Hybridisation between the British and the Spanish bluebell

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The bluebell hybrid zone in Spain

In Northern Spain populations of the British bluebell, *Hyacinthoides non-scripta*, and its Spanish sister species, *Hyacinthoides hispanica*, co-occur within close range and hybrid individuals have been noted along the Cantabrian Mountains (Fig. 1a: green triangles). Here, we focus on a hybrid zone around Ponferrada, which expands over 120km from north to south (Fig. 1b). Population structure analyses on allozyme data cluster the hybrids into one group (Fig. 1b – green triangles), though, separating them from the unmixed parental populations (yellow and blue points).

Gene flow is mainly mediated through pollen exchange (paternal lineage), because seed dispersal and occasionally clonal buds are only important on an imminent range. In monocots the chloroplast is usually transitioned by seeds, thus chloroplast markers are useful to track the maternal lineage. The chloroplast haplotype of *H. non-scripta* reaches into the core contact zone, whereas several *H. hispanica* haplotypes contribute only to the more southern hybrid populations (Fig. 1b – pie charts).

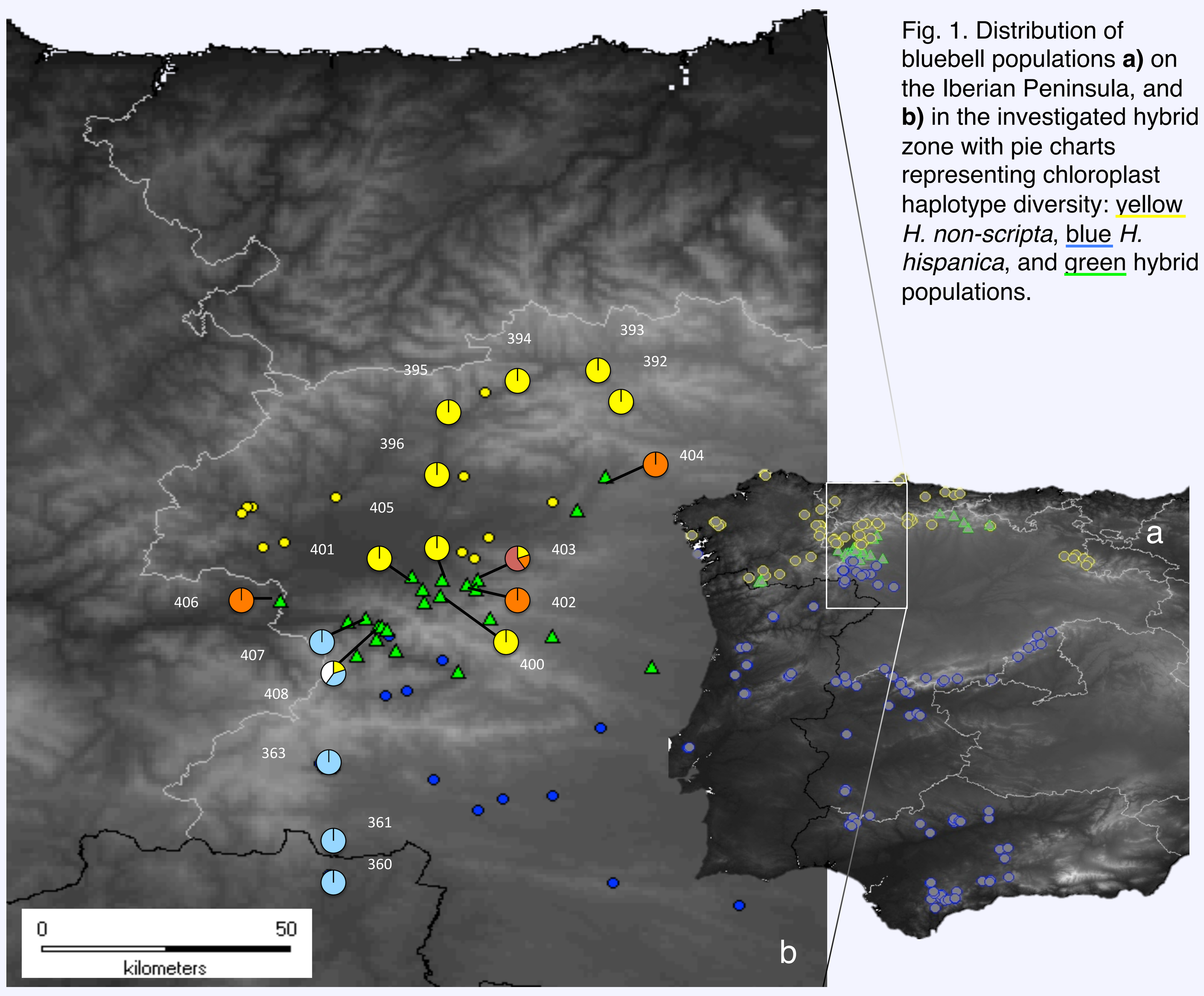
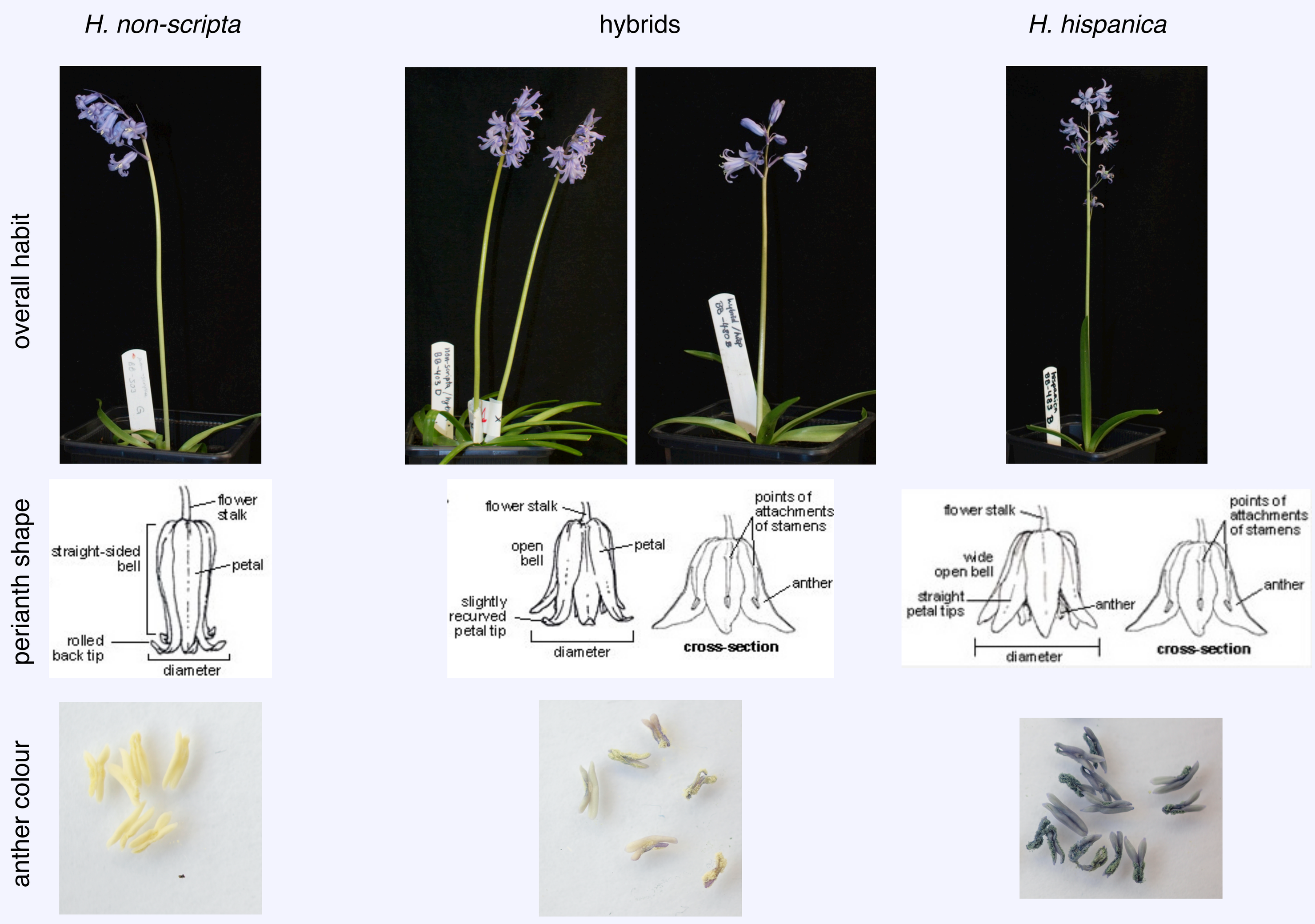


