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Pollen limitation and its effect on seed germination

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Abstract

In nature, fruit and seed production in many plants have been shown to be pollen limited. Pollen limitation is demonstrated when open-pollinated plants that are hand-supplemented (P_s) with outcross pollen produce more fruits and/or seeds than open-pollinated controls that are not hand-pollinated (P_o). There are three categories of results in such studies: $P_s > P_o$, $P_s = P_o$ and $P_s < P_o$, in which case pollen limitation indices are positive, zero and negative, respectively. In an index widely used to calculate pollen limitation, $1 - (P_o/P_s)$, the bounds for $P_s \geq P_o$ are 0 to + 1, whereas the bounds for $P_s < P_o$ are 0 to $-\infty$. The first aim of this review was to show how the pollen limitation index can be modified so that the bounds of $P_s < P_o$ are 0 and –1, whereupon the index gives equal weight to the best performer (P_s or P_o) and worst performer (P_s or P_o). In addition to seed quantity, pollen supplementation can affect seed quality, including germinability. Thus, our second aim was to summarize the results of studies that have also tested the effect of pollen limitation on seed germination. In short, the 30 case studies in 15 families, 16 genera and 18 species that we identified show that seed germination percentage increased, was not affected or decreased by pollen supplementation in 12, 11 and seven cases, respectively. The effect of pollen limitation on seed germination, which can be quite large, has not been considered in developing population growth models to determine the effect of pollen limitation on λ.

Introduction

Fruit/seed set in a high percentage of angiosperms is pollen limited (Burd, 1994; Larson and Barrett, 2000; Knight *et al.*, 2005). Pollen limitation occurs when plants that are open-pollinated plus hand-cross pollen supplemented (treatment) produce more fruits and seeds than those that are open-pollinated and not pollen supplemented (control). Ideally, *all flowers on the plant* should receive supplemental cross pollen (i.e. whole-plant supplementation) in order to avoid the problem of resource allocation to flowers on plants that are pollen supplemented from those that have not been supplemented. Furthermore, perennial plants should be monitored in successive years to determine if they incurred costs of reproduction, i.e. reduction in growth, survival and reproduction, which would indicate limitation of resources caused by pollen supplementation (Bierzychudek, 1981; Zimmerman and Pyke, 1988; Snow and Whigham, 1989; Primack and Hall, 1990; Ramsey, 1997; Ashman *et al.*, 2004). Pollen limitation includes both pollen quantity and quality (e.g. Byers, 1995; Ramsey and Vaughton, 2000; Ashman *et al.*, 2004; Colling *et al.*, 2004; Aizen and Harder, 2007; Castro *et al.*, 2008), which cannot be separated in pollen supplementation studies (Aizen and Harder, 2007). However, pollen limitation generally has referred to the *number* of fruits and/or seeds produced, and thus pollen quality limitation has not been measured (Zimmerman and Pyke, 1988).

The effects of pollen limitation (PL) on various plant life history traits (other than the number of fruits and seeds produced) have been measured in studies on pollen limitation (Knight *et al.*, 2006). Some of these studies have measured its effects on seed germination. The primary aims of this review are twofold: firstly to evaluate two indices used to measure pollen limitation, giving special attention to situations in which the value of PL is higher for open pollinated (P_o) than for open pollinated plus pollen-supplemented plants (P_s); and secondly to review the studies that have included the effect of pollen limitation on seed germination. The results of the second objective will provide some basis for an opinion about the effect of pollen limitation on population dynamics.

Pollen limitation indices

The following index often has been used to calculate pollen limitation (Larson and Barrett, 1999, 2000; Kasagi and Kudo, 2003; Asikainen and Mutikainen, 2005; Ward and Johnson, 2005; Lázaro and Traveset, 2006; Vanhoenacker *et al.*, 2006; Duan *et al.*, 2007; Merrett *et al.*, 2007; Robertson *et al.*, 2008; González-Varo *et al.*, 2009; Spigler and Chang, 2009;

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González-Varo and Traveset, 2010; Kelly *et al.*, 2007, 2010; Marten-Rodriguez and Fenster, 2010; Cursach and Rita, 2012; Delmas *et al.*, 2014; Suarez-Gonzalez and Good, 2014; Shabir *et al.*, 2015; Van Etten *et al.*, 2015):

