one or a few surrounding cell layers of endosperm remaining (Yeung and Cavey, 1988; Costa *et al.*, 2004; Finch-Savage and Leubner-Metzger, 2006). Genes expressed in the embryo-surrounding region in the endosperm (micropylar endosperm) regulate this absorption process (Costa *et al.*, 2004; Yang *et al.*, 2008). Moreover, in several dicotyledons such as Fabaceae, the endosperm is completely absorbed by the embryo during the early stage of seed maturation, and the embryo directly absorbs resources from the female parent (Zhang *et al.*, 2007). In those species, genes expressed in cotyledons communicate with the parent tissues regarding demand for resources (Weber *et al.*, 2005; Zhang *et al.*, 2007; Zhou *et al.*, 2009).

Why, then, are there three different destinies of the endosperm during seed maturation? Why does the endosperm disappear in some taxa but not in others before or during seed maturation? In particular, why does the embryo begin to absorb resources directly from the female parent even though endosperm is present?

The endosperm is triploid in the most common seed type: it consists of two maternally derived (madumnal) and one paternally derived (padumnal) nuclei (2m:1p). Haig and Westoby (1989) have proposed that the 2m:1p ratio is a consequence of the conflict between the female and male parents, as is genomic imprinting. That is, with multiple paternity, it is advantageous for the male parent to monopolize resources by its own offspring. On the other hand, equal distribution of resources is advantageous for the female parent since all offspring are equally related to the female parent. Hence, the padumnal genes in the endosperm enhance resource absorption from the female parent and the double doses of the madumnal genes is the counterstrategy by the female parent to inhibit resource absorption by the endosperm (Haig and Westoby, 1989).

On the other hand, in the embryo, the ratio of nuclei is 1m:1p and both the madumnal and padumnal genes can equally affect its growth. Hence, if the embryo directly absorbs resources from the female parent, the demand by the padumnal gene can be realized more strongly than in the case of resource absorption by the endosperm. Thus, it is possible that direct absorption by the embryo is a counterstrategy by the male parent against the triploid endosperm.

This view also provides a new insight into the evolution of the exalbuminous seeds in which the embryo does not absorb resources directly from the female parent (the second

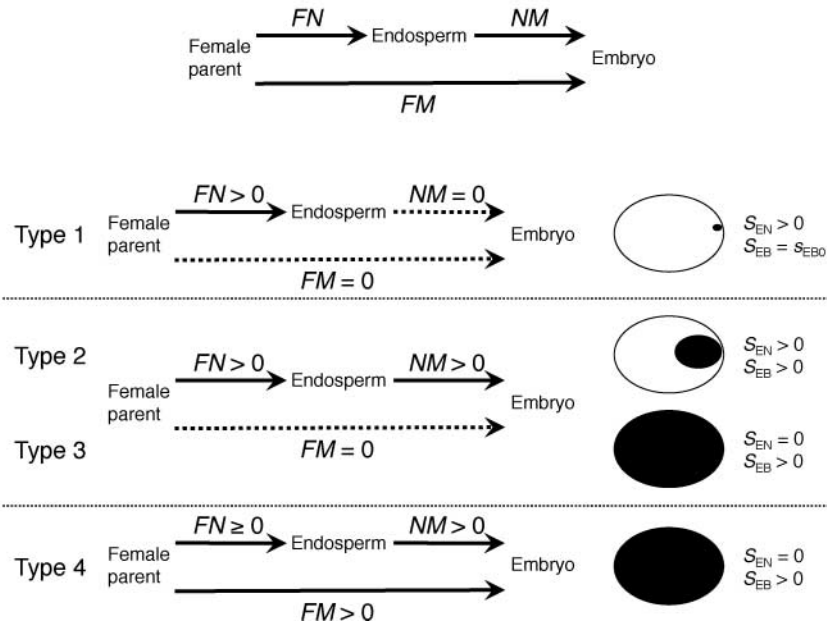
seed type above): those seeds might have evolved so that the size of the embryo becomes large while minimizing the effects of the padumnal gene on absorption of resources. That is, it has been shown that the ratio of embryo-to-seed volume has increased during the evolution of the angiosperms (Forbis *et al.*, 2002; but see Verdú, 2006), suggesting adaptive significance of having a relatively large embryo. The probable explanation for this is the rapidness in germination: Vivrette (1995) showed evidence to support this hypothesis. Given that a relatively large embryo has a certain advantage, it is advantageous for the madumnal genes to produce seeds with a relatively large embryo while minimizing the effects of the padumnal gene on the absorption of resources. One evolutionary consequence of this might be the above-stated type of exalbuminous seeds: direct absorption by the embryo is prevented as a result of the conflict.

In this paper, I develop a game-theoretic model for seed production in which the embryo, as well as the endosperm, is able to absorb resources directly (not via the endosperm) from the female parent if it completely absorbs the endosperm. The madumnal and padumnal genes simultaneously regulate the resource absorption rate by the endosperm from the female parent, that by the embryo from the endosperm, and that by the embryo from the female parent (if this occurs), their relative contributions being proportional to the genome ratios in the absorbing organs (endosperm or embryo). Using this model, I examine the conditions selecting for the three different destinies of the endosperm.

### MODEL

In the game-theoretic model presented here, the embryo, as well as the endosperm, is able to absorb resources from its female parent (with respective absorption rates  $FM$  and  $FN$ ) (Fig. 1). Direct absorption by the embryo occurs if the endosperm disappears during the development of fertilized ovules, i.e. if the embryo starts to absorb resources from the endosperm (with resource absorption rate  $NM$ ) and if the embryo completely absorbs the resources from the endosperm during development. Thus, there are four types of seed in the development of fertilized ovules (Fig. 1). In type 1 seed, the embryo does not start to absorb resources from the endosperm during the development of the fertilized ovule. The endosperm alone absorbs resources from the female parent, and the mature seed consists of a well-developed endosperm and an embryo of minimum size. In type 2 seed, the embryo starts to absorb resources from the endosperm during the development of the fertilized ovule. However, the embryo does not completely absorb the resources from the endosperm, and hence the embryo does not absorb resources directly from the female parent during development of the ovule. The mature seed then consists of an endosperm and an embryo of certain sizes. In type 3 seed, the embryo also starts to absorb resources from the endosperm, and the embryo completely absorbs the resources from the endosperm just at the end of ovule development. However, direct absorption by the embryo does not occur because ovule development has ceased by that time. The mature seed has no endosperm and consists only of an embryo. In type 4 seed, the endosperm disappears before seed maturation due to absorption by the embryo, and thereafter the endosperm absorbs resources directly from the female parent. The mature seed also has no endosperm and consists only of an embryo.

