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## Pollination ecology in *Lupinus pilosus*

### Abstract

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Pollination ecology was studied in *Lupinus pilosus* with respect to floral colour change. White-phase banner-spot flowers offered more pollen as reward to foraging pollinators than purple-phase flowers did. The changes in colour and reward were recognized by the pollinators. They preferred pre-change flowers and avoided post-change ones. The change in the banner spot from white to purple was enhanced by pollination. Pollination experiments indicate that shortage in resources rather than in pollination was the limiting factor for fruit set, especially in the upper flower whorls.

### Introduction

Floral colour change is taxonomically and geographically widespread, occurring in at least 214 genera and 74 families, i.e., in at least 21 % of all angiosperm families (Weiss 1991). Pre-change flowers contain more nectar or pollen reward than post-change flowers do, are receptive, and have viable pollen, in contrast with post-change ones (Casper & La Pin 1984, Eisikowitch & Lazar 1987, Weiss 1991). Most pollinators, bees, butterflies, moths, flies, beetles, and birds (Weiss 1991), discriminate between floral colour phases, which influence their movement and behaviour. By visiting the reward-rich pre-change flowers, pollinators increase their foraging efficiency (Heinrich 1975). By visiting these young, or unpollinated, pre-change flowers they also increase the probability of pollination (Schaal & Levrich 1980, Cruzan & al. 1988, Gori 1989). As a result, floral colour change benefits both plant and pollinator.

Several aspects concerning the breeding system and flower mechanism were studied in *Lupinus pilosus* L. (e.g. by Plitmann & al. 1980, Pazy & al. 1981, Pazy 1984, Horovitz & Harding 1983, Alon 1986). However, no research has yet been done on the pollination ecology of any of the Old-World lupins, in contrast to the New-World ones (e.g., Schaal & Levrich 1980, Gori 1983, 1989, Juncosa & Webster 1989, Stead & Reid 1990, Karoly 1992).

*Lupinus pilosus* is an east Mediterranean annual species that grows in the Mediterranean regions of Israel in open fields, on a variety of soil types. It blooms in spring and

forms dense blue patches seen from a distance. The flowers are arranged in racemes made up of 5-flowered whorls. All flowers within a whorl open on the same day, with whorls maturing acropetally. The flowers are blue, with a white banner-spot on the standard petal at anthesis that turns pink, and later on purple. Pollen remains viable, and the stigma receptive, throughout the flowering time (Alon 1986). Bees visiting the flower land on the wing petals, pressing them and the keel down. As a result, some pollen is squeezed out and gathered by the bees.

The aim of the present research was to study the ecological significance of floral colour change in *Lupinus pilosus* L. both for the plant and the pollinator.

### Materials and methods

The current investigation was carried out in spring 1991, with supplementary observations in spring 1993, at Mt Carmel, Israel (35°04'E, 32°38'N).

20 plants were randomly chosen in a 1000 m<sup>2</sup> patch and bagged in fine nylon mesh that prevented visitation by insect pollinators; 10 of them served as control, the other 10 were artificially cross-pollinated on the first day of anthesis. The duration of each colour phase was recorded for each of the 5 flowers in the lowermost and of the 5 in the second lowest whorl, in each of the controls and handled plants. Fruit set was recorded for both flower whorls. Two-way analysis of variance (ANOVA) was used to test significance of the influence of flower position, by whorl, and of artificial pollination, on the duration of the various colour phases of the banner spot, and on fruit set. The significance (P) of the F statistics, for the ANOVA model, was tested by Duncan's multiple range test. R<sup>2</sup>, the proportion of the variance explained by the ANOVA model, was calculated (Sokal & Rohlf 1981).

Fresh flowers were collected in the field and brought to the laboratory for pollen weighing. The amount of pollen released by a single triggering of 10 flowers in each colour phase was weighed, as well as the total amount of their pollen content. Weighing was done with a Sartorius 2001 MP2 analytical balance, scaled to 0.1 mg.

