

Supplementary materials for MS “Pollen grain size associated with pollinator feeding strategy ” by Kai HAO et al.

Figure S1 to S4; Table S1 to S3

Figure S1.

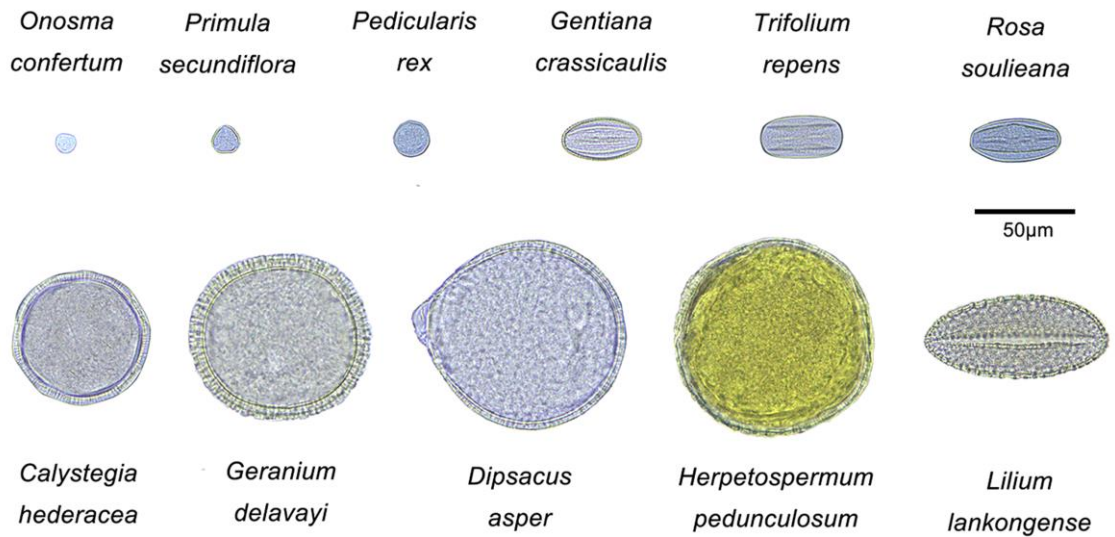


Fig. S1. Pollen samples of 11 species from Shangri-La, southwest China under a light microscope, showing pollen grain size ranging from small (upper six species) to large (bottom five species). Scale bar = 50 µm.

Figure S2



Fig. S2. Bumblebees (upper line) or solitary bees (bottom line) positively collecting pollen into corbiculae or scopae on the hind legs (arrows), leaving less pollen for fertilization. *Bombus festivus* collecting pollen from *Pedicularis cephalantha* (a); *Bombus friseanus* collecting pollen from *Pedicularis siphonantha* (b) and *Astragalus pullus* (c); halictid bee collecting pollen from *Rosa soulieana* (d); *Thalictrum delavayi* (e) and *Potentilla lancinata* (f).

