



Subindividual variation accounts for most of the variability in two reproductive traits in *Cistus ladanifer*

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ABSTRACT

There is increasing evidence of within species flower trait variability. In *Cistus ladanifer*, there is a great variation in the number of locules of the ovary (6–12). In this study, we assessed the variation in the number of ovary locules and ovule production at population, individual and intraindividual levels in 5 localities in the Madrid Region (Central Spain). We have answered the following questions: What level present larger variation? Is there a relationship between the number of ovary locules and ovule production? And if yes, does it hold across all levels? We sampled almost 500 flowers from 50 individuals for five localities. We counted the number of locules of the ovary and estimate the number of ovules per flower. The partitioning of total variation among populations, individuals and within-individuals was estimated for both variables. To analyze differences among locations, we performed general linear models. We found that within-individual variation was the main source of variation for all measured variables. Inter-individual variation was relevant only for the number of locules, and inter-population variation was almost negligible for all variables. Despite the positive correlation between the number of locules per flower and ovule production, populations with more locules did not present more ovules per flower. In summary, we found that variation in the number of locules and its relationship with ovule production is scale dependent.

1. Introduction

Plant traits have been used to investigate evolutionary strategies in plant species (Reich et al., 2003). Trait variation is essential to evolutionary processes because natural selection can only take place if there are differences in fitness among individuals which differ in phenotype (Futuyma 2009). Moreover, variation in traits across species has also been used to analyze diversity and function in biological communities (Reich et al., 2003; Wright et al., 2004; Diaz et al., 2007). Nevertheless, trait variation is relevant not only among but also within species (Mayr and Provine 1998). This within individual variation should be relevant for both evolutionary and ecological processes (Wetzel et al., 2016; Herrera 2017; Sobral et al., 2019) and therefore it has been included in the assessment of functional diversity (Herrera et al., 2015; Palacio et al., 2019). In fact, functional trait variation is integrated across multiple scales, including intraindividual scales (Messier et al., 2010; Escribano-Rocafort et al., 2017).

Within individual variation in plant vegetative traits is usually larger than that from variation in fruits and flowers (Herrera 2017). In fact,

reproductive organs usually have little variation, since are directly related to plant fitness. Therefore, they can be selected in an optimal level and therefore show little variation through a strong stabilizing selection, in contrast to the vegetative characters that affect reproductive performance indirectly (Brock and Weinig, 2007). Consequently, variation in traits such as the number of petals or ovary locules are frequently used for the classification of angiosperms at all taxonomic levels (Crane et al., 1995; Michelangeli, 2000; Sambamurty, 2010).

However, variation in flower traits within species is not rare and it has been reported among populations, among individuals of the same population and even within individuals (Herrera 2009; Żywiec et al., 2012, and references therein). The origin of such a large variability could be genetic, the result of phenotypic plasticity, position within the plant, or even related with development instability (Obeso 2004; Herrera 2009). The existence of such variation could be subjected to phenotypic selection, and several studies have assessed the correlation of trait variation with fitness measurements. For example, variation in traits related to pollination attraction such as corolla colour, petal length, corolla size, have been assessed in relation to animal-plant

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mutualistic and antagonistic interactions (Herrera 1990; Willmer et al., 2009; Teixido et al., 2011; Hirota et al., 2013; Kulbaba et al., 2017). On the other hand, variation in androecium and gynoecium size could be more directly related to male and female fitness functions.

The *Cistaceae* family (180 sp.) presents a 3 loculated ovary except for the *Cistus* genus which present 5. Nevertheless, the species *Cistus ladanifer* presented an ovary with 6–12 locules (Muñoz Garmendia and Navarro 1993; Talavera et al., 1993). This fact suggests that there should be selective forces favouring the increase in the number of carpelar leaves in this species. This increase in the number of locules could be related to an increase in flower size and ovule production since these relationships are common among species (Endress 2014; Cucinotta et al., 2020); and both traits share the same genetic and hormonal control (Cucinotta et al., 2020). In fact, *Cistus ladanifer* presents the largest flowers and the highest number of ovules in all the *Cistaceae* family (Herrera 1992). Nevertheless, the variation in the number of locules of the ovary in this species is present among populations of the same region, among individuals of the same population and even within individuals (Serrano et al., 2001; Delgado et al., 2007; Narbona et al., 2010).