The foraging behaviour of *Apis mellifera* and *Anthophora* sp. was observed for about an hour on each day, in each site. The flowers of each banner-spot colour phase that were visited by bees were counted. Foraging time of honey-bees in single flowers of each colour phase was measured. On each observation day, the number of flowers of each colour phase at the observation site was counted, and the proportion of each phase was calculated. A  $\chi^2$  test was used, to test the hypothesis of randomness of bee visits in relation to colour stages.

Statistical analyses were carried out by SAS PC (Anonymous 1988).

### Results

*Pollen reward.* – The amount of pollen released by a single triggering (Fig. 1) was significantly different in different banner-spot colour phases of open-growing, unhan-

Table 1. Two-way ANOVA results testing the effect of flower position (either in the first or second whorl), pollination, and the interaction of both, on duration of the various colour phases of banner spot (BS) and on fruit set. – df = degrees of freedom. – F = F statistics; P = significance level of Duncan's multiple range test; R<sup>2</sup> = proportion of variance explained by the ANOVA model.

<i>Variable &amp; source of variation</i>	<i>df</i>	<i>Sum of squares</i>	<i>Mean square</i>	<i>F</i>	<i>P</i>
<b>White BS duration</b>					
Model R <sup>2</sup> = 0.54	3	23.05	7.68	14.01	< 0.0001
Error	36	19.75	0.55		
Corrected total	39	42.80			
Effect of whorl	1	6.56	6.56	11.69	0.0014
– of pollination	1	14.64	14.64	26.69	0.0001
– of whorl * pollination	1	1.85	1.85	3.37	0.0746
<b>Pink BS duration</b>					
Model R <sup>2</sup> = 0.05	3	1.09	0.36	0.63	0.5989
Error	36	20.70	0.58		
Corrected total	39	21.79			
Effect of whorl	1	0.84	0.84	1.46	0.2344
– of pollination	1	0.03	0.03	0.04	0.8360
– of whorl * pollination	1	0.23	0.23	0.39	0.5356
<b>Purple BS duration</b>					
Model R <sup>2</sup> = 0.21	3	7.17	2.39	3.21	0.0343
Error	36	26.79	0.74		
Corrected total	39	33.96			
Effect of whorl	1	2.60	2.60	3.50	0.0697
– of pollination	1	4.49	4.49	6.03	0.0190
– of whorl * pollination	1	0.08	0.08	0.11	0.7434
<b>Fruit set</b>					
Model R <sup>2</sup> = 0.38	3	5790.00	1930.00	14.01	< 0.0001
Error	36	490.00	265.55		
Corrected total	39	15350.80			
Effect of whorl	1	5290.00	5290.00	19.92	< 0.0001
– of pollination	1	490.00	490.00	1.85	0.1828
– of whorl * pollination	1	10.00	10.00	0.04	0.8472

dled flowers ( $n = 30$ ,  $F_{2,27} = 76.12$ ,  $P < 0.0001$ ,  $R^2 = 0.849$ ). The total amount of pollen in these same flowers (Fig. 1) was also significantly different, depending on banner-spot colour ( $n = 30$ ,  $F_{2,27} = 90.00$ ,  $P = 0.0001$ ,  $R^2 = 0.869$ ).

*Flower colour change.* – Under the two-way ANOVA test, the effect of artificial pollination of the flowers, on their first day of anthesis, and of their position, either in the first or second whorl, on the duration of the white banner-spot colour phase was significant (Table 1). Pollination and position both had a significant effect, but the interaction

between the two had no effect (Table 1). The white phase was shorter in the second whorl than in the first, and was shortened by pollination in both whorls (Fig. 2), so that pollination enhanced colour change.

Under the same test, the effect of artificial pollination and flower position on the duration of the pink banner-spot colour phase was not significant (Table 1). The effect of artificial pollination, but not of flower position, on the duration of the purple banner-spot colour phase was significant (Table 1). The purple banner-spot colour phase was longer in pollinated flowers (Fig 2).