Figure S3

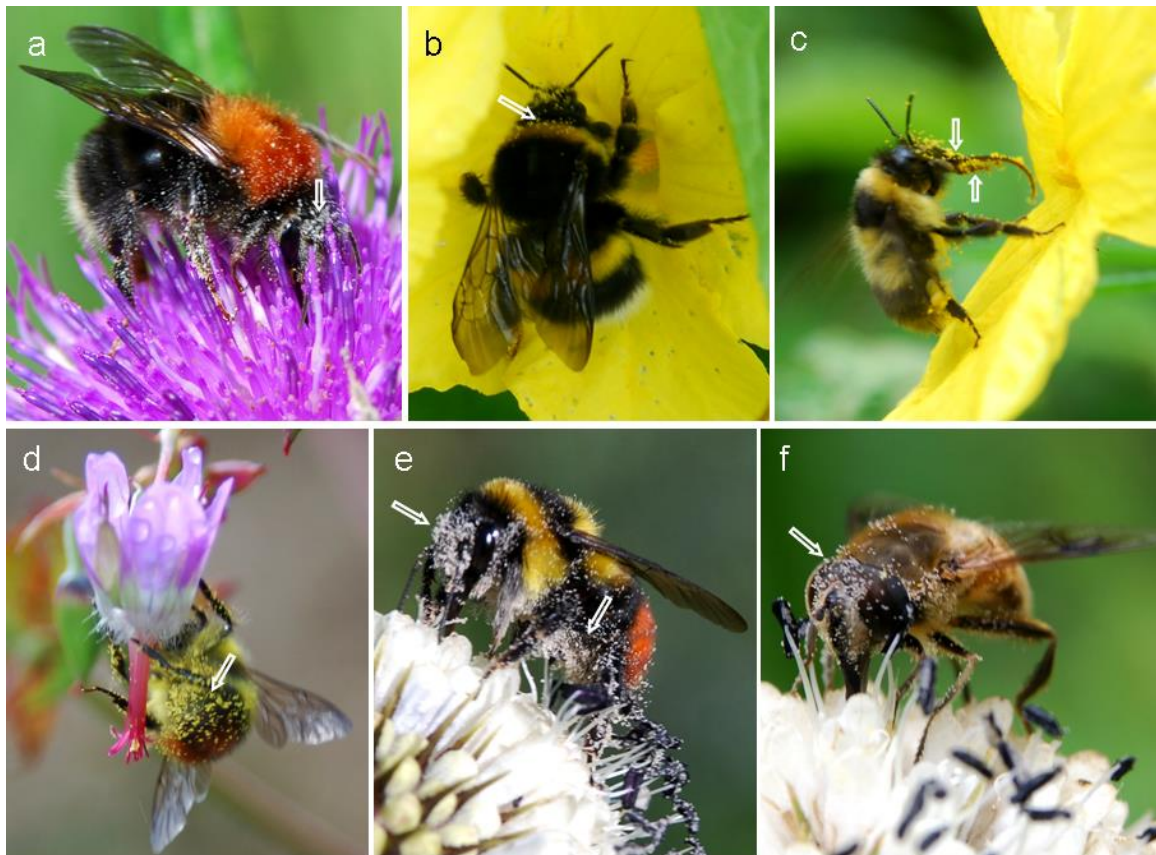


Fig. S3. Pollen grains passively placed on the floral visitors' bodies were not groomed and packed into pollen baskets, and were likely to be delivered to the next flowers visited. These plant species are characterized by relatively large pollen grains, perhaps because relaxed selection by pollen collection permits plants to produce fewer but larger pollen grains. While nectar is sucked from *Cirsium shansiense*, the white pollen accumulates passively on the head, ventral surface and legs of *Bombus festivus* (a); *Bombus lucorum* (b) and another bumblebee (c) collecting nectar from male flowers of *Herpetospermum pedunculatum*. Note that the yellow pollen grains are passively placed on the head or tongue; lots of yellow pollen from *Geranium delavayi* passively placed on the bumblebee's abdomen but not groomed (d); both the bumblebee *Bombus friseanus* (e) and a syrphid fly *Eristalis tenax* (f) rejecting white pollen grains from *Dipsacus asper*. Arrows mark passive accumulation of pollen on the pollinator body. Compared to plants shown in Figure S2, no pollen loads are visible on the bumblebees' hind legs when they are visiting plants illustrated here.