The madumnal and padumnal genes can simultaneously regulate the resource absorption rates of the endosperm and embryo,  $FN$ ,  $NM$ , and  $FM$ . Let  $FN_M$  and  $FN_P$  be the expressions by the madumnal and padumnal genes of the resource absorption rate of the



**Fig. 1.** Resource absorption rates and the destiny of the endosperm in four possible seed types.  $FN$ : the realized resource absorption rate of the endosperm from the female parent;  $NM$ : the realized resource absorption rate of the embryo from the endosperm;  $FM$ : the realized resource absorption rate of the embryo from the female parent;  $S_{EN}$ : the final size of the endosperm;  $S_{EB}$ : the final size of the embryo;  $S_{EB0}$ : the initial size of the embryo. See the text for an explanation of each type.

endosperm from the female parent,  $NM_M$  and  $NM_P$  be those of the resource absorption rate of the embryo from the endosperm, and  $FM_M$  and  $FM_P$  be those of the resource absorption rate of the embryo from the female parent. The realized resource absorption rates are determined additively by the respective maternal and paternal expressions. In the most common process of embryo sac formation, one egg cell ( $n$ ), two synergids ( $n$ ), three antipodal cells ( $n$ ), and one central cell ( $n+n$ ) are produced. The two haploid genomes of the central cell are identical to each other and identical to the haploid genome of the egg. The central cell ( $n$ ) is fertilized by a sperm ( $n$ ) and develops into the embryo ( $2n$ ), and the central cell ( $n+n$ ) is fused by a sperm ( $n$ ) and develops into the endosperm ( $3n$ ). The paternal genomes contributing to the endosperm and the embryo are identical. Thus, in the most common endosperm type, the ratio of the maternal to paternal genes is 2:1, and hence I assume the following:

$$\text{Realized resource absorption rate of the endosperm from the female parent}$$

$$FN = \frac{2FN_M + FN_P}{3}, \quad (1a)$$

$$\text{Realized resource absorption rate of the embryo from the endosperm}$$

$$NM = \frac{2NM_M + NM_P}{3}, \quad (1b)$$

Realized resource absorption rate of the embryo from the female parent

$$FM = \frac{FM_M + FM_P}{2}, \tag{1c}$$

$$FN_{\min} \leq FN_M, FN_P \leq FN_{\max},$$

$$NM_{\min} \leq NM_M, NM_P \leq NM_{\max},$$

$$FM_{\min} \leq FM_M, FM_P \leq FM_{\max}.$$

Resource absorption rates (1a) and (1b) are determined by the genes in the endosperm. This assumption is made because, in many plants, the basal endosperm transfer layer (chalazal endosperm) regulates resource absorption of the endosperm from the female parent, and the embryo-surrounding region in the endosperm (micropylar endosperm) regulates the resource absorption of the embryo from the endosperm (Costa *et al.*, 2004). Thus, the contribution of the madumnal and padumnal genes is in the ratio of 2m:1p. In contrast, the contribution to resource absorption rate (1c) is assumed to be 1:1 because the genes have a ratio of 1m:1p in the embryo. The values of  $FN_M$ ,  $FN_P$ ,  $NM_M$ ,  $NM_P$ ,  $FM_M$ , and  $FM_P$  may be positive, negative or zero: resource absorption is enhanced, inhibited or silenced if the value is positive, negative or zero, respectively. That is, the respective genes consist of an enhancer gene and an inhibitor gene, and if either is active, the other is silent (or both are silent). There should be lower ( $FN_{\min}$ ,  $NM_{\min}$ , and  $FM_{\min}$ ) and upper ( $FN_{\max}$ ,  $NM_{\max}$ , and  $FM_{\max}$ ) limits for these values because gene expression is often dose dependent (Costa *et al.*, 2004; Haig and Westoby, 1991; Scott *et al.*, 1998).

These resource absorption rates differ between mutants and the wild-type individuals. Let  $FN'_M$ ,  $FN'_P$ ,  $NM'_M$ ,  $NM'_P$ ,  $FM'_M$ , and  $FM'_P$  be the resource absorption rates expressed by the respective mutated genes. I assume that mutation occurs only in one copy of genes (diploid organisms are considered). Then, if the female parent has a mutated gene in the madumnal expression ( $FN'_M$ ,  $NM'_M$ , and/or  $FM'_M$ ), half of its ovules receive  $FN'_M$ ,  $NM'_M$ , and/or  $FM'_M$ , and hence the realized resource absorption rates are  $(2FN'_M + FN_P)/3$ ,  $(2NM'_M + NM_P)/3$ , and/or  $(FM'_M + FM_P)/2$  in half of its ovules [those in the ovules that did not receive mutated genes are given by (1a)–(1c)]. If one of the male parents has a mutated gene in the padumnal expression ( $FN'_P$ ,  $NM'_P$ , and/or  $FM'_P$ ), the realized resource absorption rates are  $(2FN_M + FN'_P)/3$ ,  $(2NM_M + NM'_P)/3$ , and/or  $(FM_M + FM'_P)/2$  in  $k/2$  of the fertilized ovules, where  $k$  is the paternity (the probability that two offspring have the same male parent) in those ovules [again, those in the ovules that did not receive mutated genes are given by (1a)–(1c)].

A female parent initiates  $N$  ovules at the beginning of reproduction. For the sake of simplicity, I assume all ovules are successfully fertilized (no pollen limitation). However, a fraction  $1 - h$  of the fertilized ovules is aborted due to developmental failure and the survival rates depend on their own resource absorption rates of endosperms and embryos. This abortion is not due to the selective abortion by the female parent, but due to the abortion cost associated with rapid growth. I assume that the survival probability is the following sigmoidal one:

$$h(FN_M, NM_M, FM_M, FN_P, NM_P, FM_P) = \frac{d}{1 + \text{bexp} \left[ c_{FN} \frac{2FN_M + FN_P}{3} + c_{NM} \frac{2NM_M + NM_P}{3} + c_{FM} \frac{FM_M + FM_P}{2} \right]},$$

where  $b$  and  $d$  are positive constants. The constants  $c_{FN}$ ,  $c_{NM}$ , and  $c_{FM}(\geq 0)$  represent the cost of overgrowth associated with the absorption of resources by the endosperm from the female parent, that associated with the absorption of resources by the embryo from the endosperm, and that associated with the absorption of resources by the embryo from the female parent, respectively.