$$PL = 1 - (P_o/P_s)[= (P_s - P_o)/P_s, \text{ see below}], \quad (1)$$

where P_o is fruit (or seed) set in open-pollinated and not pollen supplemented plants (control) and P_s is fruit (or seed) set in open-pollinated plus pollen supplemented plants (treatment). Using this index, there are three possible outcomes regarding fruit and seed production and other life history traits such as germination of seeds produced by control *vs* treatment plants: $P_s > P_o$, $P_s = P_o$ and $P_s < P_o$. As used in the often-cited paper by Larson and Barrett (2000), PL = 0.00 when there is no pollen limitation (i.e. $P_o/P_s = 1.00$ and 1.00–1.00 = 0.00) and 1.00 when fruit set with natural (open) pollination (P_o) is 0.00 (i.e. $P_o/P_s = 0.00$ and 1.00–0.00 = 1.00). However, although Larson and Barrett (2000) gave the index a lower bound of 0.00, the lower bound will be <0.00 (i.e. negative) when fruit/seed production is greater in natural (open) than in natural plus supplemental pollination [$P_o/P_s > 1.00$ and 1.00 – (>1.00) = <0.00)], i.e. when there is a negative effect of pollen supplementation on female reproduction (Campbell and Husband, 2007; Garcia-Camacho and Totland, 2009; Spigler and Chang, 2009). Thus, Larson and Barrett (2000) ignored $P_s < P_o$, equating it with $P_s = P_o$. Young and Young (1992) suggest several possible reasons why PL may be negative, including (1) pollen-supplemented flowers having a lower diversity of pollen donors (and thus pollen) than those that are open-pollinated; (2) negative effects of pollen tube growth in the style associated with high pollen density on the stigma; (3) removal of pollen from or damage to pollen-supplemented stigmas by pollen consumers; and (4) damage of stigma during supplemental pollinations.

Index (1) is appropriate for calculating pollen limitation when $P_s \geq P_o$ but not when $P_o > P_s$. Thus, whereas the bounds of the index are 0 to + 1 when $P_s \geq P_o$, they are 0 to $-\infty$ when $P_o > P_s$. In which case, the values are not an equal distance from 0 when the same highest fruit (or seed) set is achieved by best the producer (either P_s or P_o) and the same lowest fruit (or seed) set by the worst producer (either P_s or P_o). This is illustrated in the following example, in which the worst producer has a value of 75 and the best produced a value of 100.

(a) P_o (worst producer) = 75 and P_s (best producer) = 100

$$1 - (P_o/P_s) = 1 - (75/100) = +0.25$$

(b) P_o (best produced) = 100 and P_s (worst produced) = 75

$$1 - (P_o/P_s) = 1 - (100/75) = 1 - 1.33 = -0.33$$

A more meaningful way to compare pollen supplemented and open-pollinated plants is to use the following index, which gives equal weight to the best producer (P_s or P_o) and the worst producer (P_s or P_o):

$$PL = (P_s - P_o)/P_{max}\,[P_s \text{ or } P_o] \quad (2)$$

(a) P_o (worst producer) = 75 and P_s (best producer) = 100

$$(P_s - P_o)/P_s = (100 - 75)/100 = 25/100 = +0.25$$

(b) P_o (best producer) = 100 and P_s (worst producer) = 75

$$(P_s - P_o)/P_o = (75 - 100)/100 = -25/100 = -0.25$$

Index (2) will give the same answer as $PL = 1 - (P_o/P_s)$, when $P_o \leq P_s$ and the same as $PL = (P_s/P_o) - 1$, when $P_o > P_s$.

(a) $P_o \leq P_s$

$$PL = 1 - (P_o/P_s) = 1 - (75/100) = 1 - 0.75 = +0.25$$

(b) $P_o > P_s$

$$PL = (P_s/P_o) - 1 = (75/100) - 1 = 0.75 - 1.0 = -0.25$$

$$(\text{If } P_o = P_s, PL = 1 - (P_o/P_s) \text{ and } PL = (P_s/P_o) - 1 = 0.00.)$$

In addition to germination percentage, one may also wish to compare time for P_{sg} and P_{og} seeds to germinate or for seedlings to emerge (see Equation 7). In calculating time to germination or seedling emergence, such as number of days for a given percentage, e.g. 20% (t_{20}) or 50% (t_{50}) to do so (Bewley *et al.*, 2013; Soltani *et al.*, 2015), the equation to use is $1 - (P_{sg}/P_{og})$ when P_{sg} takes fewer days to germinate or emerge than P_{og} and $(P_{og}/P_{sg}) - 1$ when P_{og} takes fewer days, as illustrated below.

(a) P_{sg} (3 days) > P_{og} (4 days), i.e. pollen supplemented seeds germinate or emerge in fewer days than control seeds

$$1 - (P_{sg}/P_{og})$$

$$1 - (3/4) = +0.25$$

(b) P_{sg} (4 days) < P_{og} (3 days), i.e. control seeds germinate or emerge in fewer days than pollen supplemented seeds

$$(P_{og}/P_{sg}) - 1$$

$$(3/4) - 1 = -0.25$$

Whereas the time (t) to 50% germination or emergence of *viable* seeds is t_{50}, $1/t_{50}$ is germination rate for the 50th percentile (Joosen *et al.*, 2010; Bewley *et al.*, 2013), e.g. 1/3 days = 0.33 day^{-1}, which is faster than 1/4 days = 0.25 day^{-1}.

