

## Commentary

### Individual pollen limitation, phylogeny and selection

‘As natural selection acts by competition . . .’  
(Darwin, 1859, Ch. 14, p. 472)

Natural selection requires that some individuals perform better than others and is, therefore, conceptually closely related to the idea of competition. Darwin’s (1859) argument for evolution by natural selection makes frequent reference to competition and how its severity will vary geographically or with the players involved. Darwin also explicitly identifies competition as the cause of selection, as in the quotation above and elsewhere (e.g. Darwin, 1859, p. 205). Intuition thus suggests that increased competition should cause stronger selection (Benkman, 2013; Vanhoenacker *et al.*, 2013). Competition for mates appears nearly ubiquitous in sexually reproducing organisms and in animals is often stronger among males than females (Bateman, 1948). In seed plants, however, female success can be limited by pollen receipt (Knight *et al.*, 2005), indicating competition for mates via both female and male function. Pollen limitation of seed production has now been documented in hundreds of populations, and its severity varies over both time and space (Knight *et al.*, 2005). The natural link between competition and selection led to the proposal that greater pollen limitation should lead to stronger selection acting through female function (Johnston, 1991a,b). A meta-analysis using each of 21 species as a data point supports the hypothesis (Bartkowska & Johnston, 2015). By contrast, the very few studies comparing populations within species find that pollen limitation often fails to predict the strength of selection (Sletvold & Ågren, 2014; Bartkowska & Johnston, 2015). In this issue of *New Phytologist*, Trunschke *et al.* (pp. 1381–1389) take the phylogenetic middle ground and study selection and pollen limitation in 12 orchid species. A very nice feature of their study is that half of the species are rewarding and half are pollinated by deceit. Deceit-pollinated orchids are famously pollen limited. Pollinator-mediated selection (PMS) – that portion of selection caused by pollinators – should be most affected by pollen limitation, while other sources of selection should be less affected. The authors measure PMS as  $\Delta\beta_{\text{poll}}$ , the difference in selection gradients between naturally pollinated and fully hand-pollinated plants (Sandring & Ågren, 2009). Their overall findings support the account of increased selection with increased pollen limitation in two ways. First, the opportunity for selection, PMS and net (PMS + non-PMS) selection all increase with pollen limitation. Second, the increase in net selection with

pollen limitation is probably the result of elevated PMS because non-PMS does not increase. Here we present two ideas that might further elucidate the relation between pollen limitation and selection.

*‘A very nice feature of their study is that half of the species are rewarding and half are pollinated by deceit. Deceit-pollinated orchids are famously pollen limited.’*

#### Does selection act to reduce individual pollen limitation?

As conventionally measured, pollen limitation is a population property based on the average seed production,  $\bar{W}$ , of naturally (nat) and fully hand-pollinated (hp) plants and is quantified as  $PL = 1 - \bar{W}_{\text{nat}}/\bar{W}_{\text{hp}}$ . Pollen limitation thus describes the mean proportional reduction in seed number while selection describes the relationship between individual phenotype and seed number. Pollen limitation might influence selection for several reasons. First, increased pollen limitation might increase the variance in relative fitness, also known as the opportunity for selection, which sets an upper limit on selection (Arnold & Wade, 1984; Benkman, 2013). This is expected when, for example, reduced pollinator service causes a few individuals to receive the majority of visits. Second, as already suggested, pollen limitation might alter the relationship between phenotype and relative seed production (Johnston, 1991a,b). Note that a change in pollen limitation will have no influence on selection when the proportional fertility change is equivalent for all individuals (Ashman & Morgan, 2004). Selection will be altered only when pollen limitation changes the relation between phenotype and relative fitness.

