Similar reproductive success of the two petal colour polymorphisms of *Cistus ladanifer* (Cistaceae)

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Abstract
Variation in flower colour can reflect adaptive responses to selective pressures generated by pollinators. *Cistus ladanifer* is a Mediterranean shrub that displays self-incompatibility, entomophyly and polymorphism for the presence of a dark coloured blotch in the base of their petals (var. *maculatus* with blotched petals and var. *ladanifer* with unblotched petals). In order to investigate the effect of the petal blotch and geography in *C. ladanifer* reproductive success, variation in female reproductive output (flower production, fruit set, seed set) was analysed in four and three Iberian populations of var. *ladanifer* and var. *maculatus*, respectively. Additionally, we tested whether co-existence of these two *C. ladanifer* phenotypes yielded different reproductive output (ovule production, seed set) in a subset of two populations. Significant differences in flower production (56.4–229.4%), fruit set (64.9–93.8%) and seed set (82.8–98.0%) among populations were reported. Geographical and taxonomic variables did not significantly explain this variation. Significant results between floral morphs within a single population were found neither for ovule nor for seed production. Our findings suggest that the petal blotch in *C. ladanifer* should be considered to be neutral in its effect on fruit and seed production and its occurrence may simply reflect a relaxed selection under current conditions.

Keywords: *Cistaceae*, flower production, fruit set, multi-population approach, petal colour polymorphism, seed set

Introduction
It is widely accepted that many morphological characters of flowers have evolved as a result of the plant’s need to attract pollinators, which is an important component of fitness (Waser 1983; Stanton et al. 1986; Galen 1989). Pollinating animals may respond to flower characters such as size, shape, fragrance and colour as these signals usually act as indicators of quality or quantity of reward (Kaufield & Sorenson 1971; Galen 1985; Herrera 1993; Conner & Rush 1996; Franchi & Pacini 1996; Petanidoua & Lamborna 2005; Caledon-Neighme et al. 2007; Gómez & Perfectti 2010). Countless floral adaptations have arisen in response to selection from pollinators (see Fenster et al. 2004 for review). However, the increasing number of studies exploring the relative strength of selection from pollinators versus other sources (Galen 2000; Gómez 2003; Cariveau et al. 2004; Irwin 2006) is shedding light on the importance of non-pollinator selection on floral traits (Strauss & Whittall 2006). In particular, petal colour variation, involving the whole (Kay 1978; Gómez 2000) or parts (e.g. Abbott 1981; Shore & Barrett 1987; Hong & Pan 2005) of the corolla, such as whether petal spots or marks are present, has been described in a large number of plants that are pollinated by insects. There has been a widespread belief that flowers with intense coloured petals or with corollas of contrasting colours receive more pollinator visits than those with dull or not contrasting colours (Waser & Price 1981). However, new evidence indicates that floral pigments have pleiotropic functions such as protection from drought stress (Warren & Mackenzie 2001), heat stress (Coberly & Rausher 2003), herbivory (Irwin et al. 2003) or pathogen infections (Frey 2004).

Cistaceae are a medium sized family (eight genera) of about 180 entomophilous species (Arrington & Kubitzki 2003) distributed in temperate and subtropical regions of the northern hemisphere. American Cistaceae (genera *Crocanthemum*, *Hudsonia*, *Lechea*) and the Mediterranean *Fumana* have flowers with homogeneous petal colouration (yellow or dark
red). In contrast, other Cistaceae from the Mediterranean floristic region (genera *Cistus*, *Halimium*, *Helianthemum*, *Tuberaria*) contain species with blotched petals (known as maculation). A historical reconstruction of petal maculation within Cistaceae (Guzmán & Vargas 2009) shows a recurrent acquisition of the character in *Halimium*, *Helianthemum* and *Tuberaria*, but only one such occurrence in one species of *Cistus* (*C. ladanifer*).

An important ecological role is played by Cistaceae in the Mediterranean floristic region in terms of habitat occupation and plant–pollination interactions. As a result, a high number of reproductive biology results has been obtained, but they are mostly community oriented (Herrera 1987; Brandt & Gottsberger 1988; Bosch 1992; Herrera 1992; Talavera et al. 1993; Blasco & Mateu 1995; Boscaiu & Güemes 2001; Rodríguez-Pérez 2005; Mingo 2009). These studies have primarily focused on ecological and reproductive aspects, such as the phenology, reproductive success, breeding system and pollination, and all of them are based on a single population. However, these characteristics usually varied among populations (Herrera 2002). Additionally, the role of the petal blotch in the reproductive biology of Cistaceae remains unclear. *Cistus ladanifer* (gum rock-rose) relies entirely on pollinators for reproduction (Talavera et al 1993), thus different floral morphotypes could affect pollinator attractiveness and then reproductive success. In this article, we analysed the female reproductive output in flower polymorphic populations of the shrub *C. ladanifer* and examined the potential evolutionary effect of the petal blotch in their reproductive success. Specifically, we addressed (1) whether there is a significant variation in flower production, fruit set and seed set and, if so, whether this variation is correlated with taxonomy and geography and (2) whether the two flower colour morphs differ within the same population with respect to female reproductive output, estimated as ovule production and seed set.