In the present study, we assessed the variation in the number of locules of the ovary and its relationship with the number of ovules at several ecological levels in a group of populations of *Cistus ladanifer* (*Cistaceae*) from a Mediterranean scrubland in Central Spain. We have selected these traits because variation in the number of ovary locules is usually low, and have a direct impact on fitness through ovule production, which makes more intriguing the large variation found in this species. We hypothesize that flowers with more locules will produce more ovules, and that this relationship will hold for intra-individual, intra-population and among population levels. We expect strong variation in the number of ovary locules among and within individuals as reported in many populations from this area. But not necessarily among populations since the fragmentation is not strong and the variation in altitude and precipitation should be very low.

We specifically attempt to answer the following questions: How important are intra-individual, interindividual and inter-population variance in number of ovary locules? Is there a relationship among the number of ovary locules and the number of ovules produced? And if so, are they consistent across organization scales?

2. Methods

2.1. Study area and species

Cistus ladanifer L. is a Mediterranean shrub species distributed through the south of France, Portugal, and Spain, and the north of Morocco and Algeria (Demoly et al., 1993). This species is an obligate seeder with a strong dependence upon seeds available in the soil bank for post-fire seedling establishment a reproductive adaptation to the recurrent fires that affect many Mediterranean shrub communities (Troumbis and Trabaud 1986). *C. ladanifer* produces many fruits (capsules) with a variable number of locules (6–12) (Fig. 1). Within each fruit there is many seeds (700–900; Acosta et al., 1997), which remains inside during several weeks before they are released with the opening of the valvae (Talavera et al., 1993). Flowers are large (7.2–10.5 cm; Teixido and Valladares 2014) and short lived (usually less than one day; Teixido and Valladares 2015).

We collected fruits from natural patches of *C. ladanifer* with scattered trees of Mediterranean Holm-oak (*Quercus rotundifolia* Lam. 1785) in five different localities in the northwest of Madrid Region (Table 1.). Soils are mainly acidic coarse-grained sands from the erosion of granite rocks. Climate is continental Mediterranean with dry summers and wet winters.

2.2. Sampling

Sampling work takes place in May 2013, during the flowering period. Within each locality, we randomly selected 10 *C. ladanifer* individuals with open flowers. Within each individual, two main branches were selected at random from the four lowest positions in the main stem. From each branch 5 recently open flowers were collected but per 7 branches it was only possible to collect 4 flowers.

Flowers were conserved in alcohol 70% before dissected under the

Table 1

List of sampled locations, geographical coordinates, and altitude.

Location	Symbol	Altitude	Coordinates	
Colmenarejo	C	869	4° 2'11.98"W	40° 33'36.50"N
El Escorial	E	906	4° 6'11.65"W	40° 32'30.09"N
Galapagar	G	922	4° 1'34.33"W	40° 35'8.57"N
Torrelodones	T	848	3° 58'54.74"W	40° 34'47.96"N
Valdemorillo	V	880	4° 7'0.11"W	40° 30'38.33"N



Fig. 1. Plant habit, flower and fruits of *Cistus ladanifer*. (A) Flowering plant. (B) Flower with a pollinator insect (coleoptera). (C) Opened capsule with eight locules. (D) Fruits with seven, eight, nine and ten locules (from top to bottom). All photographs by Juan A. Delgado.

stereomicroscope to count the number of locules of the ovary and the number of ovules from a randomly selected locule. To count ovules, they were dispersed in water to prevent them from sticking together. The total number of ovules per flower were estimated by multiplying the number of ovules of selected locule by the number of locules in the ovary.

2.3. Statistical analyses

To assess how the variation in the number of locules, number of ovules per locule and number of ovules per flower, is explained by each of the hierarchical scales defined above, we used a hierarchical variance partitioning analysis (Borcard et al., 1992).

To assess variation in the number of locules per flower, the number of ovules per locule and the number of ovules per flower we used General linear models with “population”, “plant” (nested in “population”) and “branch” (nested in “plant”) as main factors. We also included an additional analysis of the variation in the number of ovules per flower using the number of locules as a covariate.