Figure S4

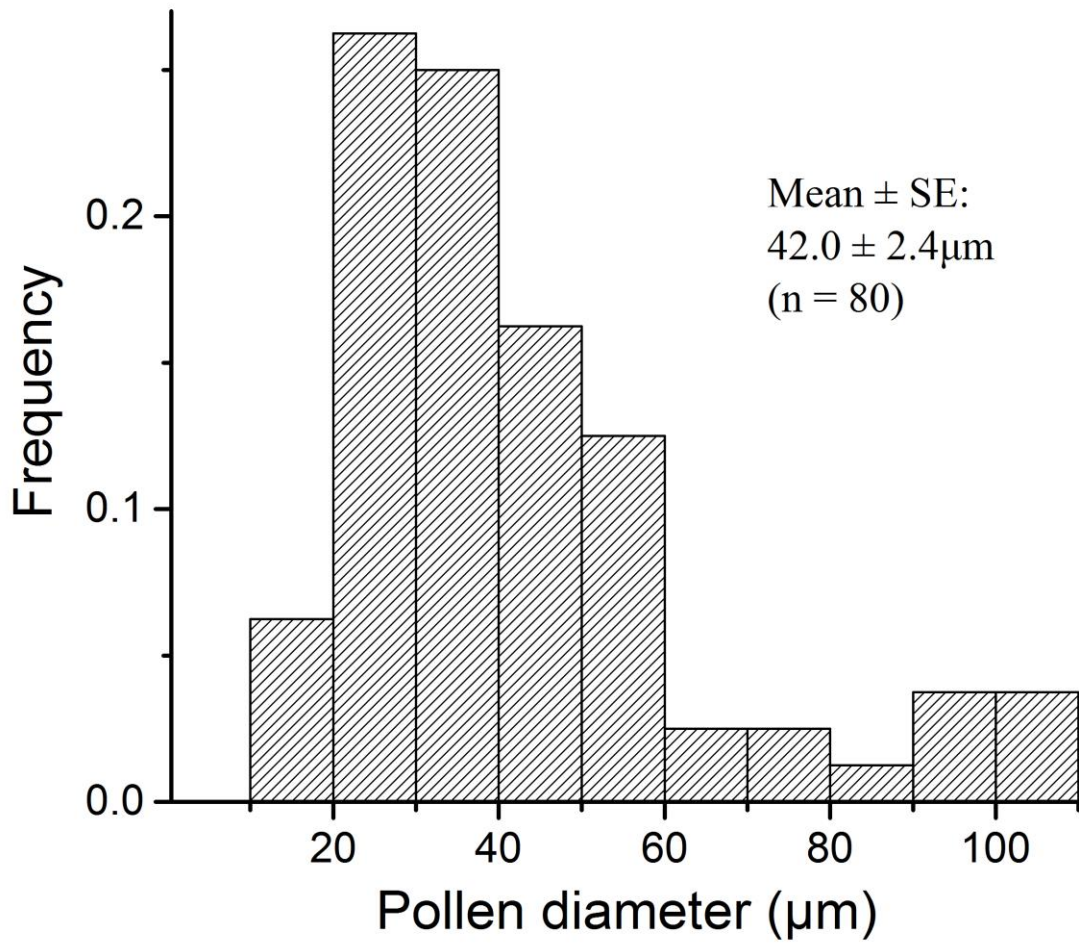


Fig. S4. Variation in pollen diameter in the 80 flowering species from natural communities in Shangri-La field station, southwest China.

Table S1. Three hypotheses proposed for the evolution of pollen grain size and collected evidence of interspecific comparisons of pollen-related traits in the studied plant communities. A null (allometry) hypothesis and two functional hypotheses including pre-pollination pollinator foraging and post-pollination stylar-interaction are considered.

Hypothesis	Evidence		
	Correlation between flower size and grain size	Association between pollen foraging and grain size	Correlation between style length and grain size
Allometry	Support	Reject	Support
Pollinator foraging	Neither reject nor support	Support	Neither reject nor support
Stylar interactions	Neither reject nor support	Reject	Support

Table S2. Dataset of floral traits (mean value) and number of sampled flowers per species, pollen grain diameter and pollen production per flower, pollinator foraging pollen behaviour and observation period in 80 flowering species at Shangri-La field station, southwest China, and DNA sequence code downloaded from the Genebank (NCBI).

Table S3. The results of trait correlations from 21 publications between pollen size and style length or stigma depth, and/or flower size, or pollen number. The phylogenetic independent contrast (PIC) analysis and measurement of flower size were involved only in a few lineages.

Table S2. Dataset of floral traits (mean value) and number of sampled flowers per species, pollen grain diameter and pollen production per flower, pollinator foraging pollen behaviour and observation period in 80 flowering species at Shangri-La field station, southwest China, and DNA sequence code downloaded from the Genebank (NCBI).