A female parent has  $R_0$  amount of resources that will be allocated to seed production, and the surviving ovules grow by absorbing those resources (see Appendix for a detailed explanation). The endosperm and the embryo (if it grows) start to grow at  $t=0$  and  $t = t_1(t_1 \geq 0)$ , respectively. The competition for the absorption of resources is a game: though fertilized ovules with a higher resource absorption rate have an advantage in the competition, the amount of resources absorbed by those ovules depends on the resource absorption rates of the other fertilized ovules. Ovule growth ceases and seed production is completed at  $t = T$  when the  $R_0$  resources are depleted, and the final size of seeds (the sum of the endosperm and embryo) is the size at that time.

Among the surviving ovules, those that have absorbed more resources become larger, successful seeds (see Appendix for a detailed explanation). Let  $S_{EN}$  and  $S_{EB}$  be the final sizes of the endosperm and embryo of a seed, respectively. Let  $G(S_{EN}, S_{EB})$  be the probability that one seed with certain sizes of endosperm,  $S_{EN}$ , and embryo,  $S_{EB}$ , is successfully established.  $G$  is a monotonically increasing sigmoid function that satisfies  $G(0, 0) = 0$ . I assume the following for  $G$ :

$$G(S_{EN}, S_{EB}) = \exp[-a/(S_{EN} + fS_{EB})],$$

where  $f(> 0)$  and  $a$  are the advantage of having a large embryo relative to endosperm and a positive constant, respectively.

The fitness  $\phi_M$  of a madumnal mutant whose expressions are  $FN'_M$ ,  $NM'_M$ , and  $FM'_M$ , the fitness  $\phi_P$  of a padumnal mutant whose expressions are  $FN'_P$ ,  $NM'_P$ , and  $FM'_P$ , and the fitness  $\phi_F$  of a female mutant that initiates  $(N + N')/2$  ovules are given by

$$\begin{aligned} \phi_M(FN'_M, NM'_M, FM'_M, FN_P, NM_P, FM_P, N) \\ &= h(FN'_M, NM'_M, FM'_M, FN_P, NM_P, FM_P)G(S'_{EN}, S'_{EB})\frac{N}{2}, \\ \phi_P(FN_M, NM_M, FM_M, FN'_P, NM'_P, FM'_P, N) \\ &= h(FN_M, NM_M, FM_M, FN'_P, NM'_P, FM'_P)G(S'_{EN}, S'_{EB})\frac{kN}{2}, \\ \phi_F(FN_M, NM_M, FM_M, FN_P, NM_P, FM_P, N') \\ &= h(FN_M, NM_M, FM_M, FN_P, NM_P, FM_P)G(S'_{EN}, S'_{EB})\frac{1}{2}\frac{N' + N}{2}, \end{aligned}$$

where  $S'_{EN}$  and  $S'_{EB}$  are the sizes of the endosperm and embryo of the mutants, respectively. I obtain numerically the evolutionarily stable strategy  $FN^*_M$ ,  $NM^*_M$ ,  $FM^*_M$ ,  $FN^*_P$ ,  $NM^*_P$ ,  $FM^*_P$ , and  $N^*$  that satisfies

$$\begin{aligned}
& \phi_M(FN^*_M, NM^*_M, FM^*_M, FN^*_P, NM^*_P, FM^*_P, N^*) \\
& > \phi_M(FN'_M, NM'_M, FM'_M, FN^*_P, NM^*_P, FM^*_P, N^*), \\
& \phi_P(FN^*_M, NM^*_M, FM^*_M, FN^*_P, NM^*_P, FM^*_P, N^*) \\
& > \phi_P(FN^*_M, NM^*_M, FM^*_M, FN'_P, NM'_P, FM'_P, N^*) \\
& \phi_F(FN^*_M, NM^*_M, FM^*_M, FN^*_P, NM^*_P, FM^*_P, N^*) \\
& > \phi_F(FN^*_M, NM^*_M, FM^*_M, FN^*_P, NM^*_P, FM^*_P, N'),
\end{aligned}$$

for any mutants. I examine whether a certain genotype is an ESS by invading possible mutants to the population consisting of that genotype.

