

Inheritance of pleated petals in California poppy (*Eschscholzia californica*)

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Abstract

A long-standing discrepancy among reports concerning the inheritance of the pleated petal phenotype in California poppy (*Eschscholzia californica* Cham.) is resolved. Hybridization of plants from diverse sources true-breeding for non-pleated petals (wild-type), with plants true breeding for pleated (variant form) petals and screening of the F₁, F₂, and BC populations for segregation of phenotype established single-locus inheritance for this trait. The inheritance of the pleated petal trait (*pp*) was recessive to non-pleated petals (*PP*). However, in some genetic backgrounds a partial pleated-petal phenotype was evident in some heterozygous individuals (*Pp*), suggesting incomplete penetrance and low expressivity of the non-pleated-petal trait in the heterozygous state.

Key words: Papaveraceae, *Eschscholzia californica* Cham., California poppy, pleated petals, genetics, incomplete penetrance, low expressivity

Introduction

California poppy (*Eschscholzia californica* Cham., Papaveraceae) has gained attention in recent times as a model basal eudicot species, especially for evolutionary and developmental genetic studies (Becker et al., 2005; Carlson et al., 2006; Zahn et al., 2006; Barakat et al., 2007; Orashakova et al., 2009; Pabon-Mora et al., 2012). It exhibits polymorphism in a wide range of interesting traits, including plant habit, seed dormancy, petal and pollen carotenoids, petal anthocyanins, petal pigmentation patterns, and cyanogenesis (Beatty, 1936; Douwes, 1943; Cook, 1962; Frias et al., 1975; Urbanska, 1981; Wakelin et al., 2003; Barrell et al., 2010). To facilitate the use of this plant as a model system for basal eudicot species, it is important to develop an understanding of the inheritance of these polymorphic traits.

California poppy is known for diverse bright flower colours and forms in cultivation. One variant flower form

involves pleated, fluted, or frilled petals (Figure 1), a consistent and stable trait that forms the basis of several cultivars marketed in the flower seed industry (e.g. Ballerina Fluted, Apricot Chiffon, Mission Bells). Early inheritance studies established single locus inheritance for this trait (Beatty, 1936; Douwes, 1943). Beatty (1936) concluded that plants with pleated flowers were lethal in the homozygous condition and dominant over non-pleated flowers. Following crosses between plants with pleated and non-pleated flowers, both phenotypes were observed in the F1 generation. The progeny with non-pleated flowers bred true upon self pollination, whereas the self pollinated progeny from the plants with pleated flowers segregated in a ratio of 2:1 for plants with pleated versus non-pleated flowers. In contrast, Douwes (1943) concluded that non-pleated were dominant over pleated flowers, with F2 progeny segregating in the classical Mendelian 3:1 ratio for plants with non-pleated versus pleated flowers. The current study was undertaken to resolve



Figure 1. Phenotypes of the P locus influencing pleated petals. A. Flower with pleated petals (from Bfl3); B. wild-type flower (from HurI x HurP).

the discrepancy between these reports.

Methods

Parents for crosses made in this study were plants with non-pleated flowers obtained from the cultivars 'Ivory Castle' and 'Red Chief', as well as partially inbred lines derived by 3-5 generations of sib-mating from single plants selected from naturalized populations located at Lake Benmore, South Canterbury, New Zealand (Ben; white-flowered plant) and the Hurunui River, North Canterbury, New Zealand (HurI and HurP; both yellow-flowered plants) (Barrell et al., 2010). Parents with pleated flowers (Blf3 and Blf5) were raised from the cultivar 'Ballerina Fluted'. All plants were grown under greenhouse conditions as previously described (Wakelin et al., 2003). Flowers were emasculated prior to hybridization and stigmas were covered for 48 h after pollination to exclude contamination with other pollen as described previously (Wakelin et al., 2003). First filial (F1), second filial (F2) and backcrossed (BC) progeny populations were generated for phenotypic screening of petal morphology. The F2 generation was derived by pollinating between two F1 plants, because most plants in this species are self-incompatible (Wright, 1979). For each population examined, the F1, F2 and BC progeny were derived from pooling seeds from several crosses between two-four individuals. Chi-square analysis was used to compare observed data with expected ratios, for interpretation of inheritance.

Results and Discussion

In an earlier study (Barrell et al., 2010) white-flowered 'Ben' plants were

hybridized with yellow-flowered 'Blf3' plants. Among the F2 progeny, white-flowered plants with pleated petals were recovered, and following selection and progeny testing a true breeding population ('White Frill') with these traits was recovered. All the parental lines assembled for this study bred true for either non-pleated or pleated petals (Table 1).

The F1 progeny from crosses between genotypes with non-pleated and pleated petals failed to exhibit the pleated-petal trait, although some progeny in crosses in a some genetic backgrounds exhibited a very weak to weak pleated-petal phenotype (Table 1). This establishes the dominance of the non-pleated phenotype over the pleated phenotype.