Family	Taxon	Substituted taxon (if the origin taxon was not found in Genebank)	Surface area of corolla (mm ²)	Sample size (herbarium specimen)	Style length (mm)	Sampled flowers (n)	Pollen size (um)	Sampled flowers (n)	Pollen number	Sampled flowers (n)	Pollen exposure	Grooming pollen behaviour	Main pollinator	Total observation time	Observation period	ITS	mat K	rbc L
Amaryllidaceae	<i>Allium condensatum</i>		18.2	21	5.9	1	44.0	5	41851	20	Yes	No	bumblebee	120h	2010. 08	GQ181098	HQ690342	HQ690466
Amaryllidaceae	<i>Allium macranthum</i>		53.0	21	7.6	1	38.8	5	58795	20	Yes	No	bumblebee	120h	2010. 08	GQ181072	HQ690297	KP207702
Apiaceae	<i>Heracleum candicans</i>		4.5	1	2.8	1	37.5	5			Yes	No	fly	1h	2019. 08	HQ686368	FJ986061	KY683844
Apiaceae	<i>Torilis scabra</i>		1.6	1	0.7	1	33.8	5			Yes	No	Syrphidae/ Halictidae	0.5h	2019. 08	MH712597		MK932714
Asphodelaceae	<i>Hemerocallis fulva</i>		962.2	1	47.4	1	103.9	20	41905	20	Yes	No	butterfly	4h	2018. 08	JF972939	KM360813	
Asteraceae	<i>Anaphalis nepalensis</i>		10.5	1	5.0	1	25.3	5			No	No	fly	3.5h	2019. 08	EF107657	MH116528	MH116046
Asteraceae	<i>Aster oreophilus</i>	<i>Aster amellus</i>	16.7	1	7.1	10	26.4	30	5177	20	Yes	No	Syrphidae	6h	2019. 08	LC027373	KJ746181	
Asteraceae	<i>Cirsium shansiense</i>	<i>Cirsium arvense</i>	29.8	1	30.9	9	50.2	20	7643	20	Yes	No	bumblebee	6h	2019. 08	AY254532	HQ593238	JX848406
Asteraceae	<i>Ixeridium chinense</i>	<i>Ixeridium dentatum</i>	20.4	21	9.0	1	38.3	20	3518	20	Yes	No	bumblebee	3.5h	2019. 08	AB766220		LC364387
Asteraceae	<i>Ligularia lankongensis</i>		17.5	1	6.4	1	43.3	5	2779	20	Yes	No	bumblebee	120h	2010. 08	KY979100	JF954351	JF942262
Asteraceae	<i>Ligularia dictyoneura</i>		18.0	1	9.6	7	35.3	20	1676	20	Yes	No	bumblebee	2h	2019. 08	JF976814		JF942253
Asteraceae	<i>Picris hieracioides</i>		27.6	21	9.2	1	35.3	20	2770	20	Yes	No	bumblebee	4h	2019. 08	KT249837	JN896100	KF196021
Asteraceae	<i>Saussurea costus</i>		54.0	1	15.8	1	52.5	20	11107	20	Yes	No	bumblebee	6h	2019. 08	EU239685	KX344518	JX944489
Asteraceae	<i>Taraxacum mongolicum</i>		25.3	1	8.6	10	32.8	20	3004	20	Yes	No	bees	4h	2019. 08	EF114672	JN407106	JN407256
Balsaminaceae	<i>Impatiens amplexicaulis</i>	<i>Impatiens capensis</i>	84.7	1	8.3	1	35.0	20	85000	20	No	Yes	bumblebee	1.5h	2019. 08	AY712664	MG225075	KT178138
Boraginaceae	<i>Onosma confertum</i>	<i>Onosma visianii</i>	91.0	21	15.9	10	11.8	20	710880	20	No	Yes	bumblebee	120h	2010. 08	GU827159		