Fig. 1. Distribution of bluebell populations **a)** on the Iberian Peninsula, and **b)** in the investigated hybrid zone with pie charts representing chloroplast haplotype diversity: yellow *H. non-scripta*, blue *H. hispanica*, and green hybrid populations.

In spring 2013, we visited 39 different sites in this region and collected about 400 DNA samples and 131 living plants. The DNA material will be subject to molecular studies investigating the introgression of genes. The living material is being used to explore the hybrid vigour and subject to morphological characterisation of the hybrids. The British and Spanish bluebell can be differentiated by their stalk and the directions of the flowers, as well as the perianth shape and pollen colour:



Experiments

1. Morphological analyses

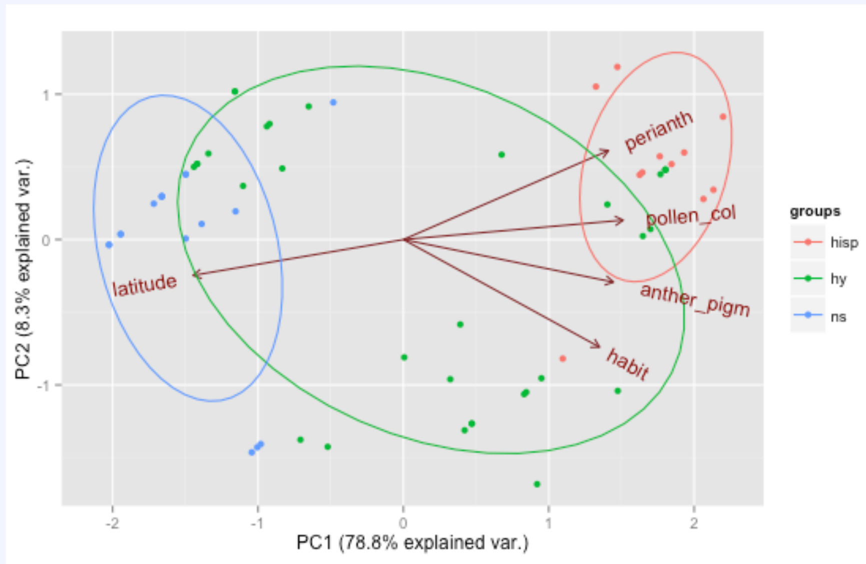
In the core zone intermediate hybrid morphologies dominate. However, during field collections we observed that the hybrid's flower morphology and pollen colour differs between the northern and southern region of the contact zone: to the north the flowers resemble more the *H. non-scripta* flowers, and to the south *H. hispanica*, respectively. To test this, we scored 60 collected individuals for their habit, perianth shape, pollen colour and degree of pigmentation in the anther epidermis. For clustering we performed a principal component analysis including the latitude.

2. Crossing and germination experiments

To test the fitness of bluebell hybrids and the frequency of F1-hybrid formation we performed crosses between individuals from different populations for either parent taxa, or the hybrids. A number of individuals were also pollinated with their own pollen to test the presence of self-incompatibility in bluebells and hybrids. Successful seed production, yet alone, does not proof viability. Thus a random subset of 10 seeds per functioning cross was germinated.

Results

The principal component analysis scatterplot shows that the given parameter were able to differentiate both parent species. Hybrid individuals seem morphologically either close to each parent or intermediate.