*Fruit set.* – Under the test, the effect of flower position on fruit set was significant (Table 1), the fruit set being lower in the second than in the first whorl (Fig. 3). Fruit set, in both whorls, tended to be higher in artificially pollinated flowers than in untreated ones (Fig. 3), but the differences were not significant (Table 1).

*Foraging behaviour of pollinators.* – Pollen gathering honey-bees were the major pollinators of *Lupinus pilosus*. Solitary bees and flower beetles were also found foraging on the flowers. In 4 of 5 counts, honey-bees and *Anthophora* sp. significantly preferred the white banner-spot colour phase flowers and avoided the purple ones (Table 2). The pink

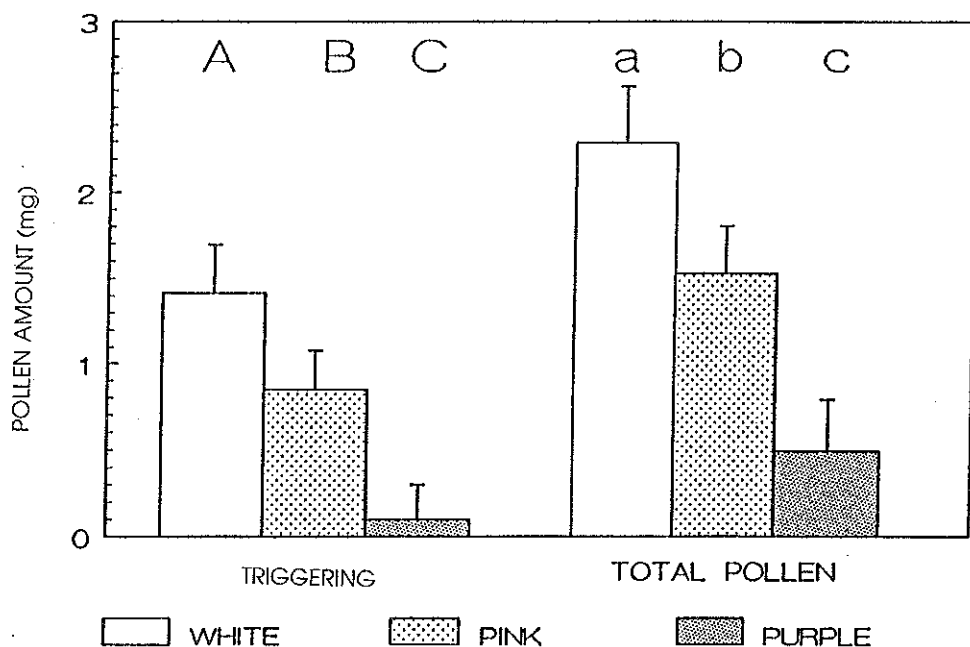


Fig. 1. The weight (mg) of pollen released per triggered *Lupinus pilosus* flower, and the total weight of pollen in the flower, in each banner-spot colour phase. – Vertical bars represent standard deviation, equivalent columns with different letters are significantly different ( $P < 0.05$ ).

colour phase, being an intermediate transit phase, received more visits than expected in some cases, and in other cases less.

The mean time spent by honey-bees foraging on flowers of various banner-spot colour phase flowers was  $15.4 \pm 15$  sec on the white ( $n = 30$ ),  $16.3 \pm 16.6$  sec on the pink ( $n = 34$ ), and  $8.0 \pm 5.1$  sec on the purple phase ( $n = 19$ ). The difference seems large, but it is not significant ( $F_{2,80} = 2.26, P = 0.1106$ ). However, when the white and pink flowers are treated as one group, since the foraging time spent on them was almost identical, the mean foraging time was  $15.9 \pm 16.0$  sec ( $n = 64$ ) on white/pink flowers, and the difference with the purple flowers becomes significant ( $F_{1,81} = 4.51, P < 0.05$ ).

