

Lethals can be estimated from doubled haploid *Larix decidua* female gametophytes

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Abstract

We present a novel way of estimating embryo lethals using doubled haploid (dihaploid or DH) female gametophytes grown in tissue culture. This DH-based lethals estimate is hypothesized to be *lower* than the more common method where lethals are estimated from one generation of selfing. The chief reasoning is the early mortality spike observed for selfed embryos of larch and other genera in the Pinaceae. This early mortality spike occurs at the stage when diploid embryos embed into haploid female gametophyte tissue. However, this spike stage is missing for DH embryos which grow in a petri dish thus the DH lethals estimate is hypothesized to be lower compared to selfed seed counts. This DH lethals estimate is based on 20,000 haploid female gametophytes cultures from which two surviving diploidized embryos were recovered. Lethals based on diploidized haploids have not been reported for a conifer previously because, aside from larch, only a few conifer species continue female gametophyte development in absence of pollen. Our chief finding is that, contrary to our hypothesis, the DH-based lethals estimate was similar to lethals estimated obtained from selfed seeds in published studies. Thus the doubled-haploid female gametophyte method is novel method for estimating embryo lethals in conifer species where diploidization can be conducted on a massive scale.

Keywords

Conifers; Deleterious alleles; Mutation load; Gametophytes; Diplohaplontic life cycle; Selfing

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1 Introduction

Creating dihaploid genotypes offer a novel approach to estimating lethals in conifers which are a well-studied model for embryo lethal systems (Figure 1). A doubled haploid female gametophyte becomes a homozygous embryo only if no lethals are present in its haplotype. Thus the proportion of dihaploid embryos surviving the diploidization process provides a direct estimate of lethals and this estimate is defined here as LE-di. This contrasts with the more common approach of estimating lethals from one generation of selfed seeds from an outbred adult, defined here as LE-self (i.e. Sorensen 1969, Williams and Savolainen 1995; Williams et al. 1998).

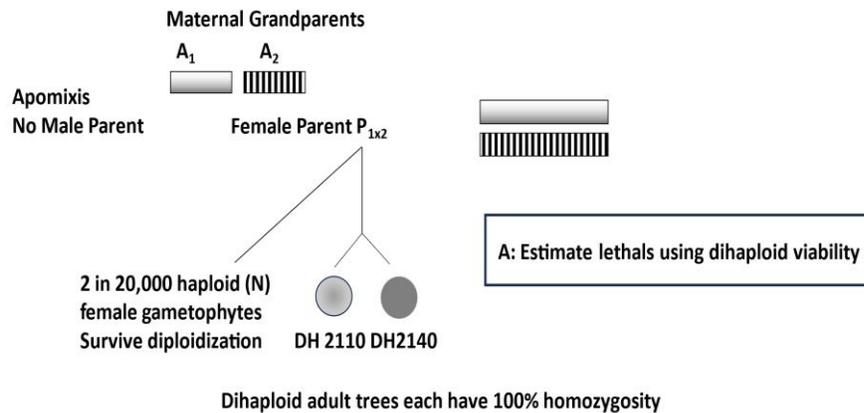


Figure 1. Schematic diagram of pedigree and its female haploid gametophytes used to estimate lethals from dihaploid *Larix decidua* tissue cultures.

To date, *Larix decidua* adults are reported to carry up to 10 lethal factors based on one generation of selfing (Table 2; Kosinski 1982, 1987; Slobodnik and Guttenberger 2005). Similarly, 10.3 lethal factors were reported for *Larix laricina* (Park and Fowler 1982).

Lethals estimated from dihaploids (LE-di) are hypothesized to be lower than these estimates obtained from selfed seeds (LE-self). As shown in Figure 2, our reasoning is that selfed seeds in larch and other Pinaceae genera typically show a sharp mortality spike at the early embryogeny stage during seed development. During this spike stage, a single dominant embryo embeds into its female gametophyte, making contact between the fertilized embryo and its female gametophyte in pines (Koski 1971; Williams 2008) and in other conifers, including larch (Owens and Blake 1985). Death to selfed embryos at this early embryogeny stage produces an empty seed coat, not a replacement embryo. Selfed seeds typically produce high proportions of empty seeds at this and other stages too. Empty seed counts are the basis for lethals estimation when using selfed seeds.