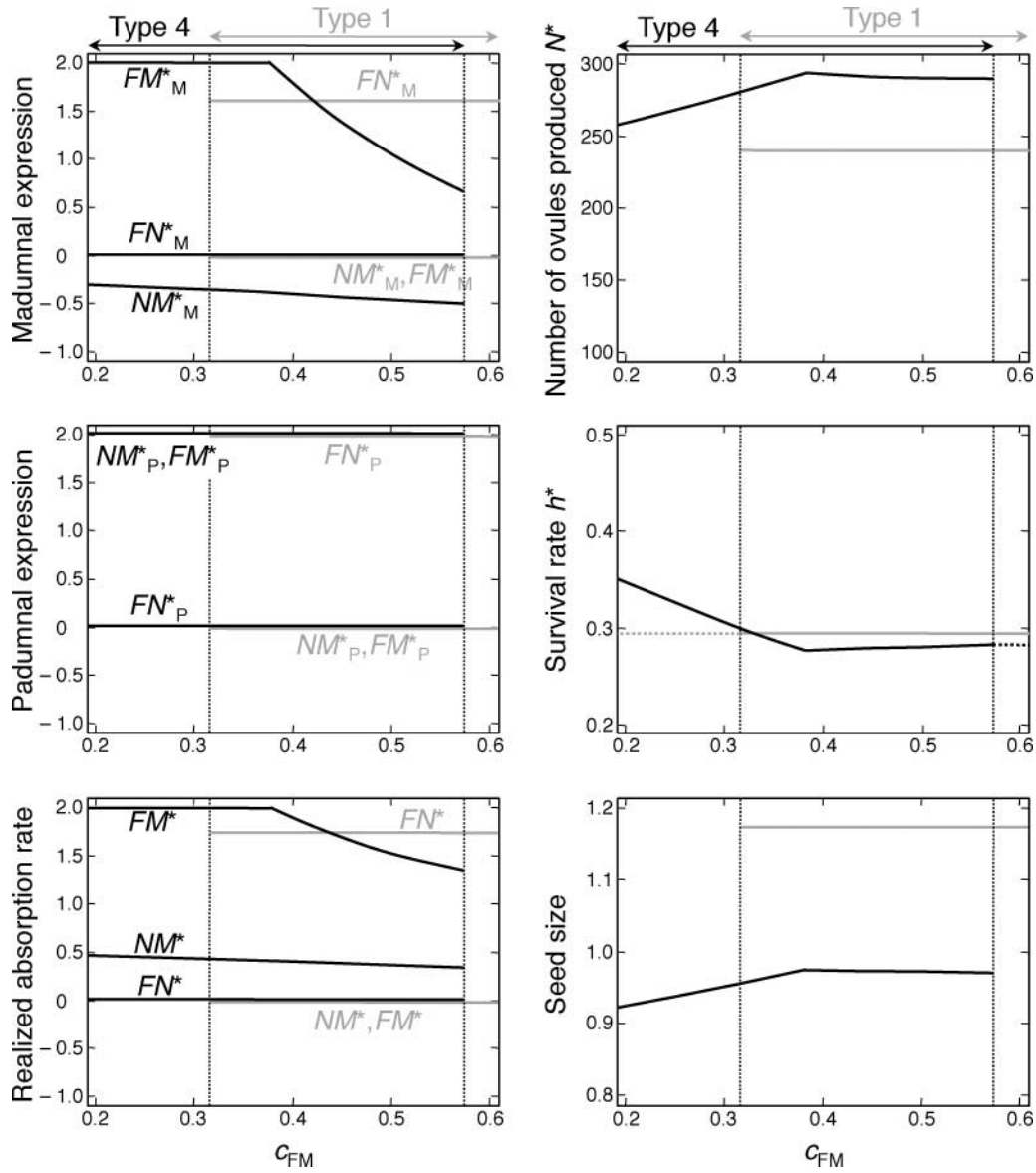
## RESULTS

Figures 2–4 show the dependences of the ESS realized absorption rates  $FN^*$ ,  $NM^*$ , and  $FM^*$ , the ESS madumnal expressions  $FN^*_M$ ,  $NM^*_M$ , and  $FM^*_M$ , the ESS padumnal expressions  $FN^*_P$ ,  $NM^*_P$ , and  $FM^*_P$ , the ESS number of ovules initially produced  $N^*$ , the ESS survival rate of fertilized ovules  $h^*$ , and the ESS size of a seed (sum of the endosperm and embryo size). The values of  $c_{NM}$  and  $f$  differ in these figures but those of the other parameters are the same. In the ESS, type 1, 3, and 4 seeds can be realized, but type 2 seed is not the ESS. Two seed types are the ESS in some parameter regions.

Type 1 seed is the ESS ( $FN^* > 0$  and  $NM^* = FM^* = 0$ ) if  $c_{NM}$  and  $c_{FM}$  are high relative to  $c_{FN}$  (Fig. 2) or if  $f$  is small (Fig. 3). In this ESS, the embryo does not start to grow during development of the ovule because of its high growth cost ( $c_{NM}$  and  $c_{FM}$ ) or the low advantage of having a large embryo ( $f$ ).  $FN^*_P > FN^*_M$ , which implies that the padumnal gene enhances the absorption of resources from the female parent more strongly than the madumnal gene because the advantage of the monopolization of the resources is greater for the former.  $NM^*_M = FM^*_M = NM^*_P = FM^*_P = 0$  so that  $NM^* = FM^* = 0$  is realized. Where  $c_{FM}$  is less than about 0.33 in Fig. 3, type 1 seed can be the ESS even though the survival rate of the fertilized ovules during development is lower in the populations occupied by this seed type than in the populations occupied by type 4 seed.

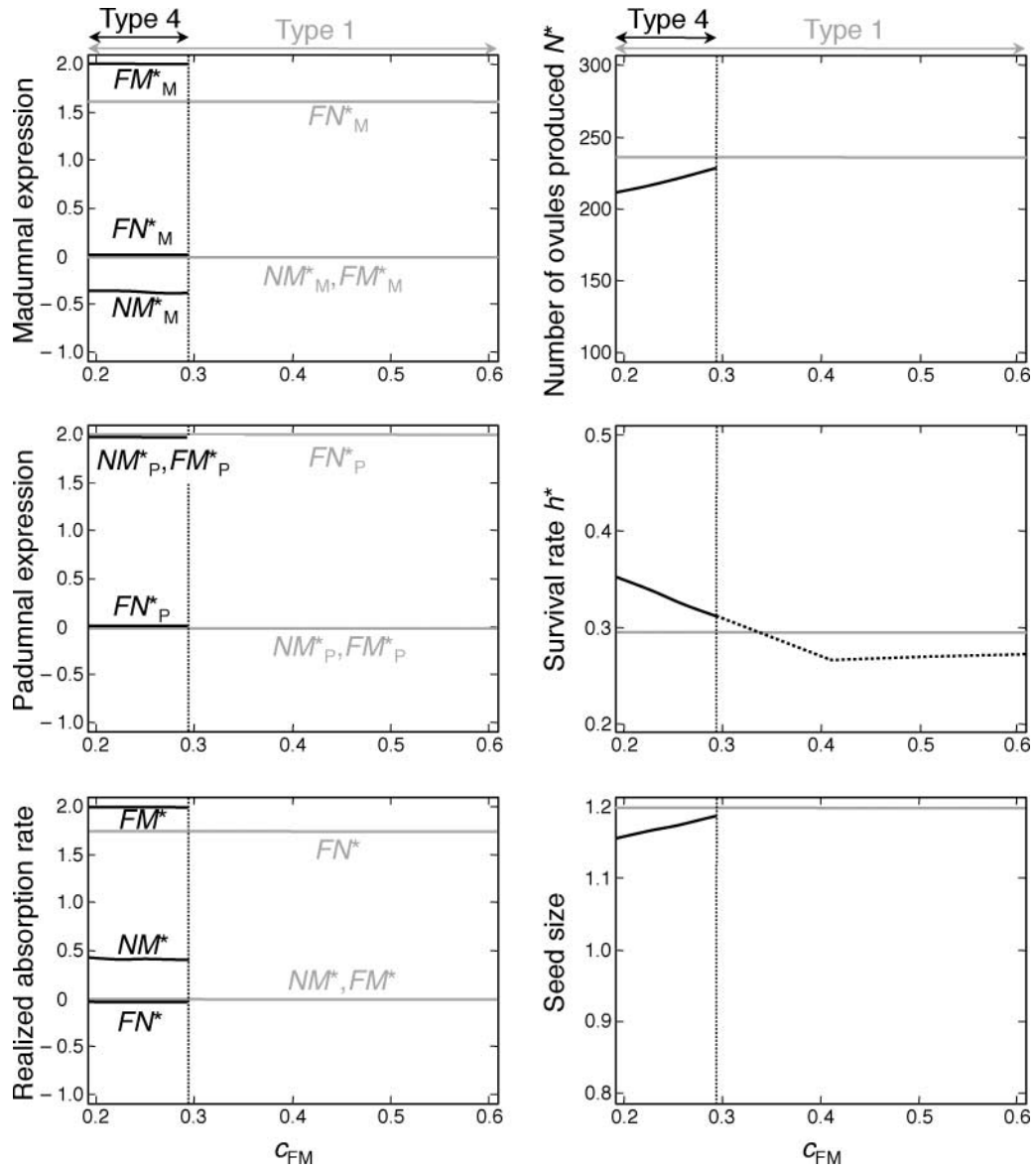
Type 3 seed is the ESS ( $NM^* > FN^* > 0$  and  $FM^* = 0$ ) if  $c_{NM}$  is low relative to  $c_{FN}$ ,  $c_{FM}$  is high relative to  $c_{FN}$ , and  $f$  is large (Fig. 4). In this ESS, the embryo starts to grow during development of the ovule because of its low growth cost ( $c_{NM}$ ) and the great advantage of becoming large ( $f$ ). However, direct absorption by the embryo does not occur because of its high growth cost ( $c_{FM}$ ). This seed type can be the ESS in an interval of  $c_{FM}$  because  $c_{FM}$  has no effect on  $h$  of type 3 seed (in which  $FM = 0$ ) and  $NM$  and  $FN$  are constants as a function of  $c_{FM}$ . Hence,  $NM$  and  $FN$  can be balanced so that the embryo completely absorbs the resources from the endosperm just at the end of ovule development.  $FN^*_P > FN^*_M > 0$  and  $NM^*_P > NM^*_M > 0$ , which implies that the padumnal gene also enhances the absorption of resources more strongly than the madumnal gene.  $FM^*_M = FM^*_P = 0$  so that  $FM^* = 0$  is realized. Where  $c_{FM}$  is about 0.33–0.42 in Fig. 4, type 3 seed cannot be the ESS even though the survival rate of the fertilized ovules during development is higher in the populations occupied by this seed type than in the populations occupied by type 4 seed.

