

Supporting Information – Mackin *et al.* (2021)

Rapid evolution of a floral trait following acquisition of novel pollinators

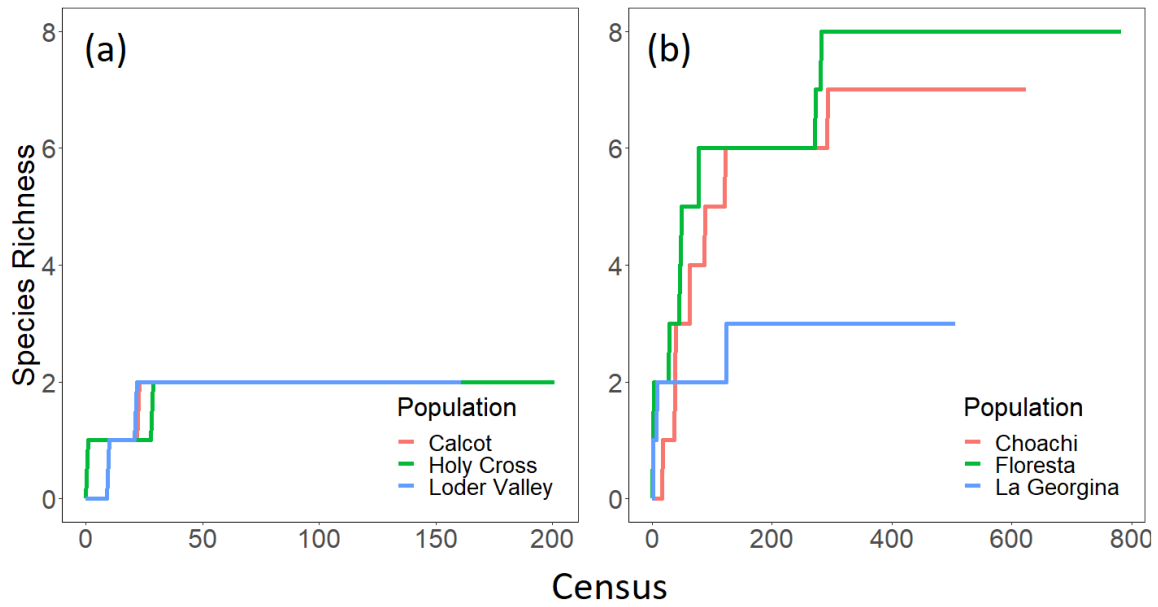


Figure S1. Species accumulation curves of pollinators in (a) the native range, where we performed between 140 and 200 pollinator censuses per population, and (b) the non-native range, with more than 500 censuses per population.

