Supplement to ‘Distinct effects of pollinator-dependence and self-incompatibility on pollen limitation in South African biodiversity hotspots’

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Contents

1. Supplementary methods

a. Literature search and data preparation

Data on pollen limitation of fruit set were obtained for biotically pollinated species in biodiverse regions of South Africa (≥ 3000 species per 10 000 km^2: Barthlott et al. 1999) from studies reporting fruit set from natural and supplemental pollination. We included both cases where hand pollination was applied to flowers from which pollinators were excluded (hand-cross pollination in breeding system experiments) and flowers also exposed to natural pollinators. We included cases where pollen limitation was estimated at the whole plant level as well as at the branch or flower level (Knight et al. 2006). The study region included two sub-regions, the Greater Cape Floristic Region (GCFR: Born et al. 2007) and an adjacent region, which we call the South Eastern Summer Rainfall Region (SESRR). The GCFR corresponds to the Cape Floristic Region and Succulent Karroo biodiversity hotspots. The SESRR is the part of South Africa’s summer rainfall region and falls almost entirely within the Maputaland-Pondoland-Albany hotspot (Mittermeier et al. 2005). We searched published literature for information on pollen limitation from South Africa using the Thompson ISI Web of Knowledge database with keywords for location – Cape, Fynbos, Renosterveld, Karoo, CFR, GCFR, South Africa – in combination with pollinat*, pollen limitation, breeding system and mating system, and searched for names of workers known to be active in the South African summer rainfall region (Johnson, SD; Peter, CI) in combination with pollinat*. We also searched a published list of African pollination studies (Rodger et al. 2004) and the thesis collection of the Bolus library at the University of Cape Town.

We included unpublished results from R. Turner, J.G. Rodger and M. van Kleunen. We excluded data from: plants in cultivation; exotic species; plants that received other treatments that may have affected resource availability; species with abiotic pollination; and South African species in diversity zones of less than 3000 species per 10 000 km^2 (Barthlott et al. 1999). Trees were also excluded to avoid biasing analyses, as they were under-represented in the South African dataset and, in the global dataset, they have higher pollen limitation than herbs and shrubs (Knight et al. 2005, Vamosi et al. 2006). Following Wolowski et al. (2014), cases where fruit-set from pollen supplementation was less than half that of natural pollination were excluded as this likely indicates experimental error.

Pollen limitation calculated from fruit set as PL = ln[supplemental pollination/natural pollination]) (Knight et al. 2005, Vamosi et al. (2006) was used as the response variable in analyses. We used average pollen limitation values from the global dataset in Vamosi et al. (2006) provided by the authors for all species except those occurring in South Africa. Where treatments were replicated in time or across subsets of populations within the same site and flowering season (for instance shade and sun locations or different style morphs), data were pooled before calculating effect size. Where effect sizes were available for different years and populations, we calculated weighted average effect size, with weights being 1/variance (Knight et al. 2005). When variance was not presented in studies but data were presented as proportion of treated flowers setting fruit, SD of each pollination treatments was calculated as square root of $(p * (1 - p) / n)$. To avoid undefined values for effect size, where proportion fruit set was zero for a treatment, it was increased to 0.01. Where proportion fruit set was zero or one, SD was set to 0.001.

Breeding system classification was obtained from calculated indices as follows, where possible, and otherwise, for certain Iridaceae, expert information (pers. comm. Peter Goldblatt). Self-incompatibility (SI) was determined from the index of self-incompatibility: ISI = 1 – hand self-pollination/hand cross-pollination (Raduski et al. 2012). Species were classified as self-incompatible when ISI ≥ 0.8, and self-compatible when ISI < 0.8. Pollinator dependence (PD) was determined from the auto-fertility index: AFI = autonomous self-pollination/hand cross pollination (Schoen and Lloyd 1992). Species were classified as pollinator dependent when AFI < 0.2, and autofertile when AFI ≥ 0.2. Self-incompatible species were classified as pollinator dependent. From these a combined breeding system variable with three levels was obtained: auto-fertile; self-compatible but pollinator dependent; and self-incompatible (Table 1, main text). For the global dataset, auto-fertility data were not available but we used the self-incompatibility classification of Vamosi et al. (2006), which used the same ISI categorisation method. Although we used a slightly different cut-off for categorising pollinator-dependent versus autofertile species than was used in Wolowski et al. (2014) (AFI = 0.2 in this study versus AFI = 0.3 in Wolowski et al. 2014) this did not impact on the relationship between breeding system and pollen limitation (J.G. Rodger, unpublished results). South African species were further categorised for the following variables reflecting different aspects of pollination specialisation: floral symmetry (actinomorphic; zygomorphic) denoting phenotypic specialisation; pollinator richness (1 to 5 species – few; greater than 5 – many) denoting ecological specialisation and pollinator functional groups (one; greater than one – many) denoting functional specialisation (Ollerton et al. 2007).