3. Results

The partition of the variance in the studied fruit traits showed that there is large within plant variation (above 50%) and very low (<4%) to no variation at population scale (Fig. 2). The partition of the variance for the number of locules showed very low variation at both branch and population levels, and high at plant (46%) and flower (52%) levels. On the other hand, for the number of ovules per locule, variation was very low at population level, reached 7% at branch level, and was close to 10% at plant level with most variation (80%) at the flower level. Finally, variation for the number of ovules per flower showed also higher values at flower level (>75%), more than 20% at plant level, no variation at population level and very low variation at branch level.

The number of locules differed among populations and among plants within a population, but do not between branches of the same plant (Table 2). All studied populations presented more than 8 locules per compartment on average, with just one of them (C) averaging more than 9 locules (Fig. 3).

Results on number of ovules per locule also differed among populations and individuals from the same population and even among branches from the same individual (Table 2). Specifically, population C, which presented the larger degree of ovary compartmentation (Fig. 3), presented a lower number of ovules per locule than the other populations (Fig. 4).

Results for the number of ovules per flower indicate that differences among populations and even among individuals within the same population exist, but do not between branches within the same individual

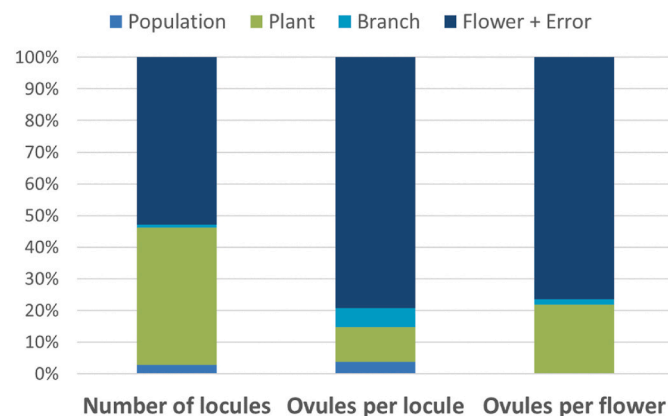


Fig. 2. Variance partitioning for the three flower variables across four ecological scales (population, individual, branch and flower).

Table 2

Results of the GLM of the number of locules, number of ovules per locule, and the number of ovules per flower in the studied populations, considering the hierarchical arrangement of ovule production. Dependent variables were log-transformed prior to analyses.

Number of locules	F	d.f.	P
Population	13.137	4	<0.001
Plant(Population)	8.938	45	<0.001
Branch(Plant(Population))	1.131	50	0.260
Number of ovules per locule			
Population	7.204	4	<0.001
Plant(Population)	2.939	45	<0.001
Branch(Plant(Population))	1.472	50	0.025
Number of ovules per flower			
Population	3.650	4	0.006
Plant(Population)	4.511	45	<0.001
Branch(Plant(Population))	1.090	50	0.322

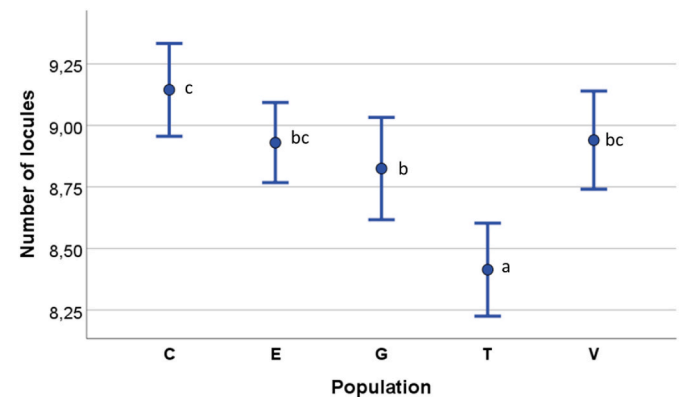


Fig. 3. Mean values and 95% confident interval of the number of locules of the ovary for the studied populations. Different letters indicate significant differences between means using posthoc Tukey test at 95% of confidence.