Another way by which pollen limitation has been calculated is the pollen percentage limitation (PPL) index (Jules and Rathcke, 1999; Castro *et al.*, 2008):

$$PPL = [100 \times (PS - C)]/PS, \quad (3)$$

where PS (=P_s) is fruit set in open-pollinated plus pollen supplemented plants and C (=P_o) is fruit set in open-pollinated plants

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that were not pollen supplemented (control). Moeller (2004), Campbell and Husband (2007), Eckert *et al.*, (2010), Moeller *et al.* (2012) and Hove *et al.* (2016) used a slightly modified version of Index (3), i.e. without the 100, and of Index (1) to calculate pollen limitation (PL):

$$PL = (P_s - P_o)/P_s \left[= 1 - \left(P_o/P_s\right)\right] \quad (4)$$

Using Indices (3) and (4) to calculate PL has the same problem as described above when PS (P_s) < C (P_o), i.e. equal weight is not given to the best [either PS (P_s) or C (P_o)] and worst [either PS (P_s) or C (P_o)] performer.

(a) C (worst performer) = 75 and PS (best performer) = 100

$$[100 \times (PS - C)]/PS = [100 \times (100 - 75)]/100 = 2500/100 = +25$$

(b) C (best performer) = 100 and PS (worst performer) = 75

$$[100 \times (PS - C)]/PS = [100 \times (75 - 100)]/75 = -2500/75 = -33$$

For Index (4), values would be 0.25 and −0.33, respectively.

The way to give equal weight to PS > C ($P_s > P_o$) (above 0) and C > PS ($P_o > P_s$) (below 0) is to put the highest of the two values (PS [P_s] or C [P_o]) in the denominator.

$$PPL = [100 \times (PS - C)]/[PS \text{ or } C]_{max} \quad (5)$$

$$PL = (P_s - P_o)/P_{max}\,[P_s \text{ or } P_o] \quad (6)$$

In which case, the range for PPL will be −100 to + 100 and for PL −1 to + 1.

Methods

We used the following index to measure the effect of pollen limitation on seed germination (PL_{germ}):

$$PL_{germ} = \left(P_{sg} - P_{og}\right)/\, P_{max}\left[P_{sg} \text{ or } P_{og}\right], \quad (7)$$

where P_{sg} is germination percentage of seeds from pollen supplemented plants, P_{og} is germination percentage of seeds from open pollinated (control) plants and P_{max} is the larger of the two values (P_{sg} or P_{og}). A positive value indicates that P_{sg} seeds germinated to a higher percentage and a negative value that P_{og} seeds germinated to a higher percentage. When $P_{sg} = P_{og}$, $PL_{germ} = 0.0$. The closer the value is to 1.0 (P_{sg}) the greater the benefit of pollen supplementation, and the closer the value is to −1.0 the greater the benefit of open pollination without pollen supplementation. We used three categories to compare germination of P_{sg} and P_{og} seeds: $P_{sg} > P_{og}$, $P_{sg} = P_{og}$ and $P_{sg} < P_{og}$. For assignment to $P_{sg} > P_{og}$, PL_{germ} had to be ≥0.10, and for assignment to $P_{sg} < P_{og}$, PL_{germ} had to be ≤ −0.10, i.e. −0.10 or more negative than −0.10. Thus, for PL_{germ} values between −0.10 and 0.10, $P_{sg} = P_{og}$.

A criticism of using this index to calculate/evaluate PL_{germ} is that for low germination percentages differences of a few percentage points between P_s and P_o can give high values (negative or positive) for PL_{germ} that may not be statistically significant. In any case, our results for PL_{germ} are based on the values for the three categories of PL_{germ} outlined above.

The results of a study by Dogterom *et al.* (2000) on the effects of pollen tetrad load on germination and on fruit and seed production in highbush blueberry cv. 'Bluecrop' (*Vaccinium corymbosum*, Ericaceae) are discussed in Appendix A. This particular study indicates, overall, that high pollen tetrad loads are advantageous for germination compared with low pollen tetrad loads. However, the study is not a pollen limitation study *per se*, primarily in that it does not compare P_s with P_o. There was no open pollination control (P_o). Thus, the results are not included in Table 2.

The results of a study by Gargano *et al.* (2017) on germination of seeds of the perennial herb *Dianthus balbisii* (Caryophyllaceae) produced via open-pollination *vs* hand-pollination over a forest-open habitat gradient at five levels of illuminance in southern Italy are briefly described in Appendix B. The authors indicate that illuminance along the ecotone gradient ranges from <500 klux to >4000 klux. However, it seems that the level of illuminance is lux rather than klux. Maximum illuminance of full sun (mid-day summer) in the middle latitudes is around 100 to 110 klux. We did not include the results of this study in Table 2, because the crossing scheme does not exactly fit the requirements for determining pollen limitation. That is, the hand-cross plants were castrated (anthers removed) before shedding pollen.