Among the F2 progeny from four independent F1 crosses, segregation of plants with non-pleated and pleated petals was consistent with the expected 3:1 ratio typical for inheritance of a single locus (Table 1). Likewise, progeny from a backcross to plants homozygous for the pleated allele segregated in the expected 1:1 ratio (Table 1). As expected, all the progeny from a backcross to plants homozygous for the non-pleated phenotype exhibited flowers with the non-pleated phenotype (Table 1).

The presence of progeny with a very weak to weak pleated-petal phenotype in the F1 progeny was also evident in some F2 and BC progeny (Table 1). This phenotype was evident as fewer pleats with shallower depth and was present on only some of the petals of flowers. It was only apparent in some individuals from crosses in a few genetic backgrounds ('Ben', 'HurP', 'Ivory Castle'). Progeny from crosses

Table 1. Inheritance of non-pleated versus pleated petals in California poppy (*Eschscholzia californica*).

Cross	Number of progeny		Expected ratio	Chi-square value ^f
	Non-pleated	Pleated		
Parents:				
Ben selfed	49	0	1:0	-
Hurl x HurP	18	0	1:0	-
Ivory Castle selfed	46	0	1:0	-
Red Chief selfed	34	0	1:0	-
Blf3 x Blf5	0	18	0:1	-
White Frill selfed	0	166	0:1	-
F1:				
Ben x (Blf3 x Blf5)	143 ^a	0	1:0	-
(Blf3 x Blf5) x Hurl	63	0	1:0	-
HurP x Blf5	48 ^b	0	1:0	-
Red Chief x White Frill	175	0	1:0	-
F2:				
(Ben x (Blf3 x Blf5)) selfed	82 ^c	39	3:1	3.59
((Blf3 x Blf5) x Hurl) selfed	55	27	3:1	2.75
(HurP x Blf5) selfed	59 ^d	28	3:1	2.19
(Red Chief x White Frill) selfed	106	37	3:1	0.06
BC:				
(Red Chief x White Frill) x White Frill	28	35	1:1	0.78
White Frill x (Red Chief x White Frill)	15	25	1:1	2.5
Ivory Castle x (Red Chief x White Frill)	118 ^e	0	1:0	-

^a52 of these progeny exhibited a very weak pleated phenotype

^b5 of these progeny exhibited a weak pleated phenotype

^c12 of these progeny exhibited a very weak pleated phenotype

^d14 of these progeny exhibited a weak pleated phenotype

^e27 of these progeny exhibited a weak pleated phenotype

^fGoodness-of-fit Chi-square values based on one degree of freedom. The critical Chi-square value at the 0.05 probability level is 3.84. The associated probability for all Chi-square values is >0.05, indicating any deviation from the expected ratio is likely to have occurred by chance.

among three independent sets of plants with the weak pleated-petal phenotypes all segregated for individuals with non-pleated petals, pleated petals and weakly pleated petals. This confirms these plants were heterozygous for the trait and suggests incomplete penetrance and low expressivity of the non-pleated trait in the heterozygous state for some genetic backgrounds. Penetrance refers to the proportion of individuals exhibiting an expected phenotype, whereas expressivity is the degree to which the phenotype is manifested (Hartwell et al., 2011). The extent to which a phenotype is penetrant or exhibits variable expressivity can be attributed to environmental conditions, stochastic effects, or modifier genes carried by the individuals (Hartwell et al., 2011). For the pleated-petal phenotype in California poppy, incomplete penetrance is indicative from the 0–36% frequency of F1 progeny exhibiting the partial phenotype. The low expressivity of the non-pleated trait is indicative from the appearance of the weak phenotype in some heterozygous individuals in a few genetic backgrounds.

This study has clarified the long-standing discrepancy among reports on the single locus inheritance of the pleated petal phenotype in California poppy (Beatty, 1936; Douwes, 1943). The recovery of plants that bred true for the pleated petal phenotype contradicts Beatty (1936), who claimed the pleated-petal trait was lethal in the homozygous condition. The previous failure to recover true breeding plants for pleated petals (Beatty, 1936) is more likely to have resulted from close linkage in coupling to recessive lethal genes. An inbreeding effect in California poppy resulting in lower seed germination and longevity of plants from closely related parents was observed by

Beatty (1936). Furthermore, markedly distorted segregation for other traits in California poppy, especially from crosses involving genetic backgrounds derived from natural populations, has been attributed to linkage to recessive lethal genes (Barrell et al., 2010). Similar to the study of Douwes (1943), this study contradicts the previous assertion that the pleated-petal phenotype is dominant over non-pleated flowers (Beatty, 1936). The incomplete penetrance and expressivity of the non-pleated trait in the heterozygous state in some genetic backgrounds might have confused the phenotypic assignment of some individuals in the study by Beatty (1936). The F2 segregation of plants with non-pleated or pleated flowers in a 3:1 ratio, as reported by Douwes (1943), was confirmed by this study and further supported by analysis of backcrossed generations and inheritance in a wide range of genetic backgrounds.

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