**Discussion**

*Mating system.* – Many annual lupines are self-compatible (Juncosa & Webster 1989). *Lupinus pilosus* was previously found to be a self-compatible autogamous species, in which triggering of the flower enhanced self-pollination (Pazy 1984, Alon 1986). Artificial cross-pollination had no effect on fruit set, indicating high efficiency of spontaneous self-pollination. The results indicate that no limitation of self-pollination can be reported for *L. pilosus*. This conclusion agrees with previous reports (Plitmann & al.

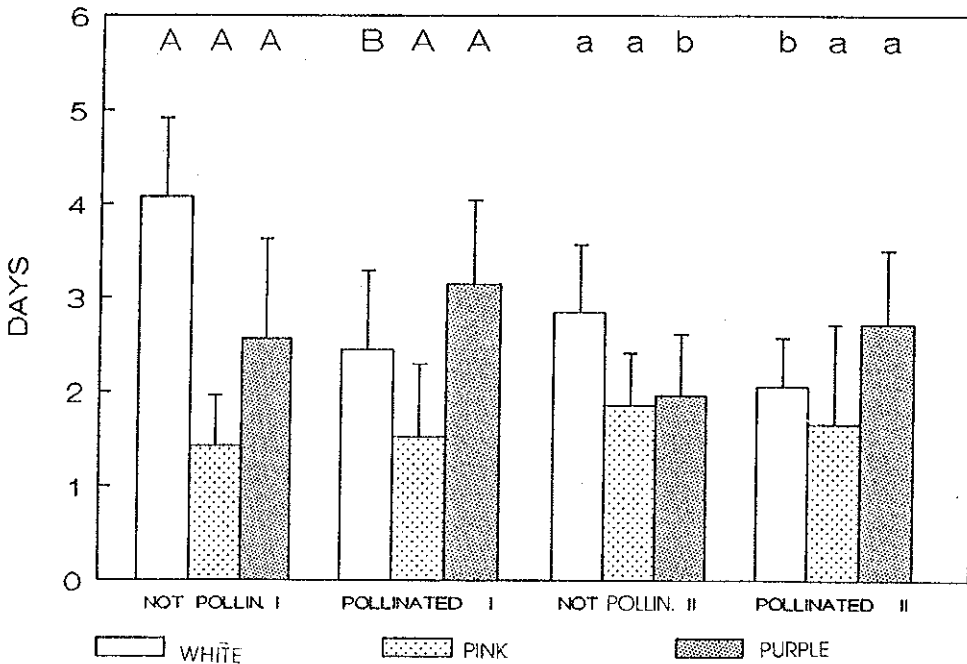


Fig. 2. The effect of artificial cross-pollination on the duration of each banner-spot colour phase (white, pink, purple) in the lowermost (I) and second lowest (II) whorl of *Lupinus pilosus* flowers. – Vertical bars represent standard deviation, equivalent columns with different letters are significantly different ( $P < 0.05$ ).

Table 2. Comparison between the observed (obs) number (n) of visited flowers of each banner spot (BS) colour phase, and the expected (exp) one. Difference ( $\Delta$ ) either not significant (ns:  $P > 0.05$ ), or significant (\*\*\*:  $P < 0.001$ ).

Date	Site	Pollinator	White BS		Pink BS		Purple BS		$\chi^2$	$\Delta$	
			n	%	n	%	n	%			
27. Mar 1991	1	honey-bee	exp	35.5	67.0	11.7	22.0	5.8	11.0	4.15	ns
			obs	35.0	66.0	16.0	30.0	2.0	4.0		
28 Mar 1991	1	honey-bee	exp	23.4	33.0	32.0	45.0	15.6	22.0	31.86	***
			obs	44.0	62.0	11	15.5	16.0	22.0		
15 Mar 1993	1	honey-bee	exp	198.5	63.0	50.4	16.0	66.1	21.0	56.36	***
			obs	249.0	79.0	60.0	19.0	6.0	2.0		
15 Mar 1993	3	honey-bee	exp	151.0	50.5	49.3	16.5	98.7	33.0	168.80	***
			obs	260.0	87.0	31.0	10.5	8.0	2.5		
15 Mar 1993	2	<i>Anthophora</i>	exp	87.6	63.0	22.2	16.0	29.2	21.0	43.91	***
			obs	123.0	79.0	15.0	19.0	1.0	2.0		