To obtain the backbone for a phylogeny for phylogenetically controlled analyses, we pruned Qian and Zhang's (2014) global family level phylogeny to include only the families for which we had data. To this tree we added within family structure from the most up to date trees available, which we searched for using Web of Science and references in entries for families on Wikipedia (See references for phylogeny below). Nomenclature and classification into families was checked at The Plant List (www.plantlist.org) on 5th of March 2015. For South African species, those in the GCFR were checked against regional conspectuses (Manning and Goldblatt 2012, Snijman 2013) and those in the SESRR against the SIBIS portal of the South African National Biodiversity Institute (http://sibis.sanbi.org) except that we followed APG 3 families.

We tested for phylogenetic signal in pollen limitation by means of Abouheif's Cmean test, using the function abouheif.moran in the package adephylo with the function proxTips and the method oriAbouheif for the proximity matrix (Munkemuller et al. 2012, Jombart and Dray 2013).

b. Analyses

Pollen limitation was compared between South Africa and the Rest of the World in a one-way analysis and, for species with breeding system information, in an analysis including self-incompatibility (SI) and SI × Region. Data from a South African study where selection of species was biased towards autofertile ones with low pollen limitation were excluded from this analysis, because a lack of pollinator dependence/autofertility information for the rest of the world prevented us from controlling for this bias. Species in this study were chosen specifically because of being naturalised or introduced and non-naturalised in other regions and the study found that naturalised species had higher autofertility than non-naturalised species (van Kleunen et al. 2008). Pollen limitation data was collected as part of this study but not published (van Kleunen unpublished). Our data showed a relationship between autofertility and pollen limitation. In this respect data from van Kleunen (unpublished) was consistent with the rest of our data. However, including the van Kleunen (unpublished) data in comparisons of South Africa and the rest of the world biased pollen limitation for self-compatible species downwards, through inclusion of a large number of autofertile species. We could not control for this bias in these analyses as we lacked information on autofertility/pollinator dependence for self-compatible species in the rest of the world, so these data were excluded here. As autofertility was included as a factor in all analyses of pollen limitation within South Africa, these data was included for within South Africa analyses. As residuals of generalised least squares (GLS) showed significant phylogenetic signal ($p = 0.008$), phylogenetically controlled least squares analysis (PGLS) with the function pgls in the caper package in R (Revell, 2010) were performed from comparisons between South Africa and the rest of the world. Lambda was obtained by maximum likelihood estimation in the function pgls itself. The analysis was repeated, excluding the SESRR, to compare the GCFR specifically with the rest of the world. PGLS residuals were acceptable for homogeneity and normality. No particular outliers strongly affected results.

Utility of pollinator dependence as a predictor of pollen limitation instead of or in addition to self-incompatibility was assessed with South African data only, as pollinator dependence was not available for the global dataset. AICc values were compared for one-way GLS analyses of pollen limitation including SI only, PD only, and the combined breeding system variable incorporating SI and PD. G-tests were carried out to assess whether South Africa and the rest of the world differed in frequency of pollination specialised species for SI and SC species separately.

Effects of each of the three pollination specialisation variables on pollen limitation within South Africa were assessed in GLS, firstly in univariate analyses, and secondly controlling for combined breeding system, with the prediction that effects of other variables should be lower for auto-fertile species than the other two levels of the effect. In the univariate analysis, levels were compared with t-statistics. We report uncorrected values in the main text, as we do not view multiple testing as a problem because we had specific hypotheses about how pollen limitation should differ between levels. Nevertheless we also present values corrected by Holm's sequential Bonferroni procedure in table S5. In GLS analyses we used the VarIdent variance structure with breeding system levels used for variance categories. Within South Africa phylogenetic analysis was not necessary as GLS residuals showed no phylogenetic signal ($p > 0.5$). GLS residuals were acceptable for homogeneity and normality except that in one-way analyses residuals deviated somewhat from normality. However, as rank transformed data gave qualitatively identical results with acceptable residuals (Table S6), we are confident in our findings. To assess impact of outliers, we dropped them from analyses and inspected model output. No particular outliers strongly affected results. Additionally, we tested whether species with more specialised pollination systems were more pollen limited and if the GCFR was more pollen limited than the SESRR within South Africa in GLS analyses. This was done first in univariate analyses and then in two-way analyses also including breeding system. The two-way analyses were repeated excluding the autofertile species to gain insight into whether pollination specialisation affected the difference in pollination specialisation between PD-SC and SI species specifically. Finally, we performed G-tests to test if levels of pollination specialisation variables were related to levels of the combined breeding system variable.

c. Phylogenetic tree

The following tree includes all species includes in this study. Coding of tip names is given in the dataset itself (doi:10.5061/dryad.pt553).