Type 2 seed is not the ESS because the condition selecting for this seed type is similar to that for type 3 seed ( $c_{NM}$  is low relative to  $c_{FN}$ ,  $c_{FM}$  is high relative to  $c_{FN}$ , and  $f$  is large). Then, all resources should be moved to the embryo because the larger the embryo is, the

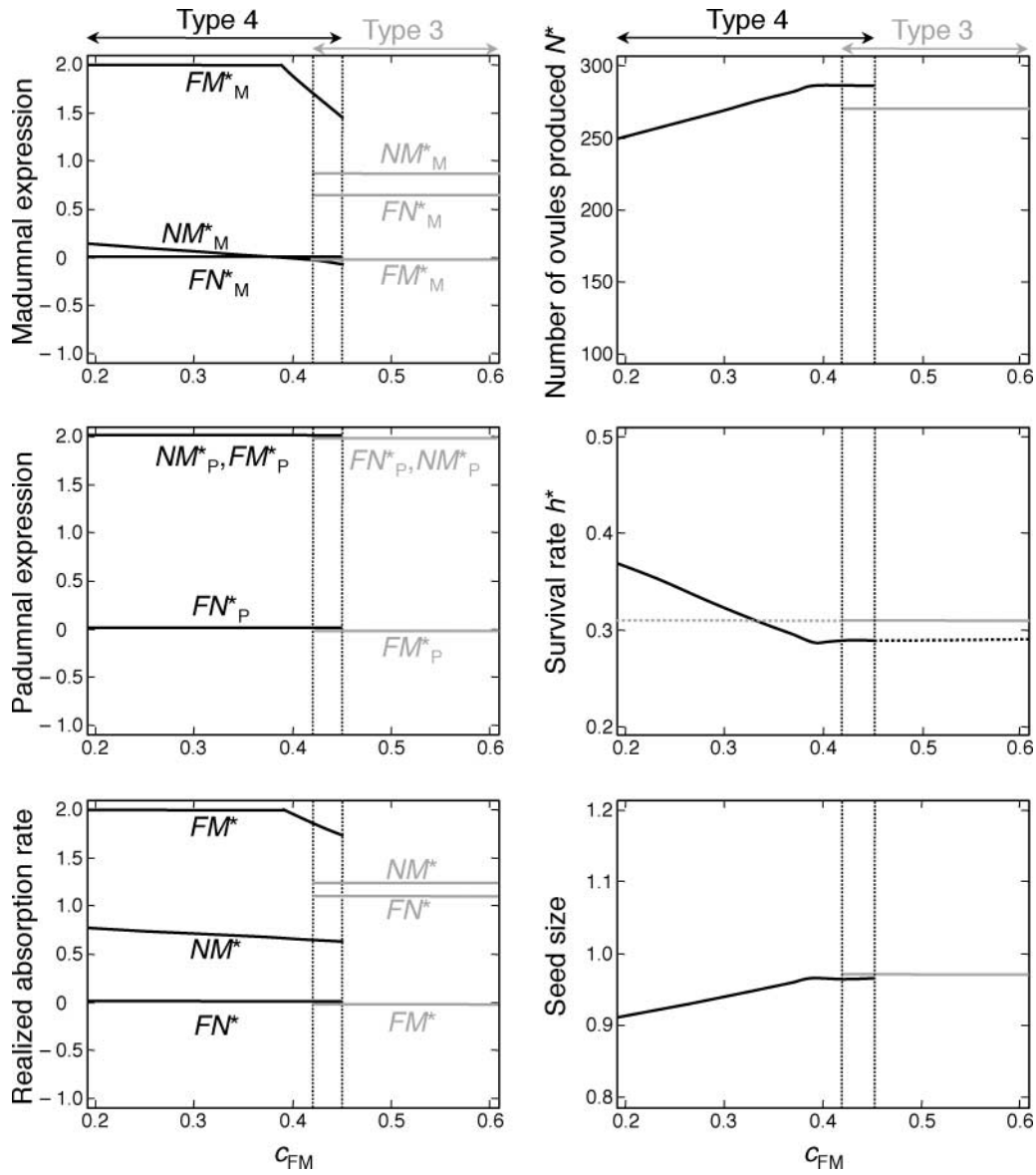


**Fig. 2.** The dependences of the ESS realized absorption rates  $FN^*$ ,  $NM^*$ , and  $FM^*$ , the ESS madumnal expressions  $FN^*_M$ ,  $NM^*_M$ , and  $FM^*_M$ , the ESS padumnal expressions  $FN^*_P$ ,  $NM^*_P$ , and  $FM^*_P$ , the ESS number of ovules initially produced  $N^*$ , the ESS survival rate of fertilized ovules  $h^*$ , and the ESS size of a seed (sum of the endosperm and embryo size) on the cost of overgrowth associated with the absorption of resources by the embryo from the female parent  $c_{FM}$ . Two seed types are the ESS in the parameter regions where two solid lines exist. The two horizontal dotted lines in the panel for survival rate show the survival rate of type 1 (grey line) and type 4 (black line) seeds during development in the populations consisting of the respective seed types (but the respective seed type is not the ESS in the respective parameter region).  $c_{FN} = 0.5$ ,  $c_{NM} = 0.5$ ,  $f = 1.3$ ,  $a = b = c = d = 1$ ,  $k = 0.5$ ,  $S_{FN0} = S_{EB0} = 0.05$ ,  $FN_{\min} = NM_{\min} = FM_{\min} = -2$ ,  $FN_{\max} = NM_{\max} = FM_{\max} = 2$ ,  $t_1 = 0.05$ , and  $R_0 = 100$ .