1980, Pazy & al. 1981, Pazy 1984, Alon 1986). In two other populations fruit set in spontaneous self-pollinated flowers was lower than in artificially self-pollinated flowers and in open-pollinated flowers (Pazy 1984, Alon 1986). However, an outcrossing rate of 10-60 % was reported for *L. pilosus* (Horovitz & Harding 1983). Similarly, no limitation of self-pollination and no differences in outcrossing rates between populations (66-78 %) were found in the American *L. nanus* subsp. *apricus* (Karoly 1992).

*Flower colour change.* – The senescence of many flowers has been shown to be influenced by pollination (Halevy 1986, Stead 1992). Flower colour change could be the result of ordinary senescence of the flower at the end of flower viability, or could take place in an earlier phase as a function of time, or as a consequence of pollination (Gori 1983). Both mechanisms are fit to direct the pollinators to flowers that are unlikely to have already been pollinated. In *Lupinus nanus*, colour change was not pollination-dependent (Juncosa & Webster 1989), while in some other lupin species the banner-spot colour change was partly influenced by pollination (Wainwright 1978, Schaal & Levrich 1980, Stead & Reid 1990). In *L. pilosus* banner-spot colour change, from white to purple, occurred in unpollinated flowers but was enhanced by pollination. Thus both ageing and pollination causes floral colour change in *L. pilosus*.

*Reduction of reward.* – Colour by itself is a meaningless information to bees unless it is reinforced by the stimulus of reward (Waddington 1983). Because of the foraging activity of pollen-gathering pollinators, a continuous reduction in the amount of pollen takes place during the flowering period in each single flower. In *Lupinus argenteus*, a correlation was found between flower age and the amount of pollen, and experimental work proved that, because of the higher amount of pollen, pollinators preferred pre-change flowers (Gori 1983). The results presented here demonstrate a correlation, in *L. pilosus*, between the total amount of pollen in the flower and the amount offered to the pollinator at a single visit on one hand, and the banner-spot colour phase on the other. Pre-change flowers with white banner spot had more pollen than post-change flowers

with purple banner spot. This difference is the basis for the discrimination of the two colour types of flowers by the pollinators. The pollinators' preference for white-spotted flowers shows that an age-dependent colour-change mechanism is not necessarily inferior to a pollination-induced mechanism in signalling the amount of reward in colour-changing flowers.

*Flower attractiveness and pollinator activity.* – Retaining old fertilized flowers is an energy expense, and therefore when it occurs it is supposed to confer a reproductive advantage in compensation (Gori 1983, 1989). Retaining old flowers increased the attractiveness of the inflorescence to pollinators in some of the American lupins (Wainwright 1978, Schaal & Levrich 1980), as it does in other genera (Weiss 1991). Since *Lupinus pilosus* grows in dense patches, retaining pollinated old flowers will likely increase the attractiveness of both the single inflorescence and the whole patch, thus increasing the probability of the plants to be discovered from a distance.

Retaining old fertilized flowers increases the total flower number but decreases the proportion of unfertilized flowers. Thus in spite of increasing the total attractiveness of the inflorescence it may reduce the chance of young, unfertilized flowers to be pollinated. A colour change that directs pollinators to young, rewarding flowers and helps them avoid old or pollinated ones will increase the chance of pollination for the young flowers (Gori 1983).