(((Hydrocharitaceae:1,Araceae:1)Alismatales:1,(((((Tril_cate:1,Tril_erec:1):1,Tril_gran:1)Melanthiaceae:1,((Alstroemeriaceae:1,(Colc_colo:1,Uvul_sess:1)Colchicaceae:1):1,(Eryt_umbi:1,Lili_aura:1)Liliaceae:1):1):1,(Blandfordiaceae:1,(((((((Free_leic:1,Free_occi:1):1,((Romu_rose:1,Romu_cruc:1,Romu_sabu:1):1,((Geis_infl:1,((Hesp_acut:1,Hesp_vagi:1):1,Hesp_falc:1):1):1,(((Spar_bulb:1,Spar_eleg:1):1,((Dier_drac:1,Dier_lute:1):1,Trit_dist:1):1):1,(((Babi_angu:1,Babi_odor:1):1,((Babi_avic:1,Babi_hirs:1):1,(Babi_ring_auss:1,Babi_ring_ring:1):1):1):1,(Chas_aeth:1,Chas_flor:1):1):1):1):1):1):1,(Ther_spic:1,(Wats_lepi:1,Wats_meri:1):1):1,((Glad_alat:1,Glad_meli:1):1,(Glad_lili:1,Glad_long:1):1):1):1,Aris_wood:1):1,((Iris_cris:1,(Iris_fulv:1,((Iris_tena_gorm:1,Iris_tena_tena:1):1,Iris_vers:1):1):1):1,((Ferr_cris:1,Ferr_unci:1):1,(Mora_eleg:1,Mora_flac:1,Mora_mino:1,Mora_tulb:1,Mora_vill:1):1):1):1)Iridaceae:1,(((Aloe_plur:1,Aloe_prui:1,Aloe_spec:1):1,(Asph_aest:1,Asph_albu:1):1)Xanthorrhoeaceae:1,((((Brun_bosm:1,Brun_lito:1,Brun_undu:1):1,Neri_sarn:1):1,((Narc_asso:1,Narc_hisp:1):1,(((Cliv_gard:1,Cliv_mini:1):1,Haem_sang:1):1,(Cyrt_brev:1,(Cyrt_guth:1,Cyrt_vent:1):1):1):1):1)Amaryllidaceae:1,(((Albu_seto:1,Orni_thyr:1):1,Scil_hyac:1):1,(Agav_mcke:1,Yucc_elat:1):1):1):1):1):1,((((((Comp_falc:1,Tolu_vari:1,Iono_utri:1)Oncidinae:1,Oeco_macu:1):1,((Caly_bulb:1,Aple_hyem:1,Tipu_disc:1)Calypsoinae:1,(Epid_cili:1,Myrm_tibi:1)Laeliinae:1):1):1,Epip_hell:1)Epidendroideae:1,((Pter_cath:1,((((Disa_atri:

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1,Disa_biva:1):1,Disa_tenu:1):1,Disa_unif:1,Disa_race:1):1,(Disa_fasc:1,(Disa_tene:1,Disa_pulc:1,Dis
a_drac:1):1):1):1,((Orch_masc:1,Plat_bifo:1):1,(Saty_pumi:1,(Saty_cori:1,(Saty_bico:1,Saty_erec:1):1
):1):1)Orchidinae:1):1,(Myro_coch:1,Cycl_cran:1):1)Orchidoideae:1):1,(Cypr_acau:1,Cypr_fasc:1):1)O
rchidaceae:1):1)Asparagales:1):1,(Marantaceae:1,Arecaceae:1):1):1)Nartheciidae:1,((Magnoliaceae:
1,((Asmi_obuv:1,Asmi_pygm:1):1,Uvar_mont:1)Annonacaceae:1)Magnoliales:1,((((((Telo_spec:1,Gre
v_bark:1):1,(Bank_aemu:1,(Bank_spin:1,Bank_eric:1):1):1):1,(Prot_caff:1,((Prot_comp:1,Prot_long:1)
:1,((Prot_laur:1,Prot_magn:1):1,(Prot_punt:1,Prot_repe:1):1):1):1):1):1,(Pers_moll:1,Pers_rigi:1):1)Pr
oteaceae:1,(Papaveraceae:1,(Berberidaceae:1,((((Anem_hepa:1,Clem_soci:1):1,Hepa_nobi:1):1,Hell_
foet:1):1,Anem_thal:1)Ranunculaceae:1):1):1):1,(((Hamamelidaceae:1,(Crassulaceae:1,Saxifragaceae
:1):1)Saxifragales:1,(((Erod_paul:1,Gera_macu:1)Geranaceae:1,(Staphyleaceae:1,((Onagraceae:1,(((D
ipl_hirs:1,Dipl_orbi:1):1,Lyth_sali:1)Lythraceae:1,Melastomataceae:1):1)Myrtales:1,((Cleomaceae:1,(
Alli_Peti:1,Card_angu:1)Brassicaceae:1):1,(Thymelaeaceae:1,((Dipt_oblo:1,(Shor_acum:1,Shor_hems
:1,Shor_lepi:1,Shor_lepr:1,Shor_macr:1,Shor_oval:1,Shor_siam:1,Shor_sple:1):1)Dipterocarpaceae:1
,(Grew_occi:1,((Duri_gran:1,Duri_kute:1):1,(Pseu_mung:1,(Sida_malv_eleg:1,Sida_malv_malv:1):1):1
):1)Malvaceae:1):1)Malvales:1):1):1):1)Malvidae:1,(Zygophyllaceae:1,((Celastraceae:1,(((Oxal_pesc:1
,(Oxal_purp:1,(Oxal_olig:1,((Oxal_nidu:1,Oxal_eckl:1):1,(Oxal_oreo:1,(Oxal_ambl:1,(Oxal_comp:1,(O
xal_gift:1,(Oxal_glab:1,(Oxal_nata:1,Oxal_tenu:1):1):1):1):1):1):1):1):1):1):1,Oxal_squa:1)Oxalidaceae
:1,((Erythroxylaceae:1,Hypericaceae:1):1,((Sali_lant:1,Sali_myrt:1)Salicaceae:1,Euphorbiaceae:1):1):1
):1):1,(((Bego_invo:1,Bego_tond:1,Bego_urop:1)Begoniaceae:1,(Rhamnaceae:1,(Rubu_cham:1,(Prun
_maha:1,(Amel_amer:1,Sorb_aucu:1):1):1)Rosaceae:1)Rosales:1):1,(Polygalaceae:1,((((Aspa_bifl:1,A
spa_unif:1):1,(((Cyti_scop:1,Cyti_stri:1):1,Reta_spha:1):1,Geni_mons:1):1):1,Geof_deco:1,((Pult_den
s:1,(Dill_hisp:1,Dill_unci:1):1):1,(Erio_dist:1,(Anth_vuln:1,Lath_vern:1):1):1):1):1,(Cera_sili:1,((Pros_g
lan:1,(Inga_dens:1,Inga_mort:1,Inga_oers:1,Inga_punc:1,Inga_sier:1):1):1,Cham_line:1):1):1)Fabace
ae:1)Fabales:1):1)Fabidae:1):1)Rosidae:1)Superrosidae:1,((Loranthaceae:1,(Plumbaginaceae:1,((Mon
tiaceae:1,((((Carn_giga:1,Pach_prin:1):1,Loph_scho:1):1,Sten_thur:1):1,(Fero_cyli:1,Fero_wisl:1):1)C
actaceae:1):1,((Sile_acau:1,Sile_dioi:1,Sile_doug:1,Sile_lati:1,Sile_stoc:1,Sile_virg:1):1,Stel_pube:1)C
aryophyllaceae:1):0.5):0.5):1,((((Ardi_esca:1,Anag_mone:1):1,(Prim_fari:1,Prim_sieb:1,Prim_veri:1):
1)Primulaceae:1,((Rori_dent:1,Rori_gorg:1)Roridulaceae:1,((Ipopm_aggr:1,(Lina_bico:1,(Lept_jeps:1,
Phlo_drum:1):1):1)Polemoniaceae:1,((Kalm_lati:1,(Eric_mult:1,(Eric_brac:1,Eric_chlo:1,Eric_shan:1):
1):1):1,(Andr_poli:1,(Vacc_hirt:1,Vacc_myrt:1,Vacc_oxyc:1,Vacc_small:1,Vacc_ulig:1,Vacc_viti:1):1):1
)Ericaceae:1):1):1):1,((Bruniaceae:1,(Caprifoliaceae:1,(Apiaceae:1,(((Lobe_carn:1,Lobe_siph:1):1,(Wa
hl_cusp:1,Wahl_kreb:1):1)Campanulaceae:1,((Acou_runc:1,Gerb_aura:1):1,(Euth_gram:1,(Soli_cana:
1,Soli_junc:1):1):1)Asteraceae:1)Asterales:1):1):1):1,(Garryaceae:1,(((((Saba_angu:1,Chir_kreb:1):1,S
eba_gran:1)Gentianaceae:1,((Ascl_syri:1,Mand_pent:1):1,Neri_olea:1)Apocynaceae:1):1,(Oxya_pyri:
1,((Pent_angu:1,Pent_prun:1):1,Psyc_suerr:1):1)Rubiaceae:1)Gentianales:1,(((Caly_coll:1,Ipom_wolc
:1)Convolvulaceae:1,(Lyci_cest:1,(Phys_long:1,Sola_caro:1):1)Solanaceae:1):1,(Oleaceae:1,((Coll_spa
r:1,Coll_vern:1)Plantaginaceae:1,(Lentibulariaceae:1,(((Cord_mari:1,Agal_stri:1):1,((Mela_arve:1,Mel
a_prat:1):1,Euph_wilk:1):1)Orobanchaceae:1,((Stilbaceae:1,Scrophulariaceae:1):1,((Glec_hede:1,Ne
pe_cata:1):1,(Sync_dens:1,Sync_macr:1,Sync_rotu:1):1)Lamiaceae:1):1):1):1):1):1):1):1):1):1)Asterid
ae:1)Pentapetalae:1):1)Eudicotyledoneae:1):1):1;
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d. Sources for phylogeny