**Fig. 3.** The dependences of the ESS realized absorption rates  $FN^*$ ,  $NM^*$ , and  $FM^*$ , the ESS madumnal expressions  $FN^*_M$ ,  $NM^*_M$ , and  $FM^*_M$ , the ESS padumnal expressions  $FN^*_P$ ,  $NM^*_P$ , and  $FM^*_P$ , the ESS number of ovules initially produced  $N^*$ , the ESS survival rate of fertilized ovules  $h^*$ , and the ESS size of a seed (sum of the endosperm and embryo size) on the cost of overgrowth associated with the absorption of resources by the embryo from the female parent  $c_{FM}$ . As Fig. 2 except that  $f=1$ .



**Fig. 4.** The dependences of the ESS realized absorption rates  $FN^*$ ,  $NM^*$ , and  $FM^*$ , the ESS madumnal expressions  $FN^*_M$ ,  $NM^*_M$ , and  $FM^*_M$ , the ESS padumnal expressions  $FN^*_P$ ,  $NM^*_P$ , and  $FM^*_P$ , the ESS number of ovules initially produced  $N^*$ , the ESS survival rate of fertilized ovules  $h^*$ , and the ESS size of a seed (sum of the endosperm and embryo size) on the cost of overgrowth associated with the absorption of resources by the embryo from the female parent  $c_{FM}$ . As Fig. 2 except that  $c_{NM} = 0.2$ .

greater advantage due to large  $f$ . Thus, type 3 seed, not type 2 seed, is always the ESS under this condition.

Type 4 seed is the ESS ( $FM^* > NM^* > 0$  and  $FN^* = 0$ ) if  $c_{FM}$  is low relative to  $c_{FN}$  (Figs. 2–4). In this ESS, the endosperm does not grow because of its high growth cost ( $c_{FN}$ ). Instead, direct absorption by the embryo from the female parent occurs because of its low growth cost ( $c_{FM}$ ). This seed type can be the ESS even if there is a low (or no) advantage ( $f$ ) of having a large embryo.  $FM^*_M = FM^*_P$  in broad parameter regions, which implies that both the madumnal and padumnal genes equally enhance direct absorption of resources by the embryo in these regions.  $FM^*_P > FM^*_M$  in the other regions and  $NM^*_P > NM^*_M$  in all regions, which implies that the padumnal genes enhance the absorption of resources more strongly than the madumnal genes.  $FN^*_M = FN^*_P = 0$  so that  $FN^* = 0$  is realized. Where  $c_{FM}$  is about 0.29–0.33 in Fig. 3, type 4 seed cannot be the ESS even though the survival rate of the fertilized ovules during development is higher in the populations occupied by this seed type than in the populations occupied by type 1 seed.

## DISCUSSION

### Effects of overgrowth and of the advantage of having a large embryo

The present model shows that three types of seeds can be the ESS dependent on the costs of overgrowth associated with the absorption of resources by the endosperm from the female parent ( $c_{FN}$ ), by the embryo from the endosperm ( $c_{NM}$ ), and by the embryo from the female parent ( $c_{FM}$ ), and the advantage of having a large embryo relative to the endosperm ( $f$ ). Type 1 seed suffers the cost  $c_{FN}$  but not the costs  $c_{NM}$  and  $c_{FM}$ , and does not benefit from a large value of  $f$ . Type 3 seed suffers the costs  $c_{FN}$  and  $c_{NM}$  but not the cost  $c_{FM}$ , and benefits from a large value of  $f$ . Type 4 seed suffers the costs  $c_{NM}$  and  $c_{FM}$  (particularly  $c_{FM}$ ) but not the cost  $c_{FN}$ , and benefits from a large value of  $f$ . Hence, the ESS seed type depends on the relative values of these parameters (Figs. 2–4).

The costs of overgrowth  $c_{FN}$  and  $c_{NM}$  may exist in many plants; that is, the balance of genomes in endosperm is critical for normal seed maturation, and genomic imbalance often results in the abortion of developing seeds (Haig and Westoby, 1991; Scott *et al.*, 1998; Carputo *et al.*, 1999; Vinckenoog *et al.*, 2003; Costa *et al.*, 2004). For example, in *Arabidopsis thaliana*, micropylar peripheral endosperm and chalazal endosperm overgrow in seeds with high paternal excess ( $2X \times 6X$ ), resulting in abortion of seeds (Scott *et al.*, 1998). On the other hand, it is not clear whether the cost of overgrowth  $c_{FM}$  exists in real plants. In legumes, the number of cells in the cotyledon determines the genetic difference in seed size (Hirshfield *et al.*, 1993; Sexton *et al.*, 1997; Munier-Jolain and Ney, 1998; Munier-Jolain *et al.*, 2008). Here, maternal genotype influences the mitotic rate during the cell division phase to achieve differences in cell number found in the cotyledons (Lemontey *et al.*, 2000). Also, paternal effects were observed on volume and number of cotyledon cells and on individual seed weight (Duc *et al.*, 2001). Thus, the madumnal and padumnal genes in the cotyledon may determine the number of cells in the cotyledon. Future studies should examine whether overgrowth of the cotyledon (excess cell division) entails costs as assumed in the present model.

An advantage of having an embryo that is large relative to the endosperm is significant for the evolution of type 3 seed. One possible advantage may be rapidness in germination: Vivrette (1995) found that species with seeds containing embryos that were large relative to the endosperm germinated more rapidly than those with seeds containing embryos that

were small relative to the endosperm. Vivrette reasoned that, in the latter seeds, the endosperm must first be absorbed by the embryo before resources can be used for growth. On the other hand, it has also been reported that early germination is advantageous (Verdú and Traveset, 2005; Abe *et al.*, 2008; De Luis *et al.*, 2009). For example, Verdú and Traveset (2005) conducted a phylogenetically controlled meta-analysis and found that early germination is advantageous in terms of seedling growth and fecundity. Early emergence benefits plants not only for a short time after germination, but has a beneficial effect for several years in perennials (De Luis *et al.*, 2009). Thus, selection for rapid germination may exist in the evolution of the relative ratio of the embryo to the endosperm.