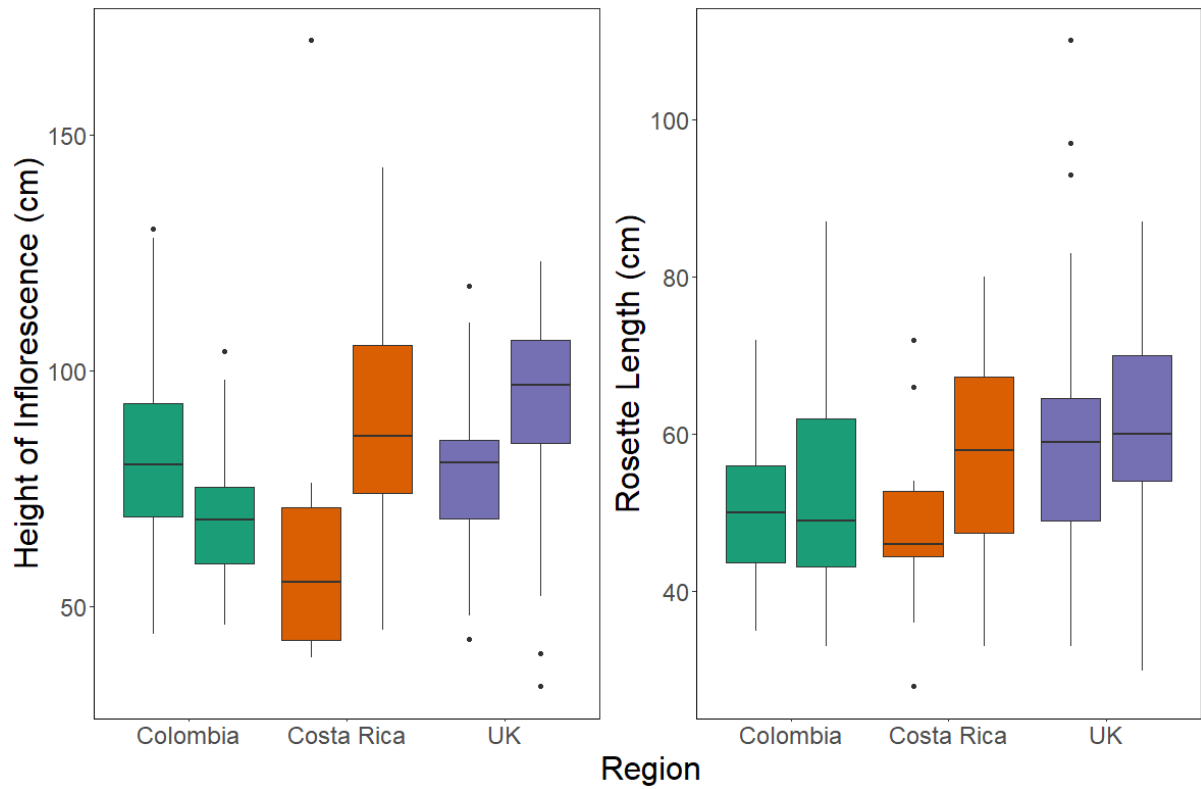


Figure S2. Comparison of the height of the inflorescence to the first flower (peduncle; left panel), and rosette length (= diameter; right panel) in introduced (Colombia and Costa Rica) and native (UK) *Digitalis purpurea* populations (N = 10 to 60 plants per population).