The stage-specific haplotype interaction has been hypothesized here to cause the stage-specific mortality spike typically observed during selfed seed development (i.e. Koski 1971; Williams 2008). The interaction between an egg cell and its female gametophyte can be traced to the male haplotype from the fertilizing pollen grain (Figure 2). Note too that the embryo and female gametophyte inside a seed share a genetically identical female haplotype. What is different is the male haplotype contributed by the pollen grain and this holds true even if the embryo is self-pollinated.

multiple embryos, or polyembryony. The fertilized larch seed may initially have multiple embryos although by the early embryogeny stage, only one embryo is present in the developing seed (Owens and Molder 1975).

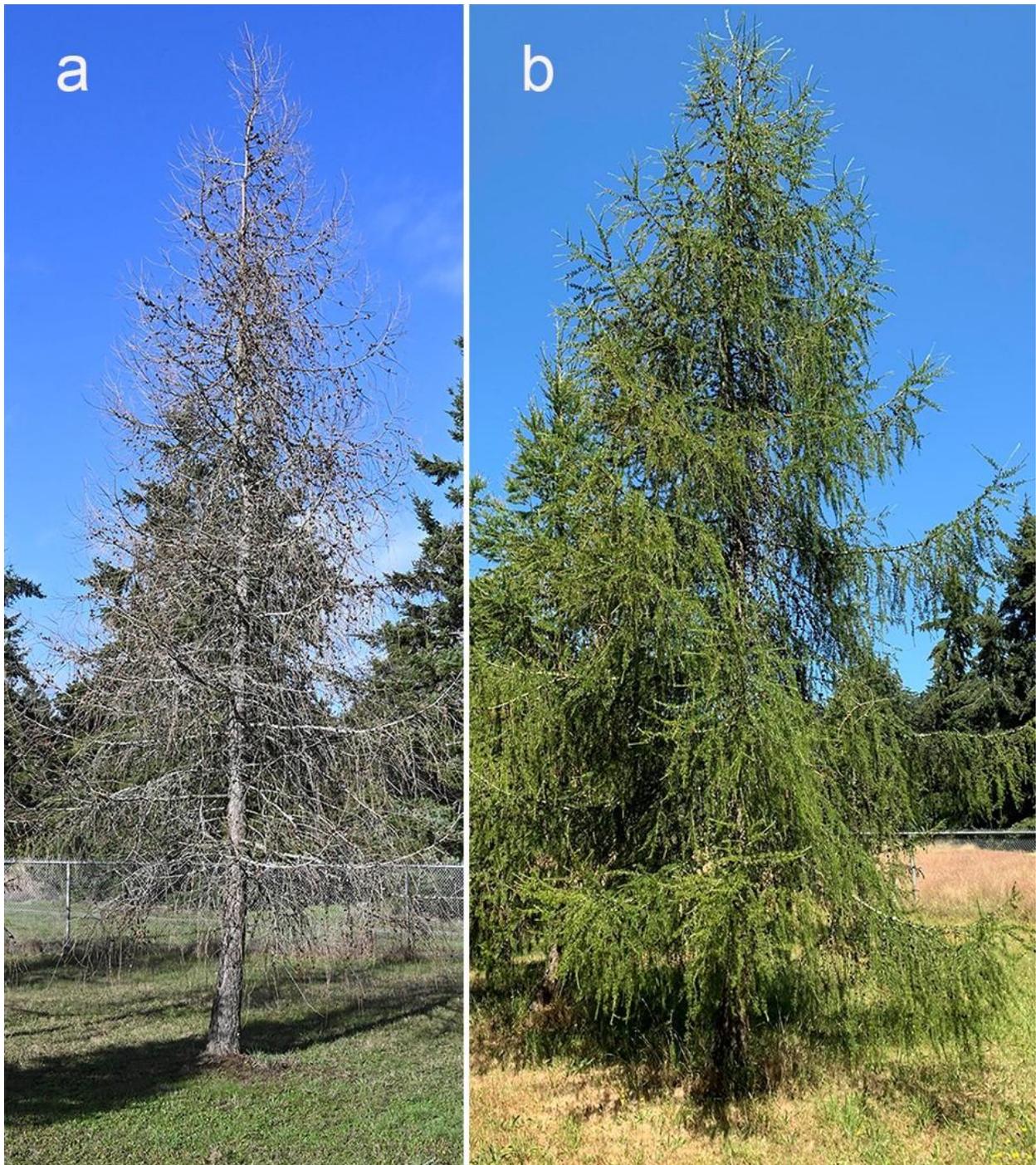


Figure 3. Photographs of the apomict survivor from a doubled female gametophyte. Note that this apomict tree has cones in early March (a) and that the same apomict tree in late July has a healthy crown (b). Tissue culture was conducted at University of New Brunswick, Fredericton N.B. Canada. Column width is 80 mm and the pixel density is 300 dpi

Few conifer species are capable of developing a haploid female gametophyte in the absence of a pollen grain. Those with this capacity include *Picea abies* (Simola and Santanen 1990) and *Larix decidua* (Nagmani and Bonga 1985). In some cases, diploidization of a female gametophyte's cells is spontaneous in culture. The resulting dihaploid (DH) embryos divide via cleavage polyembryony (von Aderkas and Anderson 1993) then develop into reproductively competent adult trees capable of reproduction and normal meiosis (von Aderkas and Williams 2025).

Larch is capable of apomixis via agamospermy, or asexual formation of embryos without fertilization (Stebbins 1950). Dihaploid (DH) larch embryos are apomicts which only have a female parent and no male parent (Figure 2). Note however that the organellar inheritance of a dihaploid larch has the same biparental pattern as a fertilized embryo. Both have male-derived chloroplast organelles and female-derived mitochondrial organelles.