Backbone (Qian and Zhang 2014)

Amaryllidaceae (Ronsted et al. 2012)

Cyrtanthus (Snijman and Meerow 2010)

Apocynaceae (Endress and Bruyns 2000)

Asparagaceae (Martínez-Azorín et al. 2011, Seberg et al. 2012)

Asteraceae (Panero and Funk 2008)

Cactaceae (Hernández-Hernández et al. 2014)

Ericaceae (Kron et al. 2012, Gillespie and Kron 2013)

Erica (Pirie et al. 2011)

Fabaceae (Wojciechowski et al. 2004)(Boatwright et al. 2008)

Iridaceae

Babiana (Schnitzler et al. 2012)

Gladiolus (Valente et al. 2012)

Iris (Wilson 2009)

Hesperantha (Goldblatt et al. 2004)

Malvaceae (Alverson et al. 1999)

Melanthiaceae

Trillium (Osaloo et al. 1999)

Orchidaceae (Chase et al. 2015, Freudenstein and Chase 2015)

Disa (Bytebier et al. 2007)

Satyrium (Van Der Niet et al. 2005)

Orobanchaceae (Mcneal et al. 2013)

Oxalidaceae *Oxalis* (Oberlander et al. 2011)

Polemoniaceae (Bell and Patterson 2000, Prather et al. 2000)

Proteaceae (Barker et al. 2007)

Banksia (Cardillo and Pratt 2013)

Protea (Valente et al. 2010)

Ranunculaceae (Wang et al. 2009)

Rosaceae (Potter et al. 2007)

Rubiaceae (Bremer and Eriksson 2009)

e. Dataset

For South Africa, pollen limitation data on fruit set was found for 101 species. Seven of these were excluded from all analyses due to being trees (5 species) and/or from regions with less than 3000 spp per 10 000 km^2 (2 species). The resulting dataset (Table S1) included 75 herbs, 19 shrubs, and 70 GCFR species and 24 SESRR species. 19 families were represented, with Iridaceae (37), Orchidaceae (14) and Amaryllidaceae (8) being most frequent (Table S1). As patterns of pollen limitation within Iridaceae were similar to the rest of the dataset (J.G. Rodger, unpublished results) and there was no phylogenetic signal in the South African dataset, dominance of Iridaceae is unlikely to bias findings.

Data collected for this study will are available at the Dryad data repository at http://dx.doi.org/10.5061/dryad.pt553.

2. Results

Pollen limitation data were obtained for 94 non-tree species from high plant diversity regions of South Africa, but only 70 had breeding system information. The total included 75 herbs, 19 shrubs, 70 GCFR species and 24 SESRR species. 19 families were represented, with Iridaceae (37), Orchidaceae (14) and Amaryllidaceae (8) being most frequent (Table S1).

Pollen limitation did not differ between South Africa and the rest of the world, either in the one-way analysis (LR = 0.27, p = 0.607) or two-way analysis (LR = 0.64, p = 0.425), in which the interaction with breeding system was also not significant (LR = 2.05, p = 0.152) (Fig 1a main text; Tables S2, S3). These findings held when comparing 1) only the GCFR to the rest of the world and 2) South Africa to lower diversity regions of the rest of the world (Tables S2, S3).

All three breeding system variables significantly affected pollen limitation in South Africa (Table S4) but the combined breeding system variable (LR = 14.04, p < 0.001) was far superior to either SI or PD alone (Δ AICc > 10, Table S4). Pollen limitation was highest for self-compatible pollinator-dependent species, lowest for autofertile species and intermediate for self-incompatible species (Fig. 1b main text; Table S5). Results were the same for the analysis on data from the GCFR alone (Tables S6-S7).

G-tests show that for self-compatible species, South Africa had a higher frequency of pollination specialised species than the rest of the world for phenotypic specialisation (floral symmetry) and ecological specialisation (pollinator species) but not functional specialisation (pollinator orders) (Tables S8-S10)

One way analyses of pollination specialisation variables showed that more plants that were more phenotypically specialised (zygomorphic) and functionally specialised (only one pollinator order) had higher pollen limitation in the South African dataset but there was no effect of ecological specialisation (pollinator richness) (Table S11). There was a near significant tendency for higher pollen limitation in the GCFR than the SESRR within South Africa (Table S11).