Brassicaceae	<i>Thlaspi arvense</i>		8.9	1	2.1	1	26.8	5			No	Yes	Halictidae	2h	2019. 08	AY254532	AF144360	JQ933501
Campanulaceae	<i>Adenophora capillaris</i>		108.1	21	25.1	9	45.1	20	20795	20	No	No	moth	18h	2009. 08, 2010. 08, 2011. 08	KF175324		KT970148
Campanulaceae	<i>Adenophora khasiana</i>		394.7	21	25.3	1	53.0	5	56097	20	Yes	No	bumblebee/ moth	18h	2009. 08, 2010. 08, 2011. 08	KF175308	KF175291	
Campanulaceae	<i>Codonopsis convolvulacea</i>		1860.3	1	9.1	9	62.3	5	94600	20	Yes	No	bees	1h	2019. 08	KY071089	KC282735	KC282764
Campanulaceae	<i>Cyananthus delavayi</i>	<i>Cyananthus macrocalyx</i>	244.4	21	12.6	1	57.3	5	16949	20	No	No	bumblebee	120h	2010. 08	JF976208.1	KC880573	KT970175
Campanulaceae	<i>Platycodon grandiflorus</i>		1336.7	1	11.9	1	50.0	5	146880	20	Yes	No	bumblebee	1.5h	2019. 08	KC282682	EU713251	AY655156
Caprifoliaceae	<i>Dipsacus asper</i>	<i>Dipsacus asperoides</i>	29.6	21	14.0	50	72.1	20	2037	20	Yes	No	bumblebee	120h	2010. 08	EU925568		KT845953
Caprifoliaceae	<i>Dipsacus chinensis</i>		20.1	1	14.7	50	102.5	reference	2465	20	Yes	No	bumblebee	120h	2010. 08	EU925569		
Caprifoliaceae	<i>Pterocephalus hookeri</i>		86.3	21	10.2	1	96.5	5	3262	20	Yes	No	bumblebee	120h	2010. 08	AY236186	AF446916	AF446946
Caryophyllaceae	<i>Psammosilene tunicoides</i>		15.8	1	3.0	1	39.0	5			Yes	No	fly	4h	2019. 08	JN589122	JN589222	JF943758
Caryophyllaceae	<i>Silene gracilicaulis</i>		103.2	21	20.9	30	50.0	20	11896	20	Yes	No	moth	12h	2017.08, 2018.08	KX757532	FJ589530	
Caryophyllaceae	<i>Silene yunnanensis</i>	<i>Silene acaulis</i>	254.5	21	19.7	30	37.0	20	24472	20	Yes	No	butterfly/ bumblebee	18h	2017.08, 2018.08, 2019.08	KX757505	MK164832	KF602214
Caryophyllaceae	<i>Stellaria yunnanensis</i>	<i>Stellaria media</i>	20.1	21	2.0	1	33.0	20	6471	20	Yes	No	Syrphidae	120h	2010. 08	JN589063	HM850779	HM850386
Convolvulaceae	<i>Calystegia hederacea</i>		1021.7	1	13.3	1	79.2	20			Yes	No	bumblebee	10h	2019. 08	MH364384		LC085875
Cucurbitaceae	<i>Herpetospermum pedunculatum</i>		1209.6	1	18.6	20	106.1	20	16500	20	Yes	No	bumblebee/ hawkmoth	> 20h	2018. 08, 2019. 08		DQ536687	DQ535818
Cucurbitaceae	<i>Thladiantha dubia</i>		505.9	1	9.5	1	46.5	5			Yes	Yes	bumblebee	> 8h	2018. 08	MH710708	DQ536740	DQ535733
Fabaceae	<i>Astragalus Pullus</i>	<i>Astragalus alpinus</i>	44.4	1	8.3	8	21.1	20	17822	10	No	Yes	bumblebee	120h	2010. 08	KX954891	KX955060	KM360658
Fabaceae	<i>Lotus corniculatus</i>		122.3	21	16.9	1	20.1	20	52601	20	No	Yes	bumblebee	6.5h	2019. 08	DQ312207	JN894450	KM360864
Fabaceae	<i>Tibetia himalaica</i>		115.0	1	5.9	1	32.5	5	2622	10	No	Yes	bumblebee	120h	2010. 08	KX021440	KX021514	JQ933348
Fabaceae	<i>Trifolium pratense</i>		29.3	21	12.8	6	57.3	5	1297	20	No	Yes	bumblebee	120h	2010. 08	AF053171	KP230743	HM850419