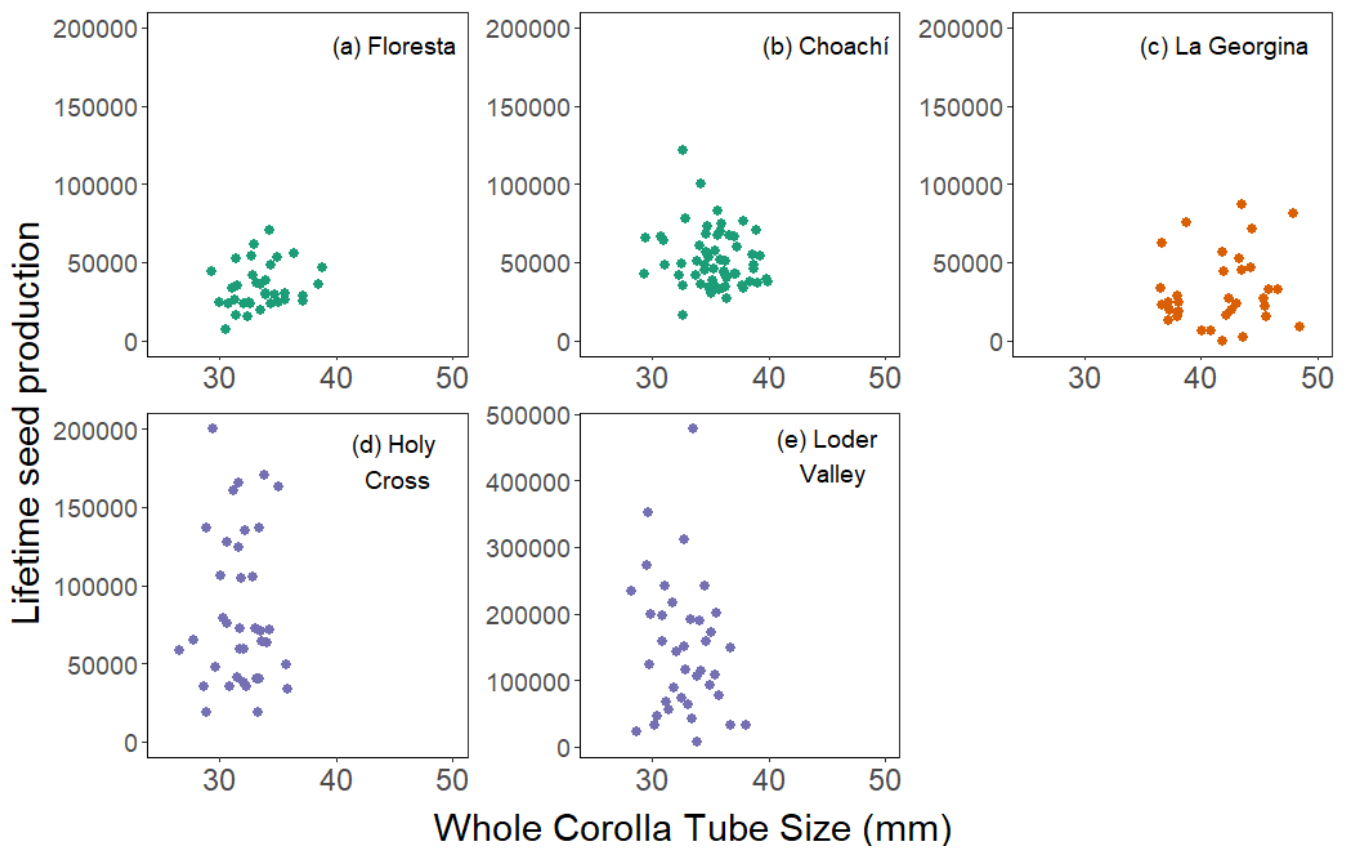


Figure S3. Relationship between the whole corolla tube size and the lifetime seed production in individual *Digitalis purpurea* plants. Whole corolla tube was measured as the geometric mean of proximal corolla tube length and width. **Non-native populations** (a) Floresta, Colombia, (b) Choachí, Colombia, and (c) La Georgina, Costa Rica. **Native populations** (d) Holy Cross and (e) Loder Valley in the UK.

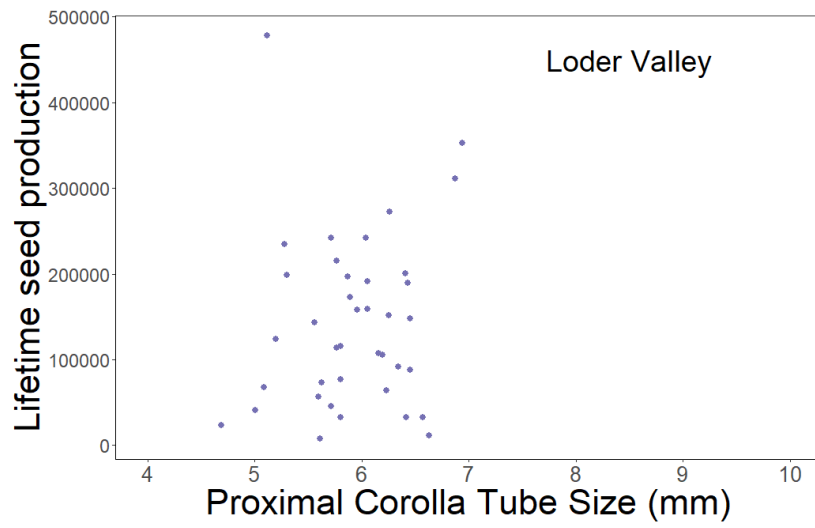


Figure S4. Relationship between the proximal corolla tube size and lifetime seed production, for the native population Loder Valley in the UK. Proximal corolla tube was measured as the geometric mean of proximal corolla tube length and width.