### Effects of genome ratio

The difference in the genome ratio between the endosperm and the embryo may play a role in the ESS seed types because the ESS is not simply the strategy by which a high survival rate of the fertilized ovules during development is realized. That is, there is a region where type 4 seed is the ESS even though the survival rate of its fertilized ovules during development is lower than that of type 3 seed in the populations occupied by type 3 seed (Fig. 4). Also, there is a region where type 4 seed, as well as type 3 seed, is the ESS even though the survival rate is lower in type 4 seed than in type 3 seed. These results may arise because type 4 seed is advantageous for the padumnal genes even if its survival rate becomes low. That is, in type 4 seed, direct absorption of resources by the embryo (whose genome ratio is  $m:p = 1:1$ ) from the female parent is realized, whereas in type 3 seed, the endosperm (whose genome ratio is  $m:p = 2:1$ ) absorbs resources from the female parent. Hence, the demand by the padumnal genes is more strongly realized in type 4 seed than in type 3 seed. Similarly, there is a region where type 4 seed cannot be the ESS even though the survival rate of the fertilized ovules during development is higher in the populations occupied by this seed type than in the populations occupied by type 1 seed (Fig. 3). In this region, madumnal mutants enhancing type 1 seed-like resource absorption invade the populations because the demand by the madumnal genes is more strongly realized in type 1 seed than in type 4 seed. Thus, type 4 seed as the ESS is restricted to the region where its survival rate is very high.

### Evolvability of seed types

There are parameter regions where two seed types are the ESS. This suggests that seed types are not always easily evolvable characteristics. For example, mutants enhancing type 4 seed-like absorption of resources may not easily invade populations occupied by type 1 seed. For type 4 seed to be realized, the resource absorption rates by the endosperm from the female parent and by the embryo from the endosperm must be regulated so that the endosperm is completely absorbed by the embryo. However, the wild type (type 1 seed) in such populations enhances the resource absorption rate by the endosperm from the female parent but inhibits the other absorption rates. Hence, evolution to type 4 seed beyond the valley in the fitness landscape may be difficult. In real plants, seed types seem to be conservative: monocotyledons produce type 1 seed, most dicotyledons produce type 3 seed, and several dicotyledons such as Fabaceae produce type 4 seed. One of the reasons for such conservation might be the evolvability of seed types.

### Test of the model

To test the present model, circumstantial evidence should be collected from two lines of research: (1) whether the relative costs of overgrowth in the three absorption routes differ among taxa with different seed types, and (2) whether the advantage of having a large embryo relative to the endosperm differs among those taxa. (1) For the first purpose, cross experiments using plants with different ploidy levels would be helpful. In any seed types, the development of the basal endosperm transfer layer (chalazal endosperm) and the embryo-surrounding region in the endosperm (micropylar endosperm) could be observed. Then, the cost of overgrowth could be compared between these organs (i.e. comparison of  $c_{FN}$  and  $c_{NM}$ ). The cost of overgrowth in the direct absorption of resources by the embryo from the female parent ( $c_{FM}$ ) was observed only for type 4 seed. However, it can at least be examined whether  $c_{FM}$  is smaller than  $c_{FN}$  and  $c_{NM}$  in type 4 seed. (2) The advantage of having a large embryo should be examined using variations in the relative size of the embryo within taxa with the same seed type. That is, seeds with a higher embryo ratio should be advantageous within taxa with type 3 seed. On the other hand, such a trend would not be observed within taxa with type 1 seed if  $c_{NM}$  were low relative to  $c_{FN}$  in those taxa.

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## APPENDIX

### Growth of an embryo and an endosperm

I assume that the endosperm starts to grow at  $t = 0$  and that the embryo, if it grows during development of the fertilized ovule, starts to grow at  $t = t_1$  ( $t_1 \geq 0$ ); there may be a time lag in their initiation. If the resources in the endosperm are completely absorbed by the embryo (say at  $t = t_2$ ), the endosperm disappears and the embryo absorbs resources directly from the female parent after that. Seed growth is completed when the resources of the female parent become exhausted (at  $t = T$ ). Here,  $T$  and  $t_2$  are not model parameters but they follow from the dynamics of resource absorption, whereas  $t_1$  is a model parameter.

The growth of the endosperm is expressed by

$$s_{\text{EN}}(0) = s_{\text{EN}0},$$

$$\frac{ds_{\text{EN}}}{dt} = \frac{2FN_M + FN_P}{3} \text{ for } t < t_1,$$

$$\frac{ds_{\text{EN}}}{dt} = \frac{2FN_M + FN_P}{3} - \frac{2NM_M + NM_P}{3} \text{ for } t_1 \leq t,$$

where  $s_{\text{EN}0}$  and  $s_{\text{EN}}(t)$  are the initial size of one endosperm and the size of that endosperm at time  $t$ , respectively. For the sake of simplicity, I assume that those absorption rates do not change with the sizes of the endosperm and the embryo, although it may be limited by the sink-strength of the offspring if its size is small (see Sakai and Harada, 2001). If  $s_{\text{EN}}(t_2) = 0$  holds for  $t_2 \leq T$ , the endosperm disappears before or at the time of seed completion  $T$ . Otherwise, the endosperm does not disappear and continues to grow until  $T$ .

The growth of the embryo is expressed by

$$s_{\text{EB}}(t_1) = s_{\text{EB}0},$$

$$\frac{ds_{\text{EB}}}{dt} = \frac{2NM_M + NM_P}{3} \text{ for } t_1 \leq t < t_2,$$

$$\frac{ds_{\text{EB}}}{dt} = \frac{FM_M + FM_P}{2} \text{ for } t_2 \leq t \leq T,$$

if the endosperm disappears, and by

$$s_{\text{EB}}(t_1) = s_{\text{EB}0},$$

$$\frac{ds_{\text{EB}}}{dt} = \frac{2NM_M + NM_P}{3} \text{ for } t_1 \leq t \leq T,$$

if the endosperm does not disappear, where  $s_{\text{EB}0}$  and  $s_{\text{EB}}(t)$  are the initial size of one embryo and the size of that embryo at time  $t$ , respectively.