In Appendix C, we give the results of a study by Pico and Retana (2003) that included the effect of hand-cross pollination *vs* open-pollination on seed germination of the short-lived Mediterranean perennial herb *Lobularia maritima* (Brassicaceae), i.e. 'PL_{germ}' = $(P_{cross(g)} - P_{og})/P_{max}$. We did not include the results of this comparison in Table 2, because the hand-cross flowers were bagged and thus not also allowed to receive pollen by open pollination.

In Appendix D, we give the results of a study by Ramsey (1995) on hand-cross pollination *vs* open-pollination in January and March on seed germination in the wet heathland species *Blandfordia grandiflora* (Blandfordiaceae) of eastern Australia. We did not include this comparison in Table 2 for the same reason given for not including *Lobularia maritima* in the Table, i.e. hand-crossed flowers were bagged and thus not allowed to receive pollen by open pollination.

Results and Discussion

We are aware of a total of 18 species whose germination has been studied in relation to pollen supplementation. One of these species is a monocot, and 17 are eudicots. For the mesic deciduous forests perennial herbaceous monocot *Arisaema triphyllum* (Araceae), Parker (1987) compared seed germination in eight treatment combinations [2 cross types (pollen supplemented, open pollinated) × 2 seed parent vigour states (healthy, infected with fungal pathogen) × 2 pollen parent vigour states (healthy, infected with fungal pathogen)] = 8 case studies. In four of the eight case studies, $P_{sg} > P_{og}$ and in the other four $P_{sg} < P_{og}$ (Table 1). Lehtila and Syrjänen (1995) compared germination percentages of P_{sg} and P_{og} seeds of *Primula veris* (Primulaceae). P_{sg} seeds germinated to 10.1% and P_{og} seeds to 14.8%. Although these differences were not statistically significant, PL_{germ} was −0.32, which meets our criterion, i.e. ≤ −0.10, for $P_{sg} < P_{og}$. P_{sg} and P_{og} seeds of *Swertia perennis* (Gentianaceae) germinated to 34.24 and 40.83%, respectively ($P < 0.05$; Lienert and Fischer, 2004). Thus, PL_{germ} was −0.21, i.e. $P_{sg} < P_{og}$. The

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Table 1. Effect of pollen supplementation and parent plant fungal disease status on PL values $[(P_s - P_o)/P_{max}]$ of seed germination in *Arisaema triphyllum* (based on data in Table 4 of Parker, 1987)

	$(P_s)^c$	$(P_s)^d$	$(P_s)^e$	$(P_s)^f$
$(P_o)^a$	−0.65	−0.26	0.11	−0.32
$(P_o)^b$	−0.40	0.20	0.47	0.13

[a]Naturally (open) pollinated healthy seed parent; [b]naturally (open) pollinated infected seed parent; [c]healthy seed parent supplemented with cross pollen from healthy donor; [d]healthy seed parent supplemented with cross pollen from infected donor; [e]infected seed parent supplemented with cross pollen with healthy donor; [f]infected seed parent supplemented with cross pollen from infected donor.

authors concluded that lower quality of the hand-outcrossed pollen (one donor, but stigma surface pollen-saturated, mostly with pollen from the donor) than of pollen from open pollination (most likely from several donors) accounted for the higher germination of P_{og} seeds; and also for the fact that $P_{og} > P_{sg}$ (although not always statistically so) for several other components of fitness. One hundred and seventy-six achenes of P_{sg} plants of *Ranunculus acris* (Ranunculaceae) sown in field plots germinated (produced seedlings), but only 107 of P_{og} plants did so. Thus, $PL_{germ} = 0.39$, i.e. $P_{sg} > P_{og}$ (Hegland and Totland, 2007). Pollen supplementation did not increase the number of viable seeds per flower or the total number of seeds per plant in *R. acris*, but it did increase achene mass and percentage of sown seeds that germinated (produced seedlings). Thus, the authors suggested that pollen quality may be a stronger element of pollen limitation in this species than pollen quantity.

