Seed predation heterogeneity in the loculate fruits of a Mediterranean bushy plant

JUAN A. DELGADO1,2, JOSÉ M. SERRANO2, FRANCISCO LÓPEZ2 & FRANCISCO J. ACOSTA2

1Instituto Madrileño de Investigación y Desarrollo Rural Agrario y Alimentario (IMIDRA), Finca El Encín, Alcalá de Henares, Spain, and 2Departamento de Ecología, Facultad de Biología, Universidad Complutense de Madrid, Madrid, Spain

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Abstract
The effects of the number of locules per fruit on seed predation in a Mediterranean woody plant (Cistus ladanifer) were analysed. Plants produced fruits with a similar number of locules (fruits with a small, intermediate or large number of locules). Nevertheless, there were no differences in the number of fruits produced or predated per plant. Different types of fruits did not differ in size, seed weight, fruit to wall ratio, insect predation rate or mean proportion of destroyed locules. They did differ, however, in the variation of the proportion of destroyed locules; fruits with few locules had a wider variation than fruits with a large number of locules. Our results suggest that phenotypic selection pressure on insect predation could give rise to more between-fruit variation in the seed output saved from predation in those plants producing fruits with a small number of locules.

Keywords: Cistus, insect predation, loculate fruits, phenotypic selection, seed