BOX 1. Genomic model for dihaploid (DH) embryos. Each DH embryo is 100% homozygous and has the same two alleles per locus across all loci. For each locus, only A_1A_1 or A_2A_2 genotypic classes are present. No A_1A_2 genotypic classes are present.

What is distinctive is the haplotype complement of a DH embryo. Each DH embryo has only two copies of the exact same female haplotype (Box 1). By contrast, a developing seed has one male haplotype plus two copies of the exact same female haplotype. Only in the DH embryo can we expect the absence of sexual conflict between male and female haplotypes (Haig and Wilczek 2006).

3 Materials and methods

Cell lines 2110 and 2140 originated from different haploid female gametophytes produced by a single *L. decidua* tree in Fredericton New Brunswick Canada (von Aderkas and Bonga 1993). Spontaneous diploidization of single haploid cells occurred in culture.

Poisson estimation method for lethal estimation. A doubled haploid plant's lethals are all recessive homozygotes. The original parent's lethal number can be inferred as follows using the Poisson distribution. If the total number of factors carry deleterious alleles is n and the average allele frequency is p then sampling lethal factors approximates a Poisson distribution with mean and variance, pn .

For example, the model for four lethals is calculated as e^{-m} or $e^{-4} = 1.83 \times 10^{-2}$ where 1 out of 55 selfed offspring are predicted to have no lethals. Similarly, if the mean number of lethals (m) in the parent is 10.7 and the probability of selfed offspring which have zero (0) lethals then this can be expressed as e^{-m} or $e^{-10.7} = 2.25 \times 10^{-5}$ or 1 out of 44,356 offspring. The Poisson-based predictions are fitted against the experimental data from the dihaploid tissue culture results. The estimated lethal number for dihaploids (LE-dihaploid) was compared with mean lethals reported in selfing (LE-selfs) studies of *Larix decidua*.

4 Results

Lethals estimate of 10 was obtained from dihaploid larch. Surprisingly, this value is similar to LE-selfs (Tables 1-2). Only 1 in 20,000 dihaploids survived and these

two seedlings grew into sexually mature adults (von Aderkas and Williams 2025). This method fits the predictions for an adult plant which is heterozygous for 10 lethals.