PL for germination percentage of seeds of *Silene douglasii* var. *oraria* (Caryophyllaceae), a narrow endemic of coastal grasslands of western Oregon, USA, was 0.38 and 0.43 at 15 and 40 days, respectively, after planting in a greenhouse, i.e. $P_{sg} > P_{og}$ (Brown and Kephart, 1999). In a study of the California serpentine endemic *Calystegia collina* (Convolvulaceae), germination percentage of scarified (to break physical dormancy) seeds of P_{sg} supplemented with nearby pollen, P_{sg} supplemented with far-off pollen and P_{og} did not differ (Wolf and Harrison, 2001). As germination percentages are not given, we cannot compute PL_{germ} values. Thus, based on lack of statistical differences among the three pollination categories, $P_{sg(near)} = P_{og}$ and $P_{sg(far)} = P_{og}$, i.e. no benefit of supplementation by either pollen from nearby or far-off plants. For *Petrocoptis viscosa* (Caryophyllaceae), endemic to crevices in limestone outcrops in the northwest of the Iberian Peninsula, pollen supplemented seeds germinated to 100% and open-pollinated control seeds to 94%. Days to germination for P_{sg} seeds was 8 and for P_{og} seeds was 11 (Navarro and Guitián, 2002). Thus, whereas PL_{germ} for germination percentage was 0.06 and that for days to germination was 0.27. Overall, then, $P_{sg} > P_{og}$. Scarified seeds of P_{sg} and P_{og} plants of the south Florida pine rocklands endemic species *Chamaecrista keyensis* (Fabaceae subfam. Caesalpinioideae) germinated to about 98 and 97%, respectively (Liu and Koptur, 2003). Thus, $PL_{germ} = 0.01$, i.e. $P_{sg} = P_{og}$. *Polemonium vanbruntiae* (Polemoniaceae) is a globally threatened rhizomatous herb endemic to wetlands from southern Quebec, Canada, to West Virginia, USA (extirpated from New Brunswick, Canada). Mean germination percentage of P_{sg} seeds of this species cold stratified at 4°C for 40 day was 23.7% and that of P_{og} seeds was 23.8% (Hill *et al.*, 2008); thus, $PL_{germ} = -0.004$, i.e. $P_{sg} = P_{og}$. There also was no difference in survival or seedling height/number of leaves from P_{sg} and P_{og} seeds of this species.

Eight of the 18 species are woody plants, i.e. shrubs or trees, and one is a tree-like cactus. In a pollen limitation study of two shrub species of *Salix* in northern Japan, PL_{germ} was 0.29 and 0.15 for seeds of *S. miyabeana* (Salicaceae) from site 1 in 1996 and 1997, respectively, and –0.05 at site 2 in 1997 (Tamura and Kudo, 2000). PL for germination of *S. sachalinensis* at site 1 in 1996 was 0.19. Although none of the four P_{sg} *vs* P_{og} germination percentages was statistically significant, three of them meet our criterion for $P_{sg} > P_{og}$. The other value of PL for germination, i.e. –0.05, agrees with the statistical test that $P_{sg} = P_{og}$. In the southwestern North American hot desert shrub *Flourensia cernua* (Asteraceae), P_{og} seeds germinated to 23% and P_{sg} seeds to 13%; thus, PL = –0.43 and $P_{sg} < P_{og}$ (Ferrer *et al.*, 2009). Germination for P_{sg} and P_{og} seeds of the South American shrub *Lycium cestroides* (Solanaceae) were only 2.7 and 2.5%, respectively (Aguilar and Bernadello, 2001). Thus, PL for germination was 0.07, which meets our criterion for $P_{sg} = P_{og}$, and also the percentages did not differ statistically. In general, seed germination for coastal populations of the tree-sized birches *Betula pendula* and *B. pubescens* (Betulaceae) were not pollen limited, whereas mountain populations were more pollen limited, especially *B. pubescens* (Holm, 1994). Thus, for *B. pendula* $P_{sg} = P_{og}$ and $P_{sg} > P_{og}$, respectively, and for *B. pubescens* $P_{sg} = P_{og}$ and $P_{sg} > P_{og}$, respectively. Seed germination of neither *Vaccinium myrtillus* (Ericaceae), a clonal heathland bog plant of the Northern Hemisphere, nor that of *Fuchsia perscandens* (Onagraceae), a gynodioecious species endemic to New Zealand, were pollen limited. Seeds of open-pollinated and pollen-supplemented flowers of *V. myrtillus* germinated to 42.0 and 44.4 %, respectively ($PL_{germ} = 0.054$, $P_{sg} = P_{og}$) (Jacquemart, 1997), and those of *F. perscandens* to 82.5 and 90.5% (Montgomery *et al.*, 2001), respectively ($PL_{germ} = 0.088$, $P_{sg} = P_{og}$). Seeds from open-pollinated flowers of the Sonoran Desert tree-like cactus *Lophocereus schottii* germinated to 87.0% and those of pollen-supplemented plants to 84.3%; (Holland et al., 2004), thus PL = –0.03 and $P_{sg} = P_{og}$.