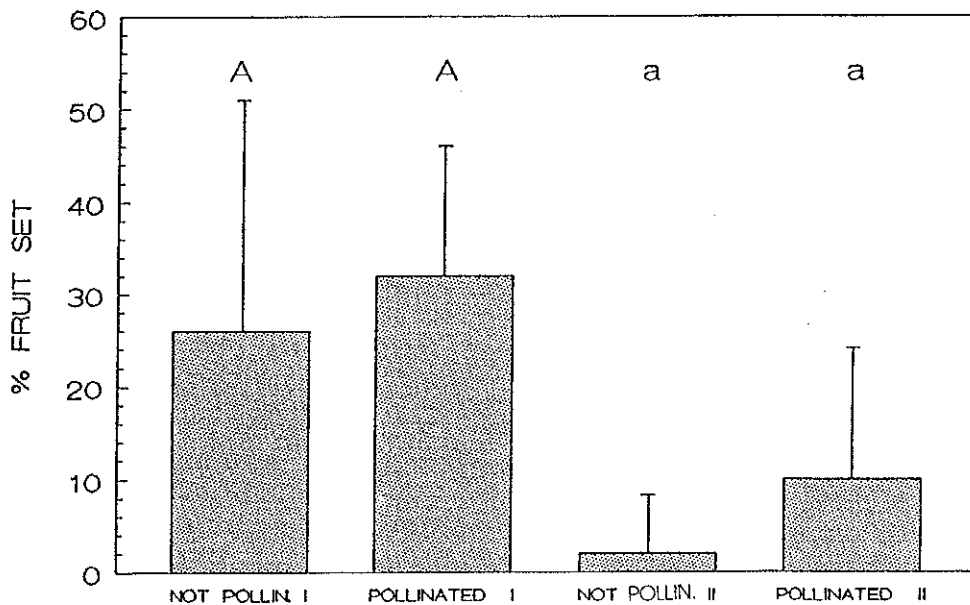


Fig. 3. The effect of artificial cross pollination on fruit set in the lowermost (I) and second lowest (II) whorl of *Lupinus pilosus* flowers. – Vertical bars represent standard deviation, columns with different letters are significantly different ( $P < 0.05$ ).

Pre-change flowers of *Lupinus pilosus*, with their white banner spot, attracted more pollinators than post-change flowers with purple banner spot. Such a selective attraction was also reported for some new world lupins (Wainwright 1978, Schaal & Levrich 1980, Gori 1983, 1989, Stead & Reid 1990). Because more pollen is offered in *L. pilosus* flowers at the white and pink banner-spot phases, honey-bees spend more time on those flowers, and thus increase the chance of pollination.

*Fruit set.* – Fruit set in artificially cross-pollinated flowers was not significantly higher than in unpollinated flowers. Thus, it can be seen that spontaneous self-pollination was effective and that pollination was not a limiting factor for fruit set.

However, the results prove that fruit set in the lowermost whorl was higher than in the second lowest one, while the upper whorls produced virtually no fruits at all (personal observations). Pazy (1984) found that 80 % of the total fruit set, in *Lupinus pilosus*, took place in the three lower flower whorls. This pattern was explained by low amounts of stigmatic exudate in the flowers of the upper whorls (Alon 1986). Similar pattern of fruit set were also found in *L. nanus* (Károly 1992). Shortage in resources may be suggested as a limiting factor for fruit production in those species of lupins, but experimental work is still needed in order to prove this assumption.

Several evolutionary explanations for the “extra” flowers and low percentage of fruit set in lupin species were suggested (Károly 1992), but none was experimentally tested. It seems that the upper flower whorls in *Lupinus pilosus* contribute to the attractiveness of the inflorescence and flowering patch, in addition to the old or pollinated flowers of the lower whorls. Increasing the low percentage of natural cross pollination and genetic variability in a mainly autogamous species was also suggested as an evolutionary driving force for the development of floral colour change in *L. pilosus* (Ne'eman & Neshet 1995).

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