Table 1. Poisson distribution method used for estimating lethals from dihaploid female gametophyte cultures of *Larix decidua*.

Lethals (LE-di) estimated from diploidized haploid female gametophytes	Required Numbers of Haploid Female Gametophyte Cultures (1/x)	Poisson value (x)
2	7	0.135335
3	20	0.049787
4	55	0.018316
5	148	0.006738
6	403	0.002479
7	1097	0.000912
8	2981	0.000335
9	8103	0.000123
10	22026	1 x 4.53999E-05
10.5	36316	1 x 2.75364E-05
10.7	44356	1 x 2.25449E-05

Table 2. Lethals estimation from one generation of selfed seeds from eight *Larix decidua* parents. Data are adapted from Kosinski (1982, 1987).

Genotype	Selfed	Wind-Pollinated	Lethals (LE-se) estimated from empty seeds
02-11	4.8	29.1	7
02-11	4.8	27.0	6
08-02	42.6	78.1	2
08-03	3.2	34.0	7
10-02	7.4	27.0	4
10-25	5.8	32.2	4
10-27	11.1	43.9	3
15-88	10.4	61.4	6
Mean	11.3	41.6	4.8

5 Discussion

This dihaploid adult tree shown in Figure 3 is the product of a doubled haploid female gametophyte. Its lethal estimate is similar to that from selfed seeds although the latter is thought to represent only a partial profile of the embryo viability loci. Note too that this DH method can have no contributions from a heterozygote advantage (e.g. Williams et al. 2001, 2003). Thus our findings here align with the mutation-selection model as the better explanation for embryo viability loci (Brandvain et al. 2023, 2024). We rejected our original hypothesis because this DH estimate is surprisingly similar to estimates reported from selfed seeds.

We also assume that all lethals are expressed solely by the embryo’s own genome (Sorensen 1974; Williams and Savolainen 1996). This seems likely in this case because *in vitro* culture of diploidized embryos lacks molecular crosstalk between a developing embryo and its female gametophyte in a developing seed.

Weaknesses of this experimental approach include the following: a) the exact same parent of DH embryos for the selfed seed comparison was not available and b) no

molecular marker genomic scan is available for the DH apomict (Figure 3) to verify its theoretical 100% homozygosity. These weaknesses could not be remedied because the DH apomict's parent is no longer alive and the DH apomict itself (Figure 3) was logged at age 34 years.

In closing, the lethals estimate of 10 was estimated using diploidized haploids larch, one of the few conifers where the haploid female gametophyte continues to develop without fertilization. This is the first reported dihaploid estimate for lethals for a conifer.