Altogether, then, the 30 case studies of the effect of pollen limitation on seed germination of 18 species in 16 genera and one monocot and 14 eudicot families yielded the following results: $P_{sg} > P_{og}$, 12; $P_{sg} = P_{og}$, 11; and $P_{sg} < P_{og}$, 7 (Table 2). Thus, pollen supplementation had a positive or negative effect on 63.3% (19) of the 30 case studies. These mixed results of studies on the effect of pollen supplementation on seed germination are in general agreement with those on the effect of pollen competition, i.e. small *vs* large pollen load placed at same position on stigma or equal pollen loads placed on stigma at different distances from the ovules (Baskin and Baskin, 2015a), and pollen source, i.e. outcross *vs* self (Baskin and Baskin, 2015b), on this life history trait. That is, seeds resulting from pollen supplementation, pollen competition and outcrossing may or may not germinate better than those resulting from open pollination (not pollen supplemented), lack of pollen competition and selfing, respectively. Furthermore, seeds from chasmogamous flowers (open, potentially outcrossed) may or may not germinate better than those from cleistogamous flowers (closed, obligately selfed) (Baskin and Baskin, 2017). In general, then, these results seem to suggest that neither amount of pollen (pollen supplementation), quality of pollen (pollen competition) nor outcross *vs* selfing mating system has a definite influence on germination of the resulting seeds.

Plant populations are demographically dependent on seeds (Bond, 1994), and a species may or may not be seed- or microsite (= establishment)-limited. It would also seem that a decrease in

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Table 2. Comparison of germination of seeds produced by flowers that were pollen supplemented plus open-pollinated (P_{sg}) with that of seeds produced by flowers that were open-pollinated only (P_{og}) (number of case studies is shown in parentheses)

Family	Species	Germination	References
Araceae	*Arisaema triphyllum*	$P_{sg} > P_{og}$ (4)	Parker, 1987
		$P_{sg} < P_{og}$ (4)	
Asteraceae	*Fluorensia cernua*	$P_{sg} < P_{og}$ (1)	Ferrer *et al*., 2009
Betulaceae	*Betula pendula*	$P_{sg} > P_{og}$ (1)	Holm, 1994
		$P_{sg} = P_{og}$ (1)	
Betulaceae	*Betula pubescens*	$P_{sg} > P_{og}$ (1)	Holm, 1994
		$P_{sg} = P_{og}$ (1)	
Cactaceae	*Lophocereus schottii*	$P_{sg} = P_{og}$ (1)	Holland *et al*., 2004
Caryophyllaceae	*Petrocoptis viscosa*	$P_{sg} > P_{og}$ (1)	Navarro and Guitián, 2001
Caryophyllaceae	*Silene douglasii* var. *oraria*	$P_{sg} > P_{og}$ (1)	Brown and Kephart, 1999
Convolvulaceae	*Calystegia collina*	$P_{sg(near)} = P_{og}$ (1)	Wolf and Harrison, 2001
		$P_{sg(far)} = P_{og}$ (1)	
Ericaceae	*Vaccinium myrtillus*	$P_{sg} = P_{og}$ (1)	Jacquemart, 1997
Fabaceae	*Chamaecrista keyensis*	$P_{sg} = P_{og}$ (1)	Liu and Koptur, 2003
Gentianaceae	*Swertia perennis*	$P_{sg} < P_{og}$ (1)	Lienert and Fischer, 2004
Onagraceae	*Fuchsia perscandens*	$P_{sg} = P_{og}$ (1)	Montgomery *et al*., 2001
Polemoniaceae	*Polemonium vanbruntiae*	$P_{sg} = P_{og}$ (1)	Hill *et al*., 2008
Primulaceae	*Primula veris*	$P_{sg} < P_{og}$ (1)	Lehtila and Syrjänen, 1995
Ranunculaceae	*Ranunculus acris*	$P_{sg} > P_{og}$ (1)	Hegland and Totland, 2007
Salicaceae	*Salix miyabeana*	$P_{sg} > P_{og}$ (2)	Tamura and Kudo, 2000
		$P_{sg} = P_{og}$ (1)	
Salicaceae	*Salix sachalinensis*	$P_{sg} > P_{og}$ (1)	Tamura and Kudo, 2000
Solanaceae	*Lycium cestroides*	$P_{sg} = P_{og}$ (1)	Aguilar and Bernadello, 2010

Summary: $P_{sg} > P_{og}$, 12; $P_{sg} = P_{og}$, 11; $P_{sg} < P_{og}$, 7.

PL would matter (i.e. have an effect on population dynamics) only if a species is limited by seeds and not be microsites. Even then, and assuming that a decrease in PL translates into an increase in number of seedlings, the 'effective' size of the population (i.e. number of reproductive individuals) will not increase unless some of the seedlings survive to adulthood. So, whether pollen limitation matters depends on its role in population growth (λ, as discussed below) [see Clark *et al*. (2007) for a review and meta-analysis of seed limitation in plant populations].

Concluding remarks and recommendations

The indices $PL = 1 - (P_o/P_s)$ and $PPL = [100 \times (PS - C)]/PS$ are not the most appropriate ones for measuring pollen limitation when the value for P_o (or C) is larger than that of P_s (or PS). Thus, we recommend use of the following index for calculating pollen limitation: $(P_s - P_o)/P_{max}$. Studies on the effect of pollen limitation on the life history trait seed germination have yielded mixed results; thus, seeds from pollen supplemented plants may be greater than, equal to or less than those from open pollinated controls.