The conventional measure of pollen limitation obscures the fact that individuals might vary in the degree to which they fall short of maximal seed production. This maximum is presumably established during development of the parent plant from its own beginning through the current season’s seed maturation. One can define individual pollen limitation, IPL, as

$$IPL(z) = 1 - \frac{W_{\text{nat}}(z)}{W_{\text{hp}}(z)},$$

where seed number  $W$  is a function of  $z$ , a vector of one or more traits. It will generally be difficult or impossible to measure IPL experimentally because of the requirement that all flowers be both

This article is a Commentary on Trunschke *et al.*, 214: 1381–1389.

naturally and hand-pollinated. Use of clonal replicates will be useful for measuring IPL of genotypes, but individuals within a clone will still differ in environmental conditions. One way around these methodological roadblocks is to estimate IPL from the ratio of expected seed numbers from the two treatments in any pollen-limitation experiment, where these expected numbers are obtained from fitted functions. These functions could allow any number of variables, curvature, etc., and should avoid overfitting. Most simply, using linear regression to estimate absolute (untransformed) seed number from phenotype (with zero mean here and throughout), we have

$$\widehat{\text{IPL}}(z) = 1 - \frac{\overline{W}_{\text{nat}} + b_{\text{nat}}z}{\overline{W}_{\text{hp}} + b_{\text{hp}}z},$$

where  $\widehat{\text{IPL}}(z)$  is an estimator of  $\text{IPL}(z)$  and  $b_{\text{nat}}$  and  $b_{\text{hp}}$  are regression coefficients from natural and hand-pollinated plants, respectively.

Does selection act to reduce IPL? Selection gradients  $\beta$  are related to the regression coefficients  $b$  as  $\beta_{\text{nat}} = b_{\text{nat}}/\overline{W}_{\text{nat}}$  and  $\beta_{\text{hp}} = b_{\text{hp}}/\overline{W}_{\text{hp}}$ . The slope of  $\widehat{\text{IPL}}(z)$  vs  $z$  will therefore have sign  $\beta_{\text{hp}} - \beta_{\text{nat}}$ , opposite to that of the pollinator-mediated selection gradient  $\Delta\beta_{\text{poll}} = \beta_{\text{nat}} - \beta_{\text{hp}}$ . These opposite signs demonstrate that total pollinator-mediated selection will generally favor phenotypes decreasing individual pollen limitation.

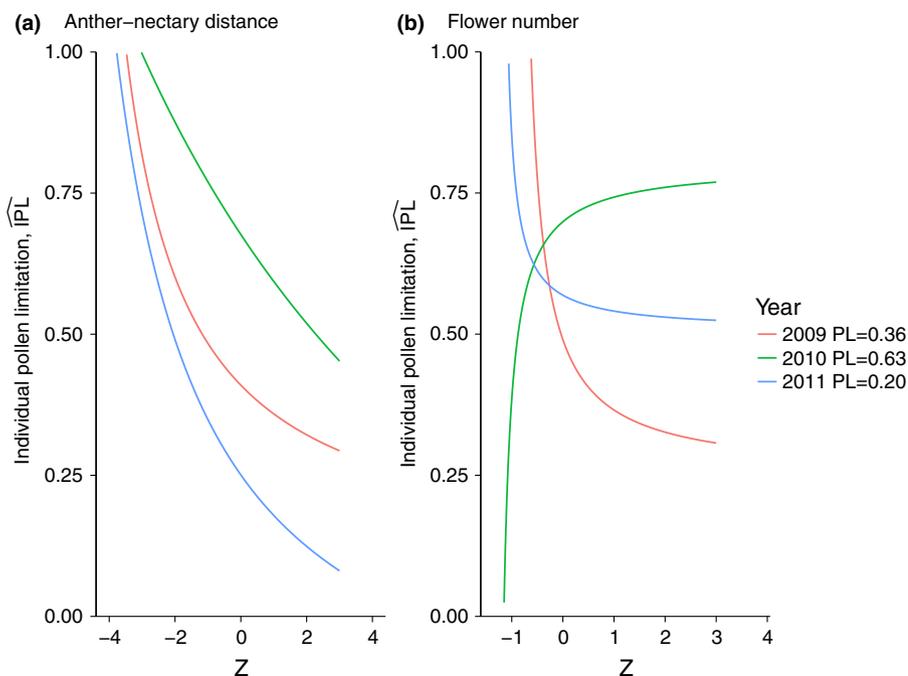
We illustrate this approach using data from a three-year selection study of six traits in *Lobelia cardinalis* (Campanulaceae) where population pollen limitation was 0.36, 0.63 and 0.20 (Bartkowska & Johnston, 2015) and individuals competed for pollen receipt (Bartkowska & Johnston, 2014). Using the formula above, we calculated IPL as  $\widehat{\text{IPL}}(z)$  across standardized trait values for six traits. Results for flower number and anther–nectary distance are shown in Fig. 1. For these traits, relative fitness increased with larger trait values, which are associated with lower individual pollen

