

SUPPLEMENTARY DATA

Table S1. Basic information of all known populations and experimental sites of studied species.

Species examined	Longitude	Latitude	Site	Note
<i>P.beesiana</i>	100.23	26.72	Heishui river, Lijiang, Yunnan, China	Pure <i>P. beesiana</i>
	101.51	27.42	Qiansuo Village, Yanyuan County, Sichuan, China	Pure <i>P. beesiana</i>
	103.18	26.09	Dongchuan County, Yunnan, China	Pure <i>P. beesiana</i>
	99.71	27.82	Lemahua, Zhongdian County, Yunnan, China	Pure <i>P. beesiana</i>
	103.80	25.50	Zhanyi, Qujing, Yunnan, China	Pure <i>P. beesiana</i>
	99.71	27.82	Haba snow mountain, Zhongdian, Yunnan, China	Pure <i>P. beesiana</i>
	101.28	27.93	Muli county, Sichuan, China	Pure <i>P. beesiana</i>
	104.26	23.38	Wenshan county, Yunnan, China	Pure <i>P. beesiana</i>
	Site 1	100.10	27.00	Wenhai village, Lijiang, Yunnan, China
<i>P.bulleyana</i>	100.85	27.28	Ninglang County, Lijiang, Yunnan	Pure <i>P. bulleyana</i>
	100.23	26.88	Baishui River, Lijiang, Yunnan, China	Pure <i>P. bulleyana</i>
Site 2	100.10	27.01	Heishui River, Lijiang, Yunnan, China	Pure <i>P. bulleyana</i>
Site 3	100.00	26.88	Lijing alpine botanical garden, Yunnan, China	<i>P.beesiana</i> and <i>P.bulleyana</i>
Site 4	100.10	27.02	Heishui river, Lijiang, Yunnan, China	<i>P.beesiana</i> and <i>P.bulleyana</i>

Table S2. Fruit set per flower and seed number per fruit for individual replicate plants within each treatment, for 16 pollination treatments.

Treatment,	Parent species ^a		Replicate	Fruit no./ Flower no.	Seed no. per fruit. ^b	
	<i>P. beesiana</i>	<i>P. bulleyana</i>				
1	P ♀	P ♂	1A	2/8	8	
			1B	1/8	25	
			1C	1/8	32	
			1D	2/8	1	
			1E	0/8	0	
2	T ♀	T ♂	2A	1/8	12	
			2B	1/8	16	
			2C	2/10	3	
			2D-E	0/19	0	
3		P ♀	P ♂	3A	1/6	5
				3B	2/7	17.5
				3C	1/6	2
				3D-E	0/12	0
4		T ♀	T ♂	4A	2/10	2.5
				4B	1/10	13
				4C	1/10	15
				4D-E	0/20	0
5	P ♀		T ♂	5A	9/9	84.67
				5B	7/9	71.18
				5C	8/8	100.86
				5D	9/9	73.63
				5E	6/8	113.5
6	P ♂		T ♀	6A	8/10	83.38
				6B	10/10	92.80
				6C	8/10	108.88
				6D	8/10	95.50
				6E	8/10	65.38
7		P ♀	T ♂	7A	10/10	108.60
				7B	8/10	99.2
				7C	10/10	76.75
				7D	9/10	79.3
				7E	10/10	85.11
8		P ♂	T ♀			

			8A	7/8	73.43
			8B	7/8	70
			8C	6/8	87.5
			8D	7/8	94.29
			8E	7/8	118
9	P ♀	T ♂			
			9A	6/9	71.83
			9B	5/9	84.4
			9C	4/9	105.25
			9D	5/9	89.75
			9E	4/8	80.5
10	P ♀	P ♂			
			10A	4/9	66.75
			10B	4/9	39.75
			10C	5/9	43.33
			10D	3/9	46
			10E	2/9	16.8
11	T ♀	T ♂			
			11A	4/9	94.75
			11B	4/9	50
			11C	6/9	28
			11D	7/9	34.71
			11E	6/9	51.33
12	T ♀	P ♂			
			12A	10/10	88.9
			12B	8/10	91.5
			12C	10/10	100.8
			12D	10/10	91.89
			12E	9/9	84
13	P ♂	P ♀			
			13A	3/10	37.33
			13B	2/10	75
			13C	2/10	30
			13D	2/10	82
			13E	1/10	36
14	P ♂	T ♀			
			14A	7/10	35.29
			14B	6/10	42
			14C	7/10	41.29
			14D	4/10	27.5
			14E	5/10	35.2
15	T ♂	P ♀			
			15A	4/10	28.5
			15B	5/10	48.4
			15C	3/10	60.33
			15D	2/10	30