Fabaceae	<i>Trifolium repens</i>		41.3	1	8.8	1	28.9	20	4256	20	No	Yes	bumblebee	120h	2010. 08	EU036988	AF522131	KM361020
Fabaceae	<i>Vicia bungei</i>		224.0	1	17.8	1	46.0	5	10858	10	No	Yes	bumblebee	120h	2010. 08	JX506192	JX505845	
Fabaceae	<i>Vicia sepium</i>		80.7	1	10.1	6	46.8	5	11100	10	No	Yes	bumblebee	120h	2010. 08	MH711085	JN896140	KF602195
Gentianaceae	<i>Gentiana crassicaulis</i>		183.0	1	12.4	1	25.5	20	46435	20	Yes	Yes	bumblebee	120h	2010. 08	MF506957	KC861277	KJ947509
Geraniaceae	<i>Geranium delavayi</i>		76.0	21	9.9	4	92.9	20	4008	20	Yes	No	bumblebee	120h	2010. 08	MH117555		MH116194
Geraniaceae	<i>Geranium sibiricum</i>		35.9	1	2.5	1	58.6	5	665	20	Yes	No	bumblebee	120h	2010. 08	DQ309044		
Lamiaceae	<i>Clinopodium megalanthum</i>	<i>Clinopodium macrostemum</i>	27.2	1	8.2	1	44.3	5			No	No	bumblebee	120h	2010. 08	JQ669083	MK601824	MK573374
Lamiaceae	<i>Clinopodium repens</i>	<i>Clinopodium vulgare</i>	17.4	1	8.3	9	45.8	15			No	No	bumblebee	120h	2010. 08	DQ667324	AY840153	KM360725
Lamiaceae	<i>Galeopsis bifida</i>		31.5	1	9.1	1	39.0	5			No	No	bumblebee	120h	2010. 08	EU827106	JN894038	KM360796
Lamiaceae	<i>Lamium amplexicaule</i>		61.7	1	12.0	1	39.5	5			Yes	No	bumblebee	120h	2010. 08	JX073974	JN894206	Z37399
Lamiaceae	<i>Nepeta laevigata</i>	<i>Nepeta cataria</i>	43.0	1	14.6	9	37.5	20			No	Yes	bumblebee	120h	2010. 08	KF803251	KT176606	Z37421
Lamiaceae	<i>Nepeta stewartiana</i>	<i>Nepeta straussii</i>	132.9	1	18.1	1	48.5	20	5173	20	No	Yes	bumblebee	120h	2010. 08	JF301404		
Lamiaceae	<i>Origanum vulgare</i>		11.0	1	6.7	2	21.3	5	1761	10	Yes	No	bumblebee	120h	2010. 08	DQ667243	FR719087	MG223937
Lamiaceae	<i>Phlomis forrestii</i>	<i>Phlomis mongolica</i>	59.7	21	9.0	1	27.0	20	39540	20	Yes	Yes	bumblebee	> 2h	2019.08		HQ839711	HM590103
Lamiaceae	<i>Prunella vulgaris</i>		28.6	21	12.2	5	40.9	20	3753	20	No	Yes	bumblebee	120h	2010. 08	AY506653	FJ395426	AY395556
Lamiaceae	<i>Salvia przewalskii</i>		665.6	21	36.3	5	63.9	20	21116	20	No	No	bumblebee	120h	2010. 08	KC473256	KC473388	KC473329
Lamiaceae	<i>Scutellaria hypericifolia</i>	<i>Scutellaria baicalensis</i>	235.4	1	19.9	7	25.8	5	14148	20	No	No	bumblebee	120h	2010. 08	KJ025067	HQ676586	HQ676582
Lamiaceae	<i>Stachys kouyangensis</i>	<i>Stachys cretica</i>	37.1	1	11.5	9	26.6	20	24728	20	No	No	bumblebee	120h	2010. 08	KF577385	HQ911554	HQ902767
Liliaceae	<i>Lilium lankongense</i>	<i>Lilium duchartrei</i>	2337.4	1	45.4	20	80.3	20	459107	20	Yes	No	butterfly	> 20h	2017. 08, 2018. 08, 2019. 08	HM045429		HQ692259
Liliaceae	<i>Lilium lancifolium</i>		4731	1	45.2	20	91.3	20			Yes	No	butterfly	> 20h	2019.08	HQ724825	KM085639	AB034937
Orchidaceae	<i>Spiranthes sinensis</i>		16.1	1	3.0	1	55.0	5			No	No	bumblebee	> 20h	2019.08	MF286494	LT600870	JF972913
Orobanchaceae	<i>Pedicularis cephalantha</i>		206.9	1	36.4	20	36.8	20	70352	25	No	Yes	bumblebee	> 100h	2017. 08, 2018. 08, 2019. 08	JN252741	JF955087	JF942976
Orobanchaceae	<i>Pedicularis confertiflora</i>		77.7	1	24.9	20	30.3	20	36423	25	No	Yes	bumblebee	> 100h	2017. 08, 2018. 08, 2019. 08	JN252745	JX091329	JF943221