limitation (Fig. 1). More generally, among the statistically significant pollinator-mediated selection coefficients in that study, all six gradients and nine of 10 differentials showed patterns consistent with selection to decrease IPL.

Measuring IPL as a function of phenotype might provide insight beyond that obtained from measuring pollinator-mediated selection alone. For example, the shape of  $\text{IPL}(z)$  vs  $z$  reveals how phenotypes experience pollen limitation, an insight that cannot be obtained by measuring pollinator-mediated selection alone or by comparing selection in populations differing in pollen limitation at the population level. IPL will depend on how well an individual ‘predicts’ both its resources for seed production and its success in pollen receipt. Species that overproduce ovules as a bet-hedging strategy under unpredictable pollination (Burd *et al.*, 2009) might show different patterns of IPL with phenotype. Finally, IPL might vary with male fertility, an issue that has not yet been investigated for pollen limitation at either level (Johnston, 1991a).

### Phylogeny and the relation between selection and pollen limitation

Just as pollinator-mediated selection through female function increases with pollen limitation among species of wide taxonomic affiliation, Trunschke *et al.* find that it also increases among terrestrial European orchids of the tribes Orchideae (10 species) and Neottieae (two species). Interestingly, one of the species in their study, *Gymnadenia conopsea*, was previously examined in multiple years and populations, with no association detected (Sletvold & Ågren, 2014). This discrepancy cannot wholly be explained by a limited range of pollen limitation, as in *G. conopsea* it ranges from *c.* 0 to 0.6. Two other within-species studies also find no relationship (*Dactylorhiza lapponica*, Sletvold & Ågren, 2014; *L. cardinalis*, Bartkowska & Johnston, 2015). These contrasting results within and among species suggest that phylogeny might