**Material and methods**

**Species characteristics and study area**

*Cistus ladanifer* L. is an evergreen shrub native to the western Mediterranean region (Southern France, Iberian Peninsula and Northern Africa) (Demoly & Monsterrat 1993). Its numerous flowers are terminal and solitary. They are among the largest flowers of the Mediterranean (5–8 cm in diameter) and display three sepals and five white petals each having a yellowish stain at the base. In addition, the presence of a dark coloured blotch just above this stain in some individuals is used to distinguish two taxa: *C. ladanifer* var. *ladanifer* (Figure 1A) and *C. ladanifer* var. *maculatus* (Figure 1B) (Rouy & Foucaud 1895). Both varieties have the same vegetative characters and can occur in monomorphic populations or mixed in the same localities. There are also populations where the size and shade of the dark coloured blotch can be highly variable.

Gum rock-rose flowers are showy and have numerous stamens (>100) located around a large and sessile stigma. As in most Cistaceae, *C. ladanifer* shows highly polliniferous flowers that rely completely on a wide diversity of insects, mainly bees, beetles and flies, for pollination (Talavera et al. 1993). A nectar disk under the ovary which produces a small amount of nectar has also been studied (Janchen 1925; Herrera 1985; Bosch 1992). During its spring flowering phenology, *C. ladanifer* produces a high number (over 500 on large size plants) of ephemeral flowers (1–3 days). Anthesis finishes with the fall of the petals and the refolding of the sepals around the ovary. A flower that has not been fertilized will fall few days after the end of anthesis, remaining its pedicel on the plant. Fruit capsules maturation extends over the summer (Talavera et al. 1993). The number of seeds (318–1185) per fruit increases with the number of fruit valves (5–12) (Narbona et al. 2010).

Our study was carried out in 2003 and 2006 in seven Iberian locations (Table I) where *C. ladanifer* was the dominant species. Four populations were located in central Iberia (Madrid province) and other...
two in southern Iberia (Huelva province) (Table I). HIN, VAL and BOA populations were monomorphic for petal colour polymorphism while ESC, MAN, ATA and CAB were mixed populations where both varieties co-exist. In these populations individuals from the predominant variety were selected.

**Estimating female reproductive success**

In 2003, 10 individuals were randomly selected from each of seven Iberian populations of *C. ladanifer* (Table I). Natural pollination of the flowers was allowed. At the end of the flowering season, we measured the following variables: (1) number of flowers per individual (number of fruits + number of pedicels from non-pollinated flowers); (2) number of fruits per individual and (3) number of seeds and aborted ovules contained in two approximately opposite valves of three or four randomly selected fruits. To estimate the number of ovules and seeds per fruit we multiplied the mean number of seeds and aborted ovules contained in two valves by the number of fruit valves of each fruit. Subsequently, fruit set (number of fruits/number of flowers) and seed set (proportion of ovules maturing into seeds) were estimated.

Maternal fitness of colouration morphs, estimated as the number of ovules per flower and the seed set per individual, was analysed in 2006 from a subset of two populations (ESC, CAB) (Table I) where blotched and unblotted individuals cohabit. Five fruits were randomly collected from 20 individuals freely pollinated (10 individuals from var. *maculatus* and 10 individuals from var. *ladanifer*).

**Statistical analyses**

Variation in flower production, fruit set and seed set between (1) varieties and (2) geographical regions (Madrid, Huelva; Table I) was analysed using mixed-model ANOVAs, the factor “variety”/“region” was fixed and the factor “population” was random and nested within “variety”/“region”. Variation in ovule production and seed set between varieties was analysed using main effects ANOVAs. Prior to analysis, normality of variables was checked with Kolmogorov–Smirnov test and homocedasticity with Levene’s test (Day & Quinn 1989). The number of flowers was LN transformed and both fruit and seed set were arcsine transformed. All the statistical analyses were performed using the computer program STATISTICA 6.0 (Statsoft, Incorporated, Tulsa, OK).