Orobanchaceae	<i>Pedicularis densispica</i>		100.1	1	19.2	20	16.1	20	124786	25	No	Yes	bumblebee	> 100h	2017. 08, 2018. 08, 2019. 08	AY155290	JF955140	KT022733
Orobanchaceae	<i>Pedicularis dichotoma</i>		59.2	1	26.2	20	25.8	20	40489	25	No	Yes	bumblebee	> 100h	2017. 08, 2018. 08, 2019. 08	JN252754	JF955144	JF943034
Orobanchaceae	<i>Pedicularis rex</i>		288.2	1	41.3	20	20.8	20	330536	25	No	Yes	bumblebee	> 100h	2017. 08, 2018. 08, 2019. 08	MH117641	JF955275	KF011759
Orobanchaceae	<i>Pedicularis siphonantha</i>		183.7	1	64.4	20	40.5	20	23007	25	No	Yes	bumblebee	> 100h	2017. 08, 2018. 08, 2019. 08	JN252798	JF955315	JF943208
Orobanchaceae	<i>Pedicularis tricolor</i>		321.8	1	82.7	20	32.1	20	67353	25	No	Yes	bumblebee	> 100h	2017. 08, 2018. 08, 2019. 08	JN252806	JF955358	JF943252
Polygonaceae	<i>Fagopyrum esculentum</i>		4.9	1	3.8	30	44.0	20	915	20	Yes	No	fly	> 4h	2019.08	EF653685	JF829981	JN187116
Polygonaceae	<i>Polygonum macrophyllum</i>	<i>Polygonum aviculare</i>	17.5	21	3.9	6	51.5	5	1169	20	Yes	No	Halictidae	120h	2010. 08	HM357902	HM357913	AF297127
Primulaceae	<i>Androsace bulleyana</i>	<i>Androsace lactea</i>	29.1	1	1.2	1	23.8	5			No	Yes	Halictidae	> 4h	2019.08	AY275069	KF907850	
Primulaceae	<i>Primula poissonii</i>		70.5	1	7.9	60	15.9	60	21226	20	No	No	bumblebee/ butterfly	120h	2010. 08	KF934448	FJ828619	AF394973
Primulaceae	<i>Primula secundiflora</i>		244.4	1	11.3	60	15.7	60	33491	20	No	No	bumblebee/ butterfly	120h	2010. 08	AF396695	FJ828613	HM018353
Ranunculoideae	<i>Aconitum piepunense</i>	<i>Aconitum carmichaelii</i>	214.9	20	6.0	1	32.5	5	143865	20	Yes	No	bumblebee	> 4h	2019.08	AY571352	LC036464	FJ449849
Ranunculoideae	<i>Cimicifuga foetida</i>		2.9	1	2.6	1	21.8	5	23986	20	Yes	No	Syrphidae	120h	2010. 08	GQ351362		
Ranunculoideae	<i>Clematis akebioides</i>		563.9	1	9.3	1	25.1	20	195000	20	No	Yes	bumblebee	> 4h	2018.08, 2019.08	GU732574		
Ranunculoideae	<i>Thalictrum delavayi</i>		44.0	1	5.3	1	21.3	20	106750	20	Yes	Yes	Halictidae	120h	2010. 08	JX233684	MH116902	JX258352
Rosaceae	<i>Cotoneaster microphyllus</i>		36.0	1	2.5	1	26.0	5			No	Yes	bumblebee	0.5h	2019.08	JQ392380	JQ390958	JQ391295
Rosaceae	<i>Potentilla fruticosa</i>		140.9	1	3.1	1	19.3	20	104103	20	Yes	Yes	bumblebee	2h	2019.08	AF163478		U06818
Rosaceae	<i>Potentilla lancinata</i>		243.5	21	3.2	1	25.2	20	162480	20	Yes	Yes	Halictidae/ fly	120h	2010. 08	MH117676	MH116782	MH116328
Rosaceae	<i>Rosa soulieana</i>		654.2	1	5.0	1	27.8	20	264380	20	Yes	Yes	bumblebee	2h	2019.08	HM593907	FJ472529	
Rosaceae	<i>Spenceria ramalana</i>		53.0	21	4.0	1	21.9	20	154615	20	Yes	Yes	Halictidae	120h	2010. 08	MF786823		KX527087