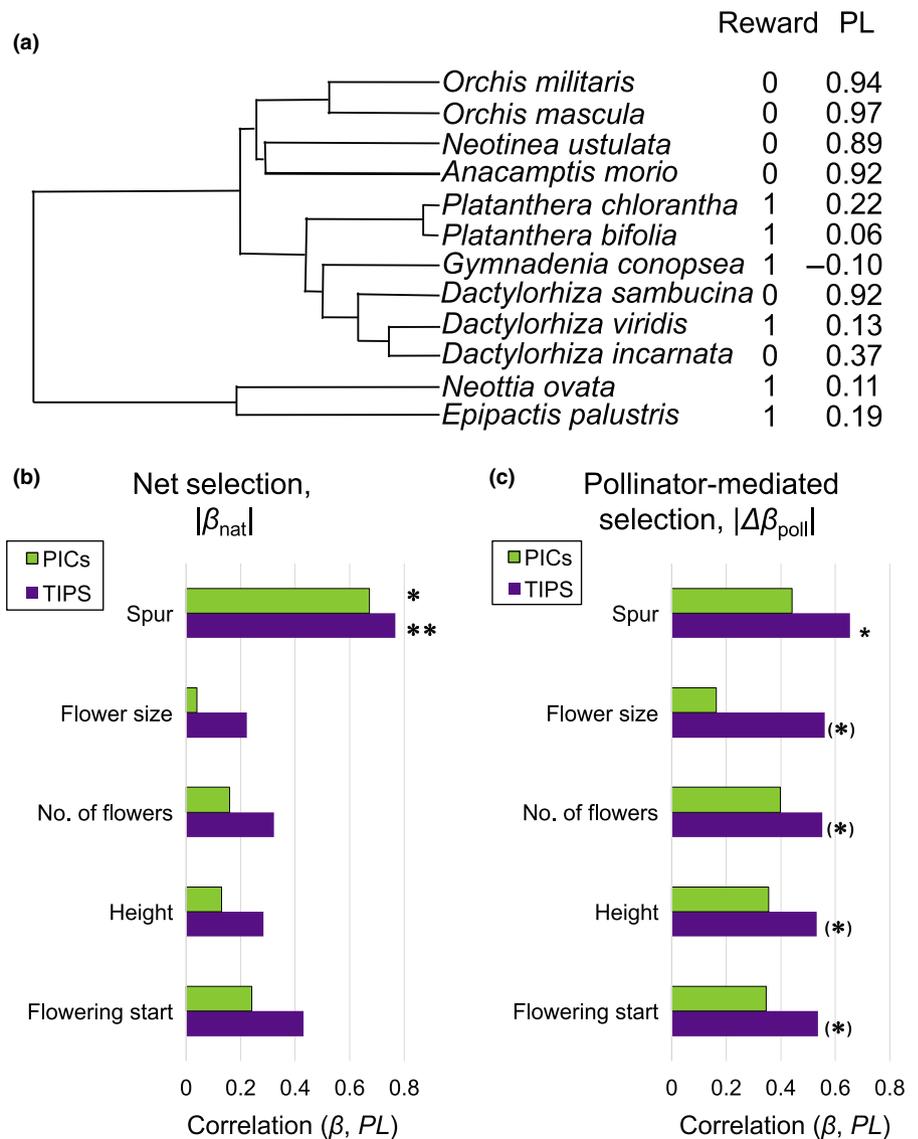
**Fig. 1** Individual-level pollen limitation vs phenotype assessed for two traits in three years in an Ontario (Canada) population of *Lobelia cardinalis*. All trait–year population combinations show selection consistent with reducing individual pollen limitation except flower number in 2010. PL, pollen limitation.

influence the relationship. This is perhaps counterintuitive, because mate competition and the opportunity for selection would seem to depend more strongly on the current pollination environment than on phylogenetic position. However, taxa certainly differ fundamentally in ways we do not understand and these might affect how selection and pollen limitation are related. In fact, a phylogenetic signal has been detected in population pollen limitation among species as well as among plant traits (Knight *et al.*, 2005).

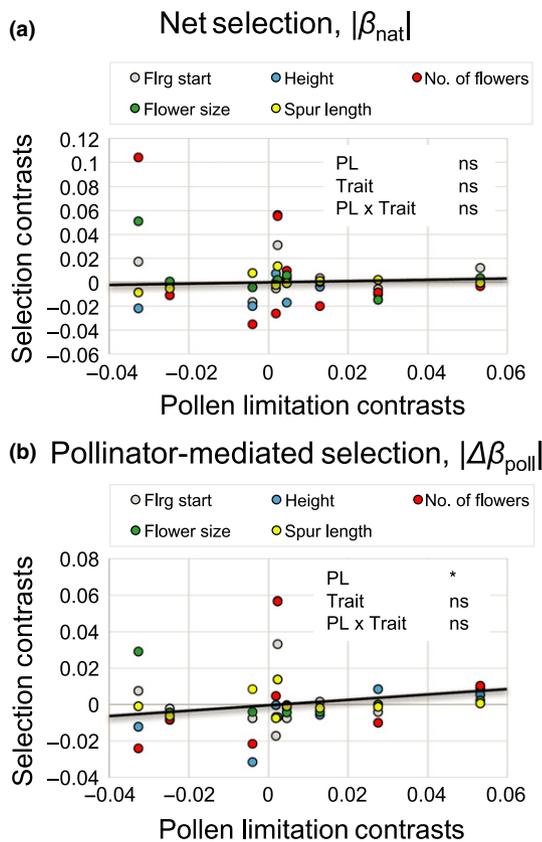
To account for shared history, we constructed a phylogeny and calculated the correlation between pollen limitation and selection through female function for the five traits studied by Trunschke *et al.* using phylogenetically independent contrasts (PICs; Paradis *et al.*, 2004). We compare these PICs correlations to those treating each species as an independent data point (TIPS analysis), as used in the regression analyses of the original study. The phylogeny shows deceit pollination and its associated high pollen limitation are unevenly distributed among clades (Fig. 2a). We find in all cases, for both net selection and pollinator-mediated selection, the PICs analyses show a weaker correlation than the TIPS analyses (Fig. 2b,