As pointed out by Baskin and Baskin (2015b) for studies on inbreeding depression, descriptions of procedures for germinating seeds in most of the 16 studies discussed in the present paper were incomplete/inappropriate for giving results that can be interpreted to the real world. Neither the study by Montgomery *et al.* (2001) on *Fuchsia perscandens* nor the one by Holland *et al.* (2004) on *Lophocereus schottii* included the light or temperature conditions under which the seeds were tested for germination. In which case, the studies cannot be repeated by others. In the study on *Lycium cestroides* by Aguilar and Bernadello (2001), seeds from open pollinated plants and open plus pollen supplemented germinated to only 2.5 and 2.7%, respectively. Assuming the non-germinated seeds were viable, the obvious reason for the very low germination percentages is that the seeds were dormant. No procedures were given for breaking dormancy or germinating the seeds. Seeds of *Chamaecrista keyensis* (Liu and Koptur, 2003) and *Calystegia collina* have physical dormancy, and in both studies dormancy was broken by scarification, which is an artificial (non-natural) way to overcome dormancy in seeds with water-impermeable seed coats (Baskin and Baskin, 2000; Zalamea *et al*., 2015). For germination of both species, $P_s = P_o$. However, one wonders if this would have been the case if dormancy had been broken naturally in the field or by a simulated natural dormancy breaking treatment such as high or fluctuating temperatures in the laboratory. In other words, did scarification equalize germination of P_s and P_o seeds that otherwise differed in ability to remain dormant/germinate under natural conditions? Two studies we judge to have been appropriately done are those on *Primula veris* by Lehtila and

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Syrjänen (1995) and on *Ranunculus acris* by Hegland and Totland (2007), who sowed seeds in the field and monitored germination (seedling recruitment).

The real significance of pollen limitation is how it affects population growth rate (λ), a global measure of fitness, but this has been measured in only a few studies. In these studies, an increase in fruit and seed production via pollen supplementation did (Bierzychudek, 1982; Parker, 1997; Price *et al.*, 2008; Law *et al.*, 2010) or did not (Ehrlén and Eriksson, 1995; Knight, 2004) translate into an increase in λ. In *Primula veris*, pollen supplementation had no effect on fruit weight and seed weight or λ (Garcia and Ehrlén, 2002). Pollen limitation appears to be influencing the demographics of *Brunsvigia radulosa* (Amaryllidaceae). Thus, with an increase in population size there was a significant decrease in PL and a significant increase in proportion of juveniles and also in number of non-predated seeds per plant (Ward and Johnson, 2005). Taken together, these correlations suggest that pollen supplementation is having an influence on the structure of the population. However, in none of the studies on population growth rate was the effect of pollen supplementation on the life history trait seed germination considered. Differences in germination between P_{sg} and P_{og} seeds were also not considered in a study by Jules and Rathcke (1999) on edge effects (resulting from fragmentation of old-growth forests) on recruitment of *Trillium ovatum* (Melanthiaceae). However, as pollen limitation values in the 16 studies on seed germination included in our opinion paper (Table 2) ranged from −0.65 to +0.47 (−65% to +47%) for this life history trait, it seems reasonable that pollen limitation for germination of P_{sg} and P_{og} seeds could have a significant effect on λ for some species.

The results of a field-sowing experiment on germination of achenes of *Ranunculus acris* by Hegland and Totland (2007) suggests that P_{sg} and P_{og} seeds can influence population dynamics differently, albeit via seed size. One-hundred and seventy-six seedlings/juveniles appeared in field plots in 2004 into which three densities of P_{sg} achenes had been sown in 2003, whereas only 107 appeared in plots into which three densities of P_{og} achenes had been sown. Thus, germination of the heavier (by 18%) P_{sg} achenes was 64% higher than that of the P_{og} achenes. Only four seedlings/juveniles were found in background plots in which the contribution from the persistent seed bank was monitored. In 2005, there were about 10% more vegetative individuals in the plots into which P_{sg} achenes had been sown than in those into which P_{og} achenes had been sown, but the difference was not significant. As the quantity of achenes produced by pollen supplemented and control plants did not differ, the authors concluded that '… pollen limitation may affect germination through seed quality [seed size], and thereby affect population dynamics'.

A long-term study by Van Etten *et al.* (2015) in New Zealand illustrates the magnitude of the negative effects of pollinator/pollen limitation can potentially have on a long-lived tree species. Pollen limitation in *Sophora microphylla* (Fabaceae) is due to reduction in native bird pollinators caused by human impact on the landscape. Consequently, reduction of pollen transfer between individuals of the species (outcrossing), together with a high selfing rate and high inbreeding depression, is having a dramatic negative impact on quality of the progeny of this self-compatible New Zealand endemic (also see Robertson *et al.*, 2011).Van Etten *et al.* (2015) stated that the high level of low quality of progeny '… could lead to cryptic recruitment failure, i.e. a decline in successful reproduction in spite of high progeny production'.