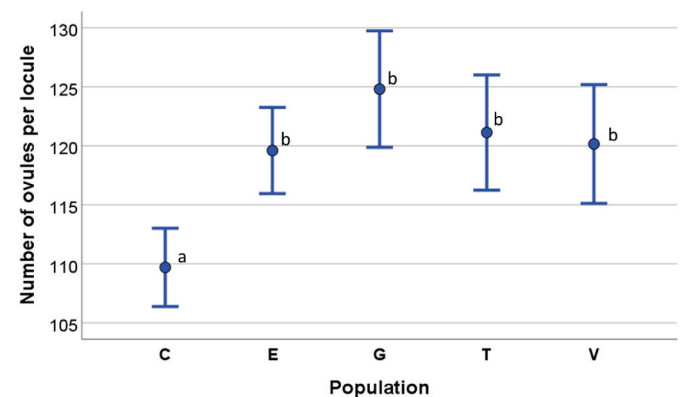


Fig. 4. Mean values and 95% confident interval of the number of ovules per ovary locules for the studied populations. Different letters indicate significant differences between means using posthoc Tukey test at 95% of confidence.

(Table 2). In our study all populations averaged more than a thousand ovules per flower. Two populations (C and T) presented lower values than the most productive population (G), with the other two populations (E and V) reaching intermediate values (Fig. 5).

The scatterplot of pooled data for the number of locules in the ovary and the number of ovules per flower show a positive relationship (Fig. 6; $r^2 = 0.334$; $F = 246.43$; $p < 0.001$), suggesting that a large compartmentation of the ovary could be related to an increase in ovule production.

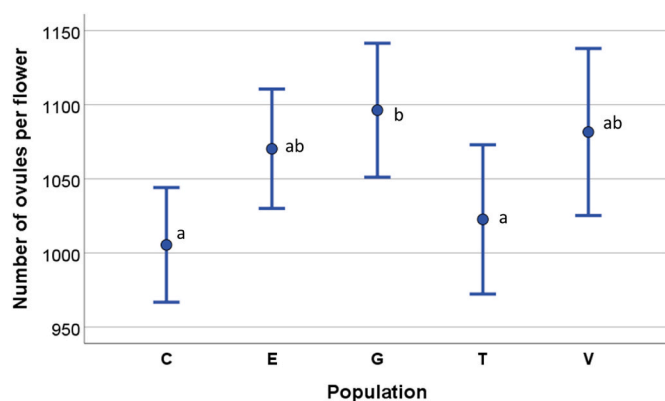


Fig. 5. Mean values and 95% confident interval of the number of ovules per flower for the studied populations. Different letters indicate significant differences between means using posthoc Tukey test at 95% of confidence.

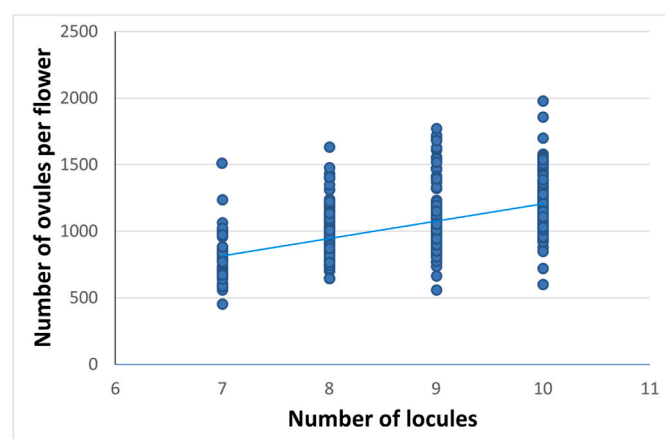


Fig. 6. Scatterplot of pooled data from the number of locules and the number of ovules per flower. Least square regression line was added to help relationship visualization.

Additional results indicate that the positive effect of the number of locules of the ovary over the number of ovules per flower can not be generalized as indicated by the positive interaction among factors and covariable (Table 3). In other words, the role of ovary compartmentation in the number of ovules per flower is not homogeneous between branches within the same individual, individuals within the same population, and between populations. More relevant is that considering ovary compartmentation (Table 3), differences among populations in the number of ovules produced per flower disappear. These results suggest that differences in the number of ovules per flower among populations could be mainly due to differences in the number of locules of the ovary.

Table 3

Results of the GLM of the number of ovules per flower in the studied populations, considering the hierarchical arrangement of ovule production and the number of locules of the ovary as covariable. Both, dependent variable and covariable were log-transformed prior to analysis.