c). Furthermore, for pollinator-mediated selection none of the PICs correlations approaches statistical significance, while all TIPS correlations are significant, or nearly so ( $P < 0.075$ ; Fig. 2c). Strikingly, the PICs analysis shows no relation between pollen limitation and net selection and no differences among traits (Fig. 3), in contrast to the TIPS analysis of covariance (ANCOVA) of Trunschke *et al.* For pollinator-mediated selection, however, PICs agrees with TIPS in finding increased selection with pollen limitation, although the relation is weaker (Fig. 3). By showing the relation holds for pollinator-mediated selection but not for net selection, the PICs analysis provides stronger support for the hypothesis.

The ideas discussed here and experimental results to date suggest several unanswered questions regarding the role of pollen limitation in causing selection. Why is the correlation between pollen limitation and PMS found among, but not within, species? How and why is the relationship changed by taking phylogeny into account? When accounting for trait correlations, does direct selection always act to reduce IPL as suggested for total selection by



**Fig. 2** Phylogeny and comparison of TIPS and phylogenetically independent contrasts (PICs) analyses of the relation between pollen limitation (PL) and selection for the 12 orchid species studied by Trunschke *et al.* (this issue of *New Phytologist*, pp. 1381–1389). (a) Phylogenetic relations. Branch lengths within the tribe Orchideae (upper 10 species) were obtained from Inda *et al.* (2012, Fig. 4). Pollinator reward (1, rewarding; 0, deceptive) and PL from Trunschke *et al.* (Supporting Information) are also shown. (b, c) TIPS vs PICs correlations between selection gradient (absolute value) and PL. Net selection gradients from naturally pollinated plants  $|\beta_{\text{nat}}|$  are analyzed in (b) and pollinator-mediated selection gradients  $|\Delta\beta_{\text{poll}}|$  are analyzed in (c). (\*),  $P < 0.075$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .



**Fig. 3** Relationship between population pollen limitation (PL) and selection using phylogenetically independent contrasts (PICs) for five traits in 12 orchid species. (a) Net selection gradients, representing naturally pollinated plants. (b) Pollinator-mediated selection gradients. Each graph contains 55 points (11 contrasts per trait). Analysis of covariance (ANCOVA) results are also shown. Data from Trunschke *et al.* (this issue of *New Phytologist*, pp. 1381–1389, Supporting Information). \*,  $P < 0.05$ ; ns, not significant.

the theory presented earlier? Finally, what is the relation between pollen limitation and male fitness? Specifically, do populations or species with increased resource limitation – and lower pollen limitation – experience stronger selection through male function, as has been suggested (Johnston, 1991a)?

## Acknowledgements

This research was supported by Discovery Grants from Natural Sciences and Engineering Research Council of Canada (NSERC) to M.O.J.

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**Key words:** competition, *Lobelia*, orchid, pollen limitation, pollinator, pollinator-mediated selection, selection.