### Competition for resource absorption

The competition for absorption of resources is described as follows. At the beginning,  $(s_{\text{EN}0} + s_{\text{EB}0})N$  resources are lost from the  $R_0$  resources to produce  $N$  ovules with an initial size of  $s_{\text{EN}0} + s_{\text{EB}0}$ . Then, for the sake of simplicity, I assume that abortion of ovules occurs immediately after fertilization;  $s_{\text{EN}0} + s_{\text{EB}0}$  resources are lost per aborted ovule, but no further resources are lost through abortion. Subsequently, the remaining  $R_0 - (s_{\text{EN}0} + s_{\text{EB}0})N$  resources decrease via absorption by the endosperms or embryos, where its rate of decrease differs between the ovules produced by a female parent having mutated genes and those fertilized by a male parent having mutated genes. The rates of decrease also differ depending on whether the endosperms of the mutant ovules (which contain mutated genes) and those of the wild-type ovules exist or not.

**Case 1: in ovules produced by a female parent having mutated genes**

When endosperms exist both in the mutant and wild-type ovules,

$$\begin{aligned} \frac{dR_1}{dt} = & \left[ \frac{1}{2} h(FN'_M, NM'_M, FM'_M, FN_P, NM_P, FM_P) \frac{2FN'_M + FN_P}{3} \right. \\ & \left. + \frac{1}{2} h(FN_M, NM_M, FM_M, FN_P, NM_P, FM_P) \frac{2FN_M + FN_P}{3} \right] N. \end{aligned}$$

When endosperms do not exist in the mutant ovules but exist in the wild-type ovules,

$$\begin{aligned} \frac{dR_2}{dt} = & \left[ \frac{1}{2} h(FN'_M, NM'_M, FM'_M, FN_P, NM_P, FM_P) \frac{FM'_M + FM_P}{2} \right. \\ & \left. + \frac{1}{2} h(FN_M, NM_M, FM_M, FN_P, NM_P, FM_P) \frac{2FN_M + FN_P}{3} \right] N. \end{aligned}$$

When endosperms exist in the mutant ovules but not in the wild-type ovules,

$$\begin{aligned} \frac{dR_3}{dt} = & \left[ \frac{1}{2} h(FN'_M, NM'_M, FM'_M, FN_P, NM_P, FM_P) \frac{2FN'_M + FN_P}{3} \right. \\ & \left. + \frac{1}{2} h(FN_M, NM_M, FM_M, FN_P, NM_P, FM_P) \frac{FM_M + FM_P}{2} \right] N. \end{aligned}$$

When endosperms do not exist either in the mutant or the wild-type ovules,

$$\begin{aligned} \frac{dR_4}{dt} = & \left[ \frac{1}{2} h(FN'_M, NM'_M, FM'_M, FN_P, NM_P, FM_P) \frac{FM'_M + FM_P}{2} \right. \\ & \left. + \frac{1}{2} h(FN_M, NM_M, FM_M, FN_P, NM_P, FM_P) \frac{FM_M + FM_P}{2} \right] N. \end{aligned}$$

**Case 2: in ovules fertilized by a male parent having mutated genes**

When endosperms exist both in the mutant and wild-type ovules,

$$\begin{aligned} \frac{dR_1}{dt} = & \left[ \frac{k}{2} h(FN_M, NM_M, FM_M, FN'_P, NM'_P, FM'_P) \frac{2FN_M + FN'_P}{3} \right. \\ & \left. + \left(1 - \frac{k}{2}\right) h(FN_M, NM_M, FM_M, FN_P, NM_P, FM_P) \frac{2FN_M + FN_P}{3} \right] N. \end{aligned}$$

When endosperms do not exist in the mutant ovules but exist in the wild-type ovules,

$$\begin{aligned} \frac{dR_2}{dt} = & \left[ \frac{k}{2} h(FN_M, NM_M, FM_M, FN'_P, NM'_P, FM'_P) \frac{FM_M + FM'_P}{2} \right. \\ & \left. + \left(1 - \frac{k}{2}\right) h(FN_M, NM_M, FM_M, FN_P, NM_P, FM_P) \frac{2FN_M + FN_P}{3} \right] N. \end{aligned}$$



When endosperms exist in the mutant ovules but not in the wild-type ovules,

$$\frac{dR_3}{dt} = \left[ \frac{k}{2} h(FN_M, NM_M, FM_M, FN'_P, NM'_P, FM'_P) \frac{2FN_M + FN'_P}{3} + \left( 1 - \frac{k}{2} \right) h(FN_M, NM_M, FM_M, FN_P, NM_P, FM_P) \frac{FM_M + FM_P}{2} \right] N.$$

When endosperms do not exist in either the mutant or the wild-type ovules,

$$\frac{dR_4}{dt} = \left[ \frac{k}{2} h(FN_M, NM_M, FM_M, FN'_P, NM'_P, FM'_P) \frac{FM_M + FM'_P}{2} + \left( 1 - \frac{k}{2} \right) h(FN_M, NM_M, FM_M, FN_P, NM_P, FM_P) \frac{FM_M + FM_P}{2} \right] N.$$

In both cases 1 and 2, there are five possible patterns in the appearance of these rates of decrease:

1.  $R_1(T) = 0$  holds [ $R_2(t)$ ,  $R_3(t)$ , and  $R_4(t)$  do not appear] if the endosperm does not disappear in either the mutant or the wild-type ovules.
2.  $R_1(t'_2) = R_2(t'_2)$  and  $R_2(T) = 0$  hold [ $R_3(t)$  and  $R_4(t)$  do not appear] if the endosperm disappears in the mutant ovule but not in the wild-type ovule, where  $t'_2$  is the time when the endosperms disappear in the mutant ovules.
3.  $R_1(t_2) = R_3(t_2)$  and  $R_3(T) = 0$  hold [ $R_2(t)$  and  $R_4(t)$  do not appear] if the endosperm disappears in the wild-type ovule but not in the mutant ovule.
4.  $R_1(t'_2) = R_2(t'_2)$ ,  $R_2(t_2) = R_4(t_2)$ , and  $R_4(T) = 0$  hold [ $R_3(t)$  does not appear] if the endosperm disappears both in the mutant and wild-type ovules, where the endosperm of the former disappears earlier.
5.  $R_1(t_2) = R_3(t_2)$ ,  $R_3(t'_2) = R_4(t'_2)$ , and  $R_4(T) = 0$  hold [ $R_2(t)$  does not appear] if the endosperm disappears both in the mutant and wild-type ovules, where the endosperm of the latter disappears earlier.

For all patterns, ovule growth ceases when the reserved resources are depleted (at  $t = T$ ) and the final sizes of the endosperm  $S_{EN}$  and the embryo  $S_{EB}$  are given by  $s_{EN}(T)$  and  $s_{EB}(T)$ , respectively.