6 References

- von Aderkas P, Bonga JM (1993) Plants from haploid tissue culture of *Larix decidua*. *Theor Appl Genet* 87: 225-228. <https://doi.org/10.1007/BF00223768>
- von Aderkas P, Williams CG (2025) Gametophytic apomixis in a gymnosperm, *Larix decidua* Mill., results in normal male. *Reforesta* 19:50-58. <https://dx.doi.org/10.21750/REFOR.19.05.126>
- Bonga JM, von Aderkas P, James D (1988) Potential application of haploid cultures of tree species. In *Genetic Manipulation of Woody Plants* (JW Hanover, DE Kathley eds). Plenum New York, NY. https://doi.org/10.1007/978-1-4613-1661-9_4
- Brandvain Y, Harkness A, Pyhajarvi T (2023) Reproductive compensation and selection among viable embryos drive evolution of polyembryony. *Am Nat* 201(5): 694-711. doi: 10.1086/723454. <https://doi.org/10.1086/723454>
- Brandvain Y, Thomson L, Pylejarvi T (2024) Early-acting inbreeding depression can evolve as an inbreeding avoidance mechanism. *Proc R Soc B* 291: 20232467. <https://doi.org/10.1098/rspb.2023.2467>
- Chowdhury CR (1962) The embryology of conifers - a review. *Phytomorphology* 12: 313-338. <https://doi.org/10.1111/j.1467-1770.1962.tb01268.x>
- Haig D, Wilczek A (2006) Sexual conflict and the alternation of haploid and diploid generations. *Philos Trans Biol Sci* 36 (1466): 335-343. <https://doi.org/10.1098/rstb.2005.1794>
- Kosinski G (1982) Genetic load in empty seeds of European larch (*Larix decidua* Mill.). *Arboretum Kornickie, Rocznik Poland XXVI*: 231-236.
- Kosinski G (1987) Empty seed production in European larch (*Larix decidua* Mill.) *Forest Ecol Manage* 19: 241-246. [https://doi.org/10.1016/0378-1127\(87\)90033-8](https://doi.org/10.1016/0378-1127(87)90033-8)
- Nagmani R, Bonga JM (1985) Embryogenesis in sub-cultured callus of *Larix decidua*. *Can J Forest Res* 15: 1088-1091. <https://doi.org/10.1139/x85-177>
- Owens, J.N. and Blake, MD (1985) Forest tree seed production. Information Report-Petawawa National Forestry Institute. Canadian Forestry Service. PI-X-53.
- Owens JN, Molder M (1979) Sexual reproduction of *Larix occidentalis*. *Can J Bot* 57: 2673-2690. <https://doi.org/10.1139/b79-317>
- Park YS, Fowler DP (1982) Effects of inbreeding and genetic variances in a natural population of tamarack (*Larix laricina* (Du Roi) K Koch) in eastern Canada. *Silv Genet* 31(1):21-26.
- Pattanavibool R, von Aderkas P, Hanhjarvi A, Simola LK, Bonga JM (1995) Diploidization in megagametophyte-derived cultures of the gymnosperm *Larix decidua*. *Theor Appl Genet* 90: 671-674. <https://doi.org/10.1007/BF00222132>
- Simola LK, Santanen A (1990) Improvement of nutrient medium for growth and embryogenesis of megagametophyte and embryo callus lines of *Picea abies*. *Physiol Plantarum* 80: 27-35. <https://doi.org/10.1034/j.1399-3054.1990.800105.x>
- Singh H (1978) Embryology of gymnosperms. Gebrüder Borntraeger, Berlin. 302 p.
- Slobodnik B, Guttenberger H (2005) Zygotic embryogenesis and empty seed formation in European larch (*L. decidua* Mill.). *Ann Forest Sci* 62: 129-134. <https://doi.org/10.1051/forest:2005004>
- Snedecor GW, Cochran WG (1978) Statistical methods. Iowa State University Press, 6th ed. Ames, IA. 593 p.
- Sorensen F (1969) Embryonic lethals in Douglas-fir, *Pseudotsuga menziesii*. *Am Nat* 103: 389-398. <https://doi.org/10.1086/282609>

- Stebbins GL (1950) Variation and evolution in plants. Columbia Univ NY 643 p. <https://doi.org/10.7312/steb94536>
- Williams CG, Savolainen O (1996) Inbreeding depression in conifers. *Forest Sci* 42(1): 102-117. <https://doi.org/10.1093/forestscience/42.1.102>
- Williams CG, Barnes RB, Nyoka I (1999) Embryonic lethal load for a neotropical conifer, *Pinus patula* Schiede and Deppe. *J Hered* 90(3): 394-398. <https://doi.org/10.1093/jhered/90.3.394>
- Williams CG (2008) Selfed embryo death in *Pinus taeda*: a phenotypic profile. *New Phytol* 178: 210-222. <https://doi.org/10.1111/j.1469-8137.2007.02359.x>
- Williams CG (2007) Re-thinking the embryo lethal system within the Pinaceae. *Can J Bot* 85 (7): 667-677. <https://doi.org/10.1139/B07-056>
- Williams CG, Zhou Y, Hall SE (2001) A chromosomal region promoting outcrossing in a conifer. *Genetics* 159: 1283-12. <https://doi.org/10.1093/genetics/159.3.1283>
- Williams CG, Auckland LD, Reynolds MM, Leach KA (2003) Overdominant lethals as part of the conifer embryo lethal system. *Heredity* 91: 584-592. <https://doi.org/10.1038/sj.hdy.6800354>