Table S1. Pollinating and non-pollinating flower visitors to *Digitalis purpurea* plants in all populations. The number and percentage of visits include non-robbing visits only. Note that the surveying effort was higher in the non-native populations; number of 3-minute pollinator census per population: Loder Valley: 161 censuses, Holy Cross: 201, Calcot Wood: 140, Choachí: 624, Floresta: 524, La Georgina: 506. See main text for more details on the methods.

Region	Population	Functional Visitor Group	Species	Pollinator?	Nectar robber	Percentage of visits	Number of Visits
UK (native)	Loder Valley	Bumblebee	<i>Bombus hortorum</i>	Yes		89.3	184
			<i>Bombus pascuorum</i>	Yes		6.3	13
		Solitary bee	<i>Andrena sp.</i>			1.0	2
		Hoverfly	<i>Episyrphus sp.</i>			3.4	7
	Holy Cross	Bumblebee	<i>Bombus hortorum</i>	Yes		83.2	407
			<i>Bombus pascuorum</i>	Yes		0.8	13
		Solitary bee	<i>Anthidium manicatum</i>			4.1	20
			<i>Lasioglossum fulvicorne</i>			1.0	5
			<i>Lasioglossum sp.</i>			3.9	19
			<i>Andrena sp.</i>			1.4	7
		Hoverfly	<i>Episyrphus sp.</i>			3.9	19
			<i>Eumerus sp.</i>			0.2	1
			<i>Epistrophe sp.</i>			0.8	4
			<i>Apis mellifera</i>			0.6	3
	Calcot Wood	Bumblebee	<i>Bombus hortorum</i>	Yes		94.6	501
			<i>Bombus pascuorum</i>	Yes		1.1	6
		Honeybee	<i>Apis mellifera</i>			1.1	6
Solitary bee		<i>Lasioglossum calcarium</i>			0.4	5	
		<i>Lasioglossum fulvicorne</i>			0.9	1	
		<i>Anthidium manicatum</i>			0.9	5	
Butterfly		<i>Pieris brassicae</i>			0.2	1	
		<i>Maniola jurtina</i>			0.8	4	

Colombia (non-native)	Choachí	Bumblebee	<i>Bombus robustus</i>	Yes	Yes	60.9	414
			<i>Bombus funebris</i>	Yes	Yes	13.4	91
			<i>Bombus rubicundus</i>	Yes	Yes	4.6	31
			<i>Bombus atratus</i>	Yes	Yes	0.4	3
		Hummingbird	<i>Aglaeactis cupripennis</i>	Yes	Yes	1.5	10
			<i>Eriocnemis vestita</i>	Yes	Yes	17.5	119
			<i>Eriocnemis cupreovertris</i>	Yes		1.8	12
		Flower Piercer	<i>Diglossa sp.</i>		Yes		
		Honeybee	<i>Apis mellifera</i>		Yes		
		Floresta	Bumblebee	<i>Bombus hortulanus</i>	Yes	Yes	87.6
<i>Bombus atratus</i>	Yes				6.2	61	
Hummingbird	<i>Coeligena bonapartei</i>		Yes		3.3	32	
	<i>Coeligena prunelli</i>		Yes	Yes	0.3	3	
	<i>Heliodoxa rubinoides</i>		Yes		0.6	6	
	<i>Coeligena torquata</i>		Yes		1.6	16	
	<i>Chrysolampis mosquitus</i>		Yes		0.4	4	
Honeybee	<i>Apis mellifera</i>			Yes			
Butterfly	<i>Pieridae sp.</i>			Yes			
Tyrant Bird	<i>Tyrannidae sp.</i>			Yes			
Costa Rica (non-native)	La Georgina	Bumblebee	<i>Bombus epiphatus</i>	Yes	Yes	72.3	518
		Hummingbird	<i>Eugenes fulgens</i>	Yes		19	136
			<i>Selaphorus flammula</i>	Yes	Yes	8.7	62

Table S2. Bee tongue length measurements from the literature (mean \pm standard deviation or standard error); N= sample size.