16	T ♂	T ♀	15E	3/10	63
			16A-E	0/32	0

^a P - pin flower, T - thrum flower, ♀ - female parent, ♂ - male parent.

^b Note that number of replicates varies from 3 to 5 for seeds per fruit, because in certain treatments no fruits were produced.

Fig. S1. Shared pollinators of both parental species. (A, B) Hawkmoth, (C, D) *Bombus richardsi*, (E, F) *Issoria lathonia* and (G, H) *Bomnus sp.* visiting *P. beesiana* and *P. bulleyana*; scale bars = 2 cm.

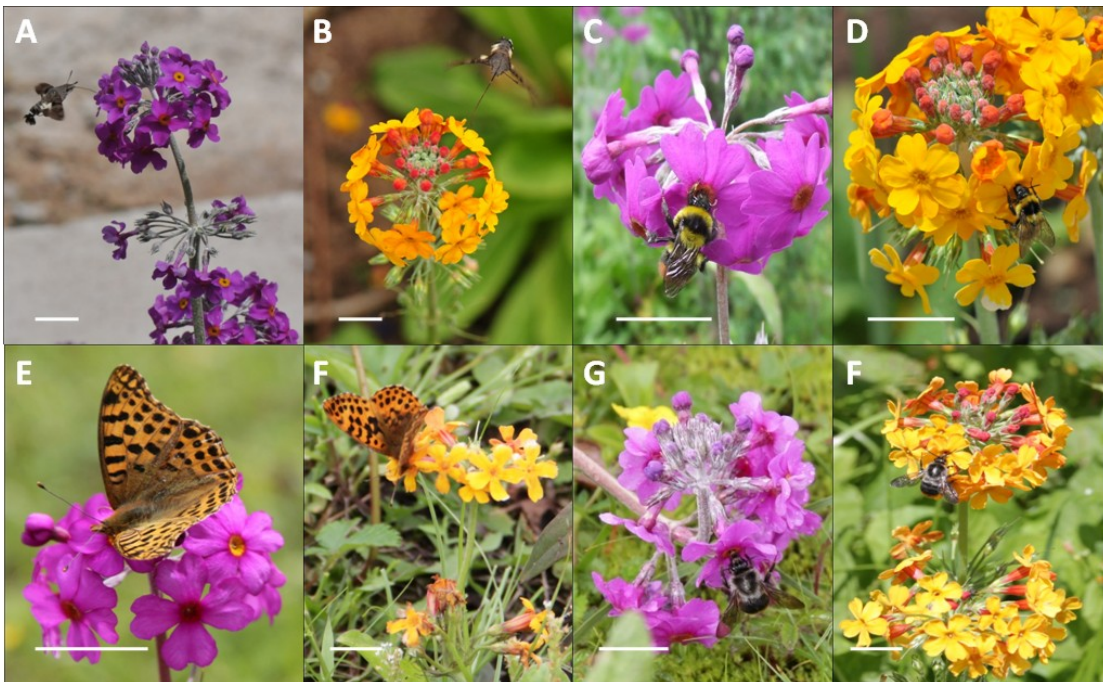


Fig. S2. Morphologies of aborted and viable seeds from four treatments observed using an X-ray imaging system. Seeds from (A) *P. beesiana* (P) ♀ × *P. beesiana* (T) ♂, (B) *P. bulleyana* (P) ♀ × *P. bulleyana* (T) ♂, and (D) *P. bulleyana* (T) ♀ × *P. beesiana* (P) ♂ show good embryos, whereas (C) *P. beesiana* (T) ♀ × *P. bulleyana* (P) ♂ all show aborted embryos.