In a two-way analysis with breeding system, including all three breeding system categories, there was a tendency for a bigger effect of pollination specialisation on PD-SC species than the other two breeding system categories, but the interaction was not significant (Fig. S1a-c; Table S12). Controlling for pollination specialisation variables, breeding system remained significant but controlling for breeding system, pollination specialisation was no longer significant (Table S12). However, in analyses excluding autofertile species, breeding system was no longer significant when controlling for phenotypic specialisation (floral symmetry) (Table S14). G-tests of contingency tables indicate that there was a higher frequency of pollination specialised species in the PD-SC than the SI category (Table S13).

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4. Supplementary Figures and Tables

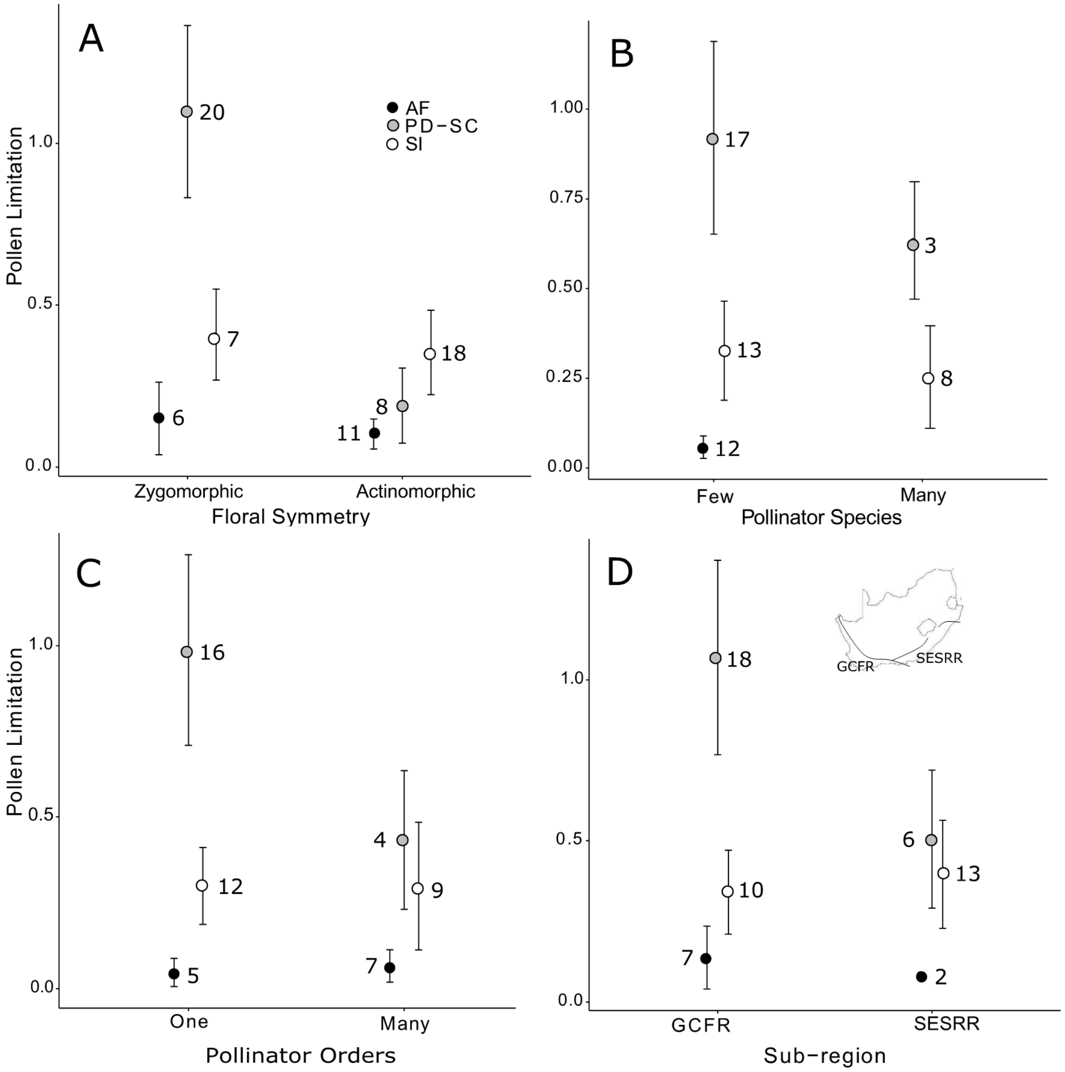

Figure S1. Relationship of pollen limitation (Mean ±SE log response ratio) with breeding system (combined SI and PD variable) and floral symmetry (a), pollinator species richness (b), pollinator orders (c) and sub-region (d), in South Africa. Numbers next to symbols represent sample size. Inset shows sub-regions of South Africa with ≥ 3000 species per 10 000km^2. AF = auto-fertile; PD-SC = pollinator-dependent self-compatible and SI = self-incompatible. GCFR = Greater Cape Floristic Region; SESRR = South-Eastern Summer Rainfall Region. Inset shows location of GCFR and SESRR in South Africa.