Region	Bumblebee Species	Tongue Length (mm)	N	Author
Native (UK)	<i>Bombus hortorum</i>	12.9 \pm 0.8 SD	10	Goulson and Darvill 2004
		11.8	n/a	Kwak 1978
	<i>Bombus pascuorum</i>	8.5 \pm 0.6 SD	10	Goulson and Darvill 2004
		8.6	n/a	Kwak 1978
Non-native (South America)	<i>Bombus atratus</i>	8.4 \pm 0.65	34	Arbulo <i>et al.</i> 2011
	<i>Bombus robustus</i>	6.9 \pm 0.55 SD	5	this study
	<i>Bombus rubicundus/hortulanus</i>	6.9 \pm 0.4 SD	10	Riveros <i>et al.</i> 2006
Non-native (Central America)	<i>Bombus ephippiatus</i>	11.1 \pm 0.34 SE	10	Del Carmen Salas-Arcos <i>et al.</i> 2019

Note: Measures for *B. rubicundus* and *B. hortulanus* are combined in the original publication.

Table S3. Directional and quadratic selection coefficients (\pm standard errors) for the whole corolla tube length and height in each population of *D. purpurea*. Values that are statistically significant from zero are indicated by * ($P < 0.05$).

Trait	Country	Population	Directional (β)	Non-Linear (γ)
Whole Corolla Tube Length	UK	Loder Valley	-0.07 ± 0.117	-0.16 ± 0.097
	UK	Holy Cross	0.07 ± 0.099	0.00 ± 0.000
	Costa Rica	La Georgina	0.20 ± 0.123	0.00 ± 0.000
	Colombia	Floresta	0.11 ± 0.072	0.00 ± 0.000
	Colombia	Choachí	0.02 ± 0.047	0.00 ± 0.000
Whole Corolla Tube Height	UK	Loder Valley	-0.15 ± 0.115	0.07 ± 0.072
	UK	Holy Cross	-0.06 ± 0.101	0.00 ± 0.000
	Costa Rica	La Georgina	0.03 ± 0.129	0.00 ± 0.000
	Colombia	Floresta	0.04 ± 0.076	0.00 ± 0.000
	Colombia	Choachí	$-0.10 \pm 0.044^*$	0.00 ± 0.000

References

- Arbulo, N., Santos, E., Salvarrey, S., Invernizzi, C. (2011). Proboscis length and resource utilization in two Uruguayan bumblebees: *Bombus atratus* Franklin and *Bombus bellicosus* Smith (Hymenoptera: Apidae). *Neotropical Entomology*, 40, 72–77. <https://doi.org/10.1590/s1519-566x2011000100010>
- del Carmen Salas-Arcos, L., Lara, C., Castillo-Guevara, C., Cuautle, M., Ornelas, J.F. (2019). “Pro-bird” floral traits discourage bumblebee visits to *Penstemon gentianoides* (Plantaginaceae), a mixed-pollinated herb. *The Science of Nature*, 106, 1. <https://doi.org/10.1007/s00114-018-1595-4>
- Goulson, D., Darvill, B. (2004). Niche overlap and diet breadth in bumblebees; are rare species more specialized in their choice of flowers? *Apidologie*, 35, 55–63. <https://doi.org/10.1051/apido:2003062>
- Kwak, M. M. (1978). Pollination, hybridization and ethological isolation of *Rhinanthus minor* and *R. serotinus* (Rhinanthoideae: Scrophulariaceae) by bumblebees (*Bombus* Latr.). *Taxon*, 145-158. <https://doi.org/10.2307/1220235>
- Riveros, A. J., Hernández, E.J., Nates-Parra G. (2006). Morphological constraints and nectar robbing in three Andean bumble bee species (Hymenoptera, Apidae, *Bombini*). *Caldasia*, 28, 111–114.