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Appendix A. Effect of outcross pollen load on seed germination in *Vaccinium corymbosum*

The study by Dogterom *et al.* (2000) includes results on the effect of a number of outcross pollen tetrads on germination. Ten, 25, 125 and 300 pollen tetrads were added to the stigmas of *Vaccinium corymbosum* cv. 'Bluecrop' plants and germination percentages and days to germination determined for the viable (large) seed progeny produced, which overall was 85%. Small and flat seeds were not viable.

We used the following index to compare all combinations of the four pollen loads on seed germination percentage:

$$P_{q(germ)} = (P_{s+} - P_{s-}/P_{max}\,[P_{s+} \text{ or } P_{s-}], \quad (A1)$$

where $P_{q(germ)}$ is the effect of pollen (P) quantity (q) on germination percentage (germ), P_{s+} is germination percentage at the highest pollen load in the two-load comparison, and P_{s-} is germination percentage at the lowest pollen load.

We compared all possible two-load combinations of the four pollen tetrad loads on days to germination (a) when P_{s+} resulted in the fastest germination (i.e. fewer days to germinate):

$$P_{q(germ)} = 1 - (P_{s+}/P_{s-}), \quad (A2)$$

and (b) when P_{s-} resulted in the fastest germination:

$$P_{q(germ)} = (P_{s+}/P_{s-}) - 1. \quad (A3)$$

In both indices, notations are the same as those in (A1).

Three categories ($P_{s-} < P_{s+}$, $P_{s-} = P_{s+}$, $P_{s-} > P_{s+}$) of the effect of pollen tetrad load on both germination percentage and days to germination were defined as described in the 'Methods' section of the present paper. The results are as follows: (a) germination percentage: $P_{s-} < P_{s+}$, 4; $P_{s-} = P_{s+}$, 2; and $P_{s-} > P_{s+}$, 0; and (b) days to germination: $P_{s-} < P_{s+}$, 1; $P_{s-} = P_{s+}$, 3; and $P_{s-} > P_{s+}$, 2. Thus, in five of the 12 two-load comparisons, seeds produced with the highest pollen tetrad load germinated best [$P_{q(germ)} \geq 0.10$]; in five of 12 comparisons seeds produced with the highest and lowest pollen tetrad load germinated equally well [$P_{q(germ)}$ between –0.10 and 0.10]; and in two of the 12 comparisons seeds produced with the lowest pollen tetrad load germinated best [$P_{q(germ)} \leq -0.10$].

Based on statistical analysis, Dogterom *et al.* (2000) showed that pollen tetrad load size had no effect on number of days to germination, whereas seeds produced from 125 and 300 tetrads germinated to significantly higher percentages than those produced by 10 tetrads. Seeds produced from 25 tetrads germinated to a slightly higher percentage (*ca* 81) than those produced with 10 tetrads (*ca* 75), but the differences were not statistically significant.

Overall, the study by Dogterom *et al.* (2000) suggests that pollen tetrad loads of 125 per stigma enhanced seed germination in highbush blueberry. The authors stated that, '… these data do suggest that pollen transfer is related to at least one fitness characteristic of highbush blueberry, percentage germination'.

Appendix B. Germination of *Dianthus balbisii* seeds produced by open-pollinated and castrated, hand-crossed flowers

The study by Gargano *et al.* (2017) described in 'Methods' compared the germination percentages of seeds of open-pollinated and castrated, hand-crossed flowers of *Dianthus balbisii* at five levels of illuminance along a forest-to-open vegetation ecotone. We used the same Index (7) and criteria for comparing germination of P_{sg} and P_{og} but with P_{sg} meaning seeds derived from castrated, hand-crossed flowers.

In three of the five comparisons, germination of seeds from hand-crossed pollination was greater than that of seeds from open-pollination, and in two of the five comparisons germination of seeds from open-pollination was equal to germination of seeds from hand-crossed pollination. It is interesting that even though only outcross pollen was used to fertilize the ovules in hand-pollinated plants, in two of the five levels of illuminance seeds from open-pollinated plants (probably a mix of self and outcross pollen) germinated equally as well as those from hand-crossed flowers.

Appendix C. Germination of seeds of *Lobularia maritima* produced by open-pollinated and bagged, hand-cross flowers

Germination of seeds produced by open-pollinated flowers was 94.9% and that of seeds produced by bagged, hand-crossed flowers was 97.9% (Pico and Retana, 2003), i.e. 'PL_{germ}' was 0.03 and thus not different.

Appendix D. Germination of seeds of *Blandfordia grandiflora* produced by open-pollinated and bagged, hand-crossed flowers

The extent of 'pollen limitation' (seed set) was much greater for plants that flowered in March (autumn) than it was for those that flowered in January (summer). However, there was no difference in germination (or seedling growth and survival) between January- and March-produced seeds (Ramsey, 1995).

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