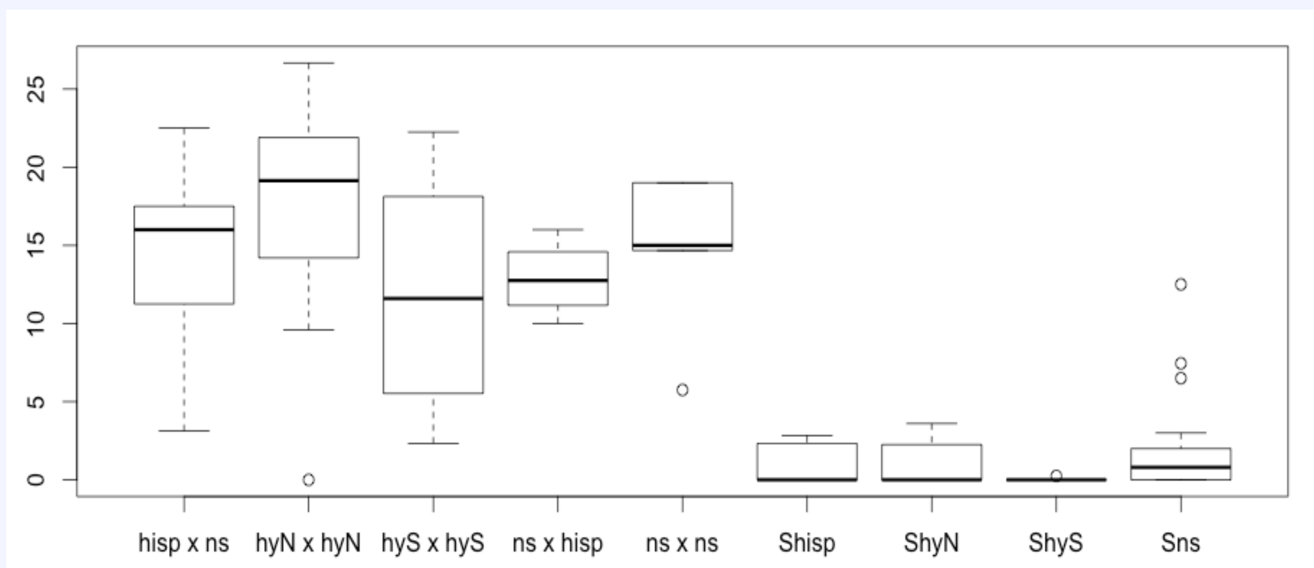


The table shows the crossing and germination results: Most outbreeding crosses resulted in flowers with produced seeds. The exception is one cross of hybrid north individuals that failed completely. The majority of selfing crosses failed. However, if fertilisation was successful there were a few seeds per capsule found. The maximum seeds per capsules in bluebells has been shown to be 25-30.

Overall, 67.22% of the seeds germinated within ten months of sowing – even seeds originating from selfing emerge into seedlings.

		seed set				germination			
		N	% crosses with no seeds	mean seeds/ seed bearing capsules	SD	N seeds	mean seedlings/ cross	SD	total % germinated by Apr'15
selfing	ns	16	50.00%	4.23	4.221	20	5.50	0.707	35.00%
	hyNorth	7	57.14%	2.70	0.819	10	8.00	NA	80.00%
	hySouth	8	87.50%	1.00	NA	NA	NA	NA	NA
	hisp	6	66.67%	2.82	0.024	20	7.00	2.828	70.00%
F1 hybrids	ns♀-hisp	4	0.00%	12.88	2.477	40	6.5	3.109	65.00%
	hisp♀-ns	5	0.00%	14.08	7.319	50	7.20	1.924	72.00%
between populations of same region	ns	6	0.00%	15.06	4.144	60	4.33	3.266	43.33%
	hyNorth	6	14.29%	20.07	4.887	60	7.83	3.061	78.33%
	hySouth	11	0.00%	12.15	6.737	90	8.11	1.764	81.11%

The box plot shows the mean number of seeds per capsule for each type of cross. Selfing has the lowest seed set. There is no significant difference between parent crosses or the hybrid crosses.



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Conclusion

- Phenotypes of either parent species are present in the hybrids.
- Bluebells have a self-incompatibility system, which is also present in the hybrids.
- There is no strong difference between the mean fitness of hybrids or *H. non-scripta*.