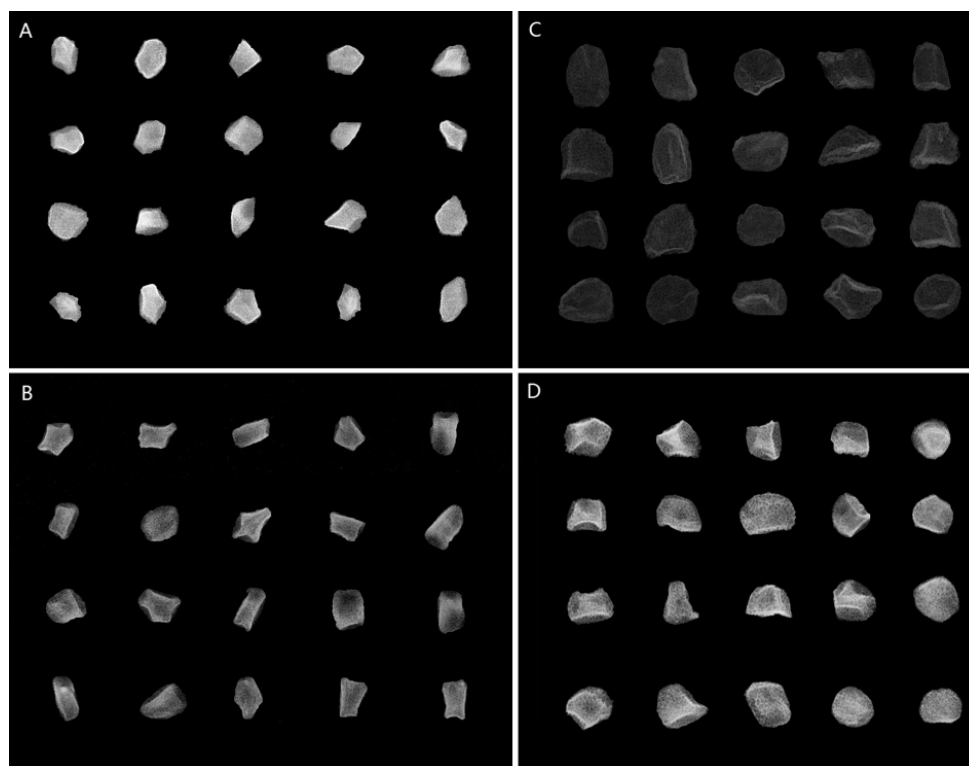


Fig. S3. Fluorescent microscope images of pollen germination and pollen tube growth. (A–C) *P. beesiana* pollen tubes growing in *P. bulleyana* style (A) 3 h, (B) 24 h, and (C) 48 h after pollen application to stigma. (D–F) *P. bulleyana* pollen tubes growing in *P. beesiana* style, (D) 3 h, (E) 24 h, and (F) 48 h after pollen application to stigma. (G–I) First pollen tubes penetrating ovules for *P. beesiana* ♀ × *P. bulleyana* ♂ after 24 hours (G) and many tubes reaching the ovules after 48 hours (I), and for *P. bulleyana* ♀ × *P. beesiana* ♂ pollen tubes have reached the ovules after 48 h (H). (J–Q) Abnormal pollen tube growth syndromes observed in heterospecific crosses: (J) callose deposition on the papilla cell of stigma surface, (K) pollen tubes coil on the surface of stigma, (L) pollen tubes curving and folding, (M) swelling on the tip of pollen tubes, (N) growth inhibition as a result of thinner tip of pollen tubes, (O) spiral curving of pollen tubes, (P, Q) callose deposition in the ovary, (R) detail of a pollen tube successfully penetrating an ovule. Scale bars: (A–F), (J–O), (R) = 200 μm; (G–I), (P, Q) = 500 μm.

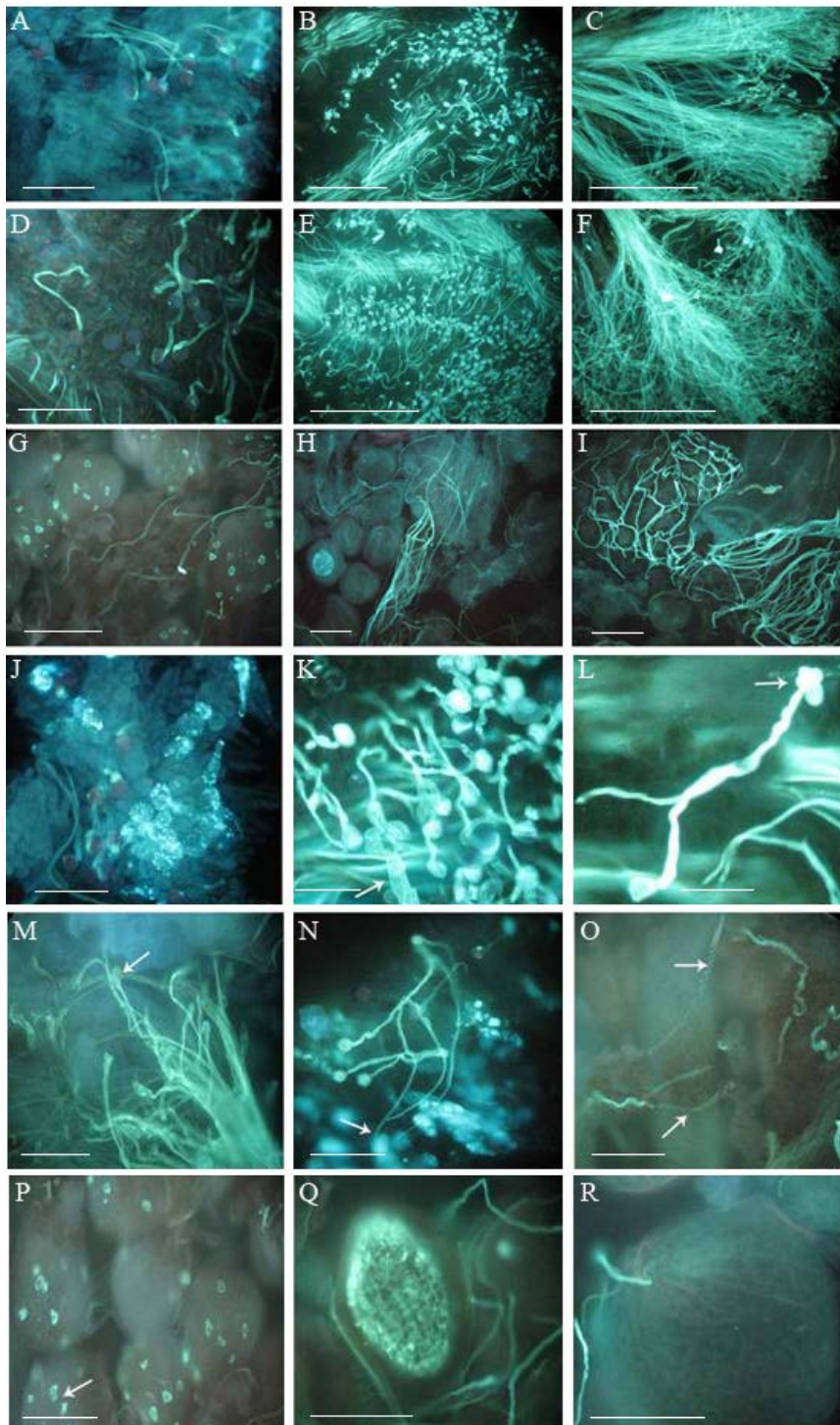


Fig. S4. Estimated number of populations (K) derived from the STRUCTURE clustering analyses. Mean probabilities of the data for 5 replicated runs (below) and ΔK (above) are plotted as a function of the number of clusters (K from 1 to 10).