Number of ovules per flower	F	d.f.	P
Population	1.086	4	0.364
Plant(Population)	2.049	44	<0.001
Branch(Plant(Population))	1.498	44	<0.028
Number of locules	109.867	1	<0.001
Number of locules x Branch(Plant(Population))	1.751	92	<0.001

4. Discussion

Populations in our study differed in the average number of ovary locules although populations were not far away and there was no significant altitude variation among them, suggesting that both, environmental and genetic variation should be low. These differences are mainly due to variation at individual and within individual levels, since variance partitioning suggests that there is almost no variation at the population level in this trait. Although variation among individuals was high for the number of ovary locules, variation within individuals was larger than 50%. This within individual variation was not associated to the hierarchical modular structure of the individual plant since branches did not differ in any of these traits. In this sense, branches are working as iterative units with almost no variation in the studied reproductive traits. In fact, variation within individuals in the ovary compartmentation was due mainly to variation among flowers (the basic modular unit). This is relevant since within plant variation for flower traits has been reported to be lower than for fruit, seed, and leaf traits and usually lower than among individual variation (Herrera 2009).

Variation in the number of ovary locules in *C. ladanifer* might be controlled at least by two quantitative loci as suggested for other species (Lippman and Tanksley, 2001; Chu et al., 2019). Therefore, variation in ovary compartmentation among individuals could be due to genetic differences among individuals. Nevertheless, there is even more variation among flowers of the same individual. This within individual variation could be due to several factors. Position within the plant, response of the developing flower to environmental conditions and developmental instability have been proposed as sources of variation (Herrera 2009). But differences could also be genetics. Accumulation of somatic mutations was suggested and partially validated by data in the genetic mosaicism hypothesis (Gill 1986; Gill et al., 1995). Nevertheless, epigenetic mosaicism, variation in gene expression within the same individual, has been proposed as a more plausible mechanism responsible of within plant variation (Herrera 2017).

Independently of the origin of these differences in the number of ovary locules, their fitness consequences might be relevant. Especially, because, in our study, there is a positive relationship among ovary compartmentation and ovule production. Flowers with more locules in the ovary produced more ovules, however, the intensity of this relationship vary within populations, individuals and even branches of the same individual. Ovary compartmentation is usually formed by the fusion of several carpels by their margins (Endress 2014). The meristem of the margin of the carpels will produce both, the placenta, and the ovules (Cucinotta et al., 2020), so the fusion of a larger number of carpels could result in a larger meristem and so more placenta extension and ovule number. Therefore, it can be suggested that increasing the number of locules in the ovary should be a plausible mechanism to increase the number of ovules produced.

Nevertheless, producing ovaries with many carpels might have their drawbacks too. For example, increasing the number of carpels over five make difficult to form a unique centralized pollen tube track, the compatum, and, therefore, the selection of male gametophytes will be hampered (Endress 2014). Another drawback of increasing locule number should suppose additional costs in terms of biomass since there is an increase in fruit wall surface (Shaanker et al., 1988). Nevertheless, the empirical evidence of these allocation trade-offs is hampered by the differences in size and available resources among populations, individuals and even within individuals.

We suggest that variation in the availability of resources among individuals and branches could produce differences in ovule investment per locule (Catalán et al., 2016). These differences produce that populations with more ovary locules were not those with more ovules per flower despite that our results suggest that the number of ovules per flower should be positively correlated to the number of locules per flower. Therefore, at the studied spatial scales, it seems that there was not any advantage in increasing the number of ovary locules which

could be associated to fitness.

Summarizing, our study suggests a high variation in the number of locules in spatially close populations. This local variation is overlapping a large-scale geographical variation which seem to be related to eco-geographical factors (Narbona et al., 2010). Therefore, local ecological factors might explain, at least partially, the among population variation found in our study. In addition, we have not found any advantage to increase the number of ovary locules in terms of ovule production at population level. Although we found a positive relationship among the number of fruit locules and ovule production, variation in ovule production among populations cannot not be explained through the number of ovary locules. Even more, other studies, have reported that despite large variation in the number of ovary locules have been found in this species, there were no associated differences in seed production, increased allocation costs, or predispersal seed predation (Acosta et al., 1997; Delgado et al., 2007). And, therefore, there is no clear signals of directional selection which could decrease variability in that trait.

Author contributions

JAD y JMS developed the conceptual framework and designed the sampling protocols of this study; JAM and MD-C collected the samples and participated in processing and interpretation; and JAD wrote the paper with contributions from all authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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