Table S1. Characterisation of the South African pollen limitation dataset. NA denotes missing data.

Variable	Category	Species
Combined Breeding System	Auto-fertile	17
	Pollinator-Dependent Self-Compatible	28
	Self-Incompatible	25
	NA	24
Floral Symmetry	Actinomorphic	46
	Zygomorphic	48
Pollinator Species Richness	Few (1-5)	58
	Many (>5)	13
	NA	23
Pollinator Functional Groups	One	46
	Many	25
	NA	23
Sub-region of South Africa (Fig S1 inset)	GCFR (Greater Cape Floristic Region)	70
	SESRR (South Eastern Summer Rainfall Region)	24
Growth-form	Herb	75
	Shrub	19
Family	Amaryllidaceae	8
	Asparagaceae	2
	Asteraceae	1
	Bruniaceae	1
	Campanulaceae	2
	Ericaceae	3
	Fabaceae	3
	Gentianaceae	1
	Iridaceae	37
	Lamiaceae	3
	Orchidaceae	14
	Oxalidaceae	6
	Plumbaginaceae	1
	Polygalaceae	1
	Proteaceae	3
	Roridulaceae	2
	Rubiaceae	2
	Stilbaceae	1
	Xanthorrhoeaceae	3

Table S2. One way comparisons of pollen limitation between biodiverse regions of South Africa and the rest of the world from phylogenetic least squares analysis (PGLS).

Datasets	N (SA)	N(ROW)	LR	P
SA vs ROW	69	154	0.27	0.607
SA vs lower diversity ROW	69	146	0.143	0.705
GCFR vs ROW	45	154	1.24	0.266

SA = South Africa, ROW = Rest of the World, GCFR = Greater Cape Floristic Region of South Africa, LR = Likelihood Ration, P = p-value.

Table S3. Comparisons of pollen limitation between biodiverse regions of South Africa and the rest of the world in analyses including breeding system from phylogenetic least squares analysis (PGLS).

Regions compared*	N (SA)	N (ROW)	Lambda	Region × Self-incompatibility			Region			Self-incompatibility		
				Effect	LR	p	Effect	LR	p	Effect	LR	p
SA vs ROW	56	143	0.375	0.41	2.05	0.152	0.30	0.64	0.425	0.17	1.59	0.207
SA vs low diversity ROW	56	135	0.210	0.35	1.58	0.209	0.26	0.57	0.450	0.21	0.260	0.610
GCFR vs ROW	35	143	0.371	0.45	1.34	0.247	0.39	1.78	0.183	0.22	2.00	0.157

SA = South Africa, ROW = Rest of world, low diversity ROW = regions of the rest of the world with species richness < 3000 species, GCFR = Greater Cape Floristic Region, SESRR = South Eastern Summer Rainfall Region, LR = Likelihood Ration, P = p-value.

Table S4. Effects of different breeding system variables on pollen limitation in biodiverse regions of South Africa, with AICc, from generalised least squares analysis (GLS). N = 70.

Effect	AICc	LR	P
Combined breeding system	132.48	14.04	< 0.001
Self-incompatibility	169.37	3.95	0.047
Pollinator dependence	144.24	9.92	0.002

LR = Likelihood Ratio, P = p-value.

Table S5. Differences in pollen limitation among breeding systems levels for combined breeding system variable in biodiverse regions of South Africa, including p-values corrected for multiple testing by Holm's sequential Bonferroni procedure, from generalised least squares analysis (GLS). N = 70.

	Self-Compatible-Pollinator Dependent				Self-Incompatible			
	Effect	t	P (uncorrected)	P (corrected)	Effect	t	P (uncorrected)	P (corrected)
Auto-fertile	0.72	3.40	0.001	0.003	0.25	2.25	0.028	0.055
Self-Compatible-Pollinator Dependent					-0.47	2.05	0.045	0.055

t = t statistic, P = p-value

Table S6. Effects of different breeding system variables on pollen limitation in South Africa's Greater Cape Floristic Region, with AICc, from generalised least squares analysis (GLS). N = 49.

Effect	AICc	LR	P
Combined breeding system	95.22	10.83	0.005
Self-incompatibility	127.75	2.46	0.117
Pollinator dependence	105.75	6.44	0.011

LR = Likelihood Ratio, P = p-value.

Table S7. Differences in pollen limitation among breeding systems levels for combined breeding system variable in South Africa's Greater Cape Floristic Region, including p-values corrected for multiple testing by Holm's sequential procedure, from generalised least squares analysis (GLS). N = 49.

	Self-Compatible-Pollinator Dependent				Self-Incompatible			
	Effect	t	P (uncorrected)	P (corrected)	Effect	t	P (uncorrected)	P (corrected)
Auto-fertile	0.81	3.10	0.003	0.010	0.22	1.76	0.081	0.081*
Self-Compatible-Pollinator Dependent					-0.59	2.12	0.039	0.079

t = t statistic, P = p-value

* Correction is not applied to p-values that are already non-significant

Table S8. Frequencies of plant species with zygomorphic versus actinomorphic flowers in South Africa and the rest of the world, with G-tests for association between factors.

Breeding System	Region	Floral Symmetry		
		Zygomorphic	Actinomorphic	
All species	South Africa	29	27	G = 3.57
	Rest of the World	53	90	P = 0.059
Self-compatible species	South Africa	24	9	G = 7.40
	Rest of the World	40	48	P = 0.007
Self-incompatible species	South Africa	5	18	G = 0.03
	Rest of the World	13	42	P = 0.856

Table S9. Frequencies of plant species with few versus many pollinator species in South Africa and the rest of the world, with G-tests for association between factors.

Breeding System	Region	Pollinator Species		
		Few	Many	
All species	South Africa	31	11	G = 9.55
	Rest of the World	50	58	P = 0.002
Self-compatible species	South Africa	20	3	G = 12.89
	Rest of the World	33	38	P < 0.001
Self-incompatible species	South Africa	11	8	G = 0.719
	Rest of the World	17	20	P = 0.39

Table S10. Frequencies of plant species with one versus many pollinator orders in South Africa and the rest of the world, with G-tests for association between factors.

Breeding System	Region	Pollinator Orders		
		One	Many	
All species	South Africa	28	14	G = 0.72
	Rest of the World	76	53	P = 0.396
Self-compatible species	South Africa	18	5	G = 0.81
	Rest of the World	52	29	P = 0.368
Self-incompatible species	South Africa	10	9	G = 1.70
	Rest of the World	24	24	P = 0.193

Table S11. Effects of pollination specialisation variables and sub-region on pollen limitation in biodiverse regions of South Africa, in separate one way analyses using PL and rank-transformed PL, from generalised least squares analysis (GLS).

Variable	N	PL		Rank PL	
		LR	P	LR	P
Floral Symmetry	94	4.22	**0.040**	4.64	**0.031**
Pollinator Richness	71	1.88	0.170	0.90	0.342
Pollinator Orders	71	5.09	**0.024**	4.10	**0.043**
Sub-region	69	3.78	0.052	3.19	0.074

N = Sample size, LR = Likelihood Ratio, P = p-value.

Table S12. Effects of pollination specialisation variables and sub-region on pollen limitation in biodiverse regions of South Africa, in analyses also including breeding system (combined SI and PD variable), from generalised least squares analysis (GLS).

Focal Variable	N	Focal Variable × Breeding System		Focal Variable		Breeding System	
		LR	P	LR	P	LR	P
Symmetry	70	3.85	0.146	0.89	0.346	13.72	**0.001**
Pollinator Richness	53	*		0.24	0.625	13.85	**0.001**
Pollinator Orders	53	3.88	0.143	0.03	0.854	12.008	**0.002**
Sub-region	56	*		0.10	0.751	11.23	**0.004**

* Interaction excluded because of low or zero sample size in some cells of analysis

N = Sample size, LR = Likelihood Ration, P = p-value.

Table S13. Frequencies of plant species with different levels of pollination specialisation in different breeding system categories, with G-tests for association between factors.

		Breeding system			
		AF	PD-SC	SI	
Floral symmetry	Zygomorphic	6	20	7	G = 11.59
	Actinomorphic	11	8	18	P = 0.003
Pollinator species	Few	12	17	13	G = 9.31
	Many	0	3	8	P = 0.009
Pollinator Orders	One	5	16	12	G = 5.25
	Many	7	4	9	P = 0.072

Table S14. Effects of pollination specialisation variables on pollen limitation in biodiverse regions of South Africa, in analyses also including breeding system, excluding autofertile species, in generalised least squares analysis (GLS).

Focal Variable	N	Focal Variable × Breeding System		Focal Variable		Breeding System	
		LR	P	LR	P	LR	P
Symmetry	53	3.19	0.074	1.31	0.253	2.39	0.122
Pollinator Richness	41	0.11	0.746	0.24	0.625	4.68	**0.031**
Pollinator Orders	41	0.90	0.343	0.111	0.740	4.83	**0.028**

N = Sample size, LR = Likelihood Ration, P = p-value.