**Results**

The mean number of flowers produced per plant ranged from 56.4 ± 13.4 (mean ± SE) in VAL to 229.4 ± 37.7 in HIN (Figure 2). The mean fruit set varied between 64.9 ± 5.6 (ATA) and 93.8 ± 1.9% (ESC) (Figure 2). Regarding the seed set, the highest percentage of ovules developed into seeds was observed in BOA (98.0 ± 0.7%) while the lowest percentage was registered in HIN (82.8 ± 1.8%) (Figure 2). Statistically significant differences in fruit set and seed set were found among populations (Table II). However, both varieties produced a similar number of flowers (203 ± 123.91 for var. *ladanifer*, 145 ± 133.63 for var. *maculatus*) and set a comparable number of fruits (76 ± 71.58% for var. *ladanifer*, 83 ± 14.28% for var. *maculatus*) and seeds (90 ± 8.76% for var. *ladanifer*, 94 ± 3.50% for var. *maculatus*). Parallel results were obtained when analysing two geographical regions (Table III), since similar number of flowers (44 ± 46 in Madrid, 47 ± 48 in Huelva), fruit set (77 ± 16.11% in Madrid, 82.18 ± 17.28% in Huelva) and seed set (43.76 ± 46.34% in Madrid, 46.75 ± 48.01% in Huelva) were found.

In 2006, individuals of *C. ladanifer* var. *maculatus* produced as many ovules and seeds as those of var. *ladanifer* in both mixed populations (CAB and ESC) (Figure 3). Consequently, the main effects ANOVA determined that variety was not a significant source of variability (Table IV). On the contrary, significant
differences in ovule production were found between both populations although these differences were not reflected in seed set (Table IV).

**Discussion**

High levels of morphological variation have previously been reported in vegetative and reproductive characters of *Cistus ladanifer* (Demoly & Montserrat 1993) but little is known about its influence in reproductive success (Narbona et al. 2010). Flower production, fruit set and seed set were significantly variable among populations of our study (Tables II and III). However, our results show no significant differences in fruit set and seed set between plants with blotched and unblotched flowers. The dependence of the gum rock-rose flower production on both age/size and location (clumped or isolated) might be also responsible for the variability found among populations (Talavera et al. 1993). Community interactions (e.g. Rathcke 1983; Gumbert & Kunze 2001) coupled with environmental factors (e.g. Hennenberg & Bruelheide 2003; Alonso et al. 2007) are potential causes of spatial and temporal variation in angiosperms.

Direct effects of flower variation on seed production are to be expected when pollinators respond differentially to flower variants, especially when a plant species relies completely on insect pollination for seed set (Schemske & Bradshaw 1999; Hodges et al. 2002). The absence of reproductive consequences of maternal fitness associated with petal colour polymorphism in *Cistus ladanifer* (results

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Table II. Results of nested ANOVAs to investigate the effect of the variety and population (nested to the variety) on flower production, fruit set and seed set in seven Iberian populations of *C. ladanifer*.

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<th>df</th>
<th>SS</th>
<th>F</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>(A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variety</td>
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<td>5810</td>
<td>2.01</td>
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</tr>
<tr>
<td>Population (variety)</td>
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<td>144.28</td>
<td>1.87</td>
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<tr>
<td>Error</td>
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<td>972.32</td>
<td></td>
<td></td>
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<tr>
<td>(B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variety</td>
<td>1</td>
<td>0.21</td>
<td>0.53</td>
<td>0.50</td>
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<td>Population (variety)</td>
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<td>10.05</td>
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</tr>
<tr>
<td>Error</td>
<td>63</td>
<td>2.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variety</td>
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<td>0.02</td>
<td>0.21</td>
<td>0.66</td>
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<tr>
<td>Population (variety)</td>
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<td>4.19</td>
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<tr>
<td>Error</td>
<td>27</td>
<td>0.52</td>
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Table III. Results of nested ANOVAs to investigate the effect of the geographical region where populations are situated and population (nested to the region) on flower production, fruit set and seed set in seven Iberian populations of *C. ladanifer*.