Thymelaeaceae	<i>Daphne aurantiaca</i>	<i>Daphne laureola</i>	61.48	1	10.6	1	31.5	5	8059	20	Yes	No	moth	1.5h	2019.08	GQ167542	JN894952	HM849946
Violaceae	<i>Viola delavayi</i>		178.3	1	1.5	3	40.0	5	18949	20	No	No	Halictidae	> 4h	2019.08	FJ002908		

Outgroup

Magnoliaceae *Tasmannia lanceolata*

AY004128 AF543735 AY298851

Table S3. The results of trait correlations from 22 publications between pollen size and style length or stigma depth, and/or flower size, or pollen number. The phylogenetic independent contrast (PIC) analysis and measurement of flower size were involved only in a few lineages.

Authors	Year	Family or taxon	PIC analysis in pollen size	Style length	Flower size	Stigma depth	Pollen number
Germeraad et al.	1968	Sonneratiaceae		No			
Baker & Baker	1979	124 families		Positive			
Ganders	1979	24 families		No			
Cruden & Miller-Ward	1981	10 families		No			
Baker & Baker	1982	Onagraceae		Positive			
Plitmann & Levin	1983	Polemoniaceae		Positive			
Cruden & Lyon	1985	Umbellifer, Crucifera, Solanaceae		No		Positive	
		Polygonaceae		Positive		Positive	
Williams & Rouse	1990	Ericaceae		Positive			
Kirk	1993	Asteraceae		Positive			
Vonhof & Harder	1995	Fabaceae	Yes				Negative
Harder	1998	11 families		Positive	Positive		Negative
Stroo	2000	64 families		Positive			
Torres	2000	Asteraceae		Positive			
Sarkissian & Harder	2001	<i>Brassica rapa</i>					Negative
Aguilar et al.	2002	Solanaceae		Positive			No
Yang & Guo	2004	Orobanchaceae		Positive			Negative
Lopez et al.	2006	Nyctaginaceae		Positive (with starch)			No
		Nyctaginaceae		No (with lipids)			No
Cruden	2009	Fabaceae		No	No	Positive	
		Proteaceae			Positive		
Bedinger et al.	2011	Solanaceae					No
Jurgens et al.	2012	Caryophylloideae	Yes	Positive			No
Wang et al.	2016	Orobanchaceae	Yes	Positive	Positive	Positive	
Wu et al.	2017	<i>Fagopyrum</i>	Yes	No		Positive	

References

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