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<th>df</th>
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<tbody>
<tr>
<td>(A)</td>
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<tr>
<td>Region</td>
<td>1</td>
<td>3.72</td>
<td>1.40</td>
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<tr>
<td>Population (region)</td>
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<td>13.26</td>
<td>5.54</td>
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</tr>
<tr>
<td>Error</td>
<td>63</td>
<td>30.15</td>
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<tr>
<td>(B)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Region</td>
<td>1</td>
<td>0.24</td>
<td>0.61</td>
<td>0.47</td>
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<tr>
<td>Population (region)</td>
<td>5</td>
<td>1.98</td>
<td>9.91</td>
<td>&lt;0.0001</td>
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<tr>
<td>Error</td>
<td>63</td>
<td>2.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Region</td>
<td>1</td>
<td>0.16</td>
<td>2.85</td>
<td>0.15</td>
</tr>
<tr>
<td>Population (Region)</td>
<td>5</td>
<td>0.28</td>
<td>2.92</td>
<td>0.03</td>
</tr>
<tr>
<td>Error</td>
<td>27</td>
<td>0.52</td>
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congruent with those of Acosta et al. (1993) suggests that pollinators do not differentially select for petal colouration when the two morphs meet in the same population. An alternative explanation of our results is that pollinators may not actually perceive the variability as humans do. In fact, the two colouration morphs of the gum rock-rose seem to show similar visual cues to pollinators, as in UV light the dark coloured blotch is imperceptible (Arias et al. 1995). If pollinators ignore the variation, morphs would be visited in proportion to their representation in the population and the pattern of mating among morphs would appear to be random.

In such a scenario a stabilizing selection of floral traits would not operate. The fact that successful mating between C. ladanifer varieties (Guzmán et al. unpublished) in mixed populations and the occurrence of hybrid individuals with intermediate size and shade blotch seems to confirm the indifference of insect pollinators to petal colour variation. Minor differences were imperceptible by our approach because flowers reached enough pollinator visits to ensure high levels of seed set (82–98%) in the seven populations (Figures 2 and 3). The question remains as to whether differences in pollinator fauna and habitat in extreme conditions (climate conditions) would change seed production. The results of the present comparative study (seven populations) show the need for (1) multiple study sites to state fine scale conclusions about the reproductive success of this widely distributed and highly polymorphic species (Narbona et al. 2010) and (2) controlling additional stages, such as pre-dispersal predation (Serrano et al. 2007), seed germination and seedling survival, to determine any pattern of reproductive success within the species.

The fact that female reproductive success alone does not account for the maintenance of flower colour variation in Cistus ladanifer populations leads us to consider additional variables not tested in this study. First, the male component (pollen) of fitness could partly explain the reproductive success, although the large amount of pollen produced per flower in the gum rock-rose suggests poor influence (but see Arista & Ortiz 2007). Second, selection pressures related to abiotic and/or non-pollinator biotic factors affecting other aspects of plant performance may allow the maintenance of C. ladanifer petal colour polymorphisms in a variable environment (Schemske & Bierzychudek 2001; Warren & Mackenzie 2001; Geber & Eckhart 2005; Irwin & Strauss 2005; Eckhart et al. 2006; Strauss & Whittall 2006; Rausher 2008). Finally, colour polymorphism in the gum rock-rose could be subject to little selection and between-population variation in the proportion of plants with blotched and unblotched petals could be maintained by drift accompanying the founding of new populations. This is partially supported by peripheral areas of the distribution of C. ladanifer, where only one flower morph is found (Guzmán et al. unpublished).

In summary, although this study provides valuable information on the lack of influence of petal colouration morphs on maternal fitness in C. ladanifer, the forces maintaining this variation across populations still remain elusive. We may gain a better understanding of the evolution of floral colour polymorphism in C. ladanifer populations from further studies examining the impact of pollinators on male functions and incorporating an assessment of as many

Table IV. Results of main effects ANOVAs of population and petal colour pattern (blotched vs. unblotched) on ovule production and female fitness, through seed set, in Cistus ladanifer.

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<th>p</th>
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<tr>
<td><strong>Ovule production</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population</td>
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<td>605,644</td>
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<td>Variety</td>
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</tr>
<tr>
<td>Error</td>
<td>192</td>
<td>11,198,009</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Seed set</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Population</td>
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<td>0.069</td>
<td>1.28</td>
<td>0.26</td>
</tr>
<tr>
<td>Variety</td>
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<td>0.0006</td>
<td>0.01</td>
<td>0.91</td>
</tr>
<tr>
<td>Error</td>
<td>192</td>
<td>10.32</td>
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Figure 3. Average (± SD) of ovule production and seed set found in two mixed C. ladanifer populations from Madrid. See statistical results in Table IV.

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sources of selection as possible. In addition, it would be particularly valuable to analysed individual fitness in the long term, using hypervariable molecular markers (fingerprinting) and genetic analysis of the metabolic pathways that lead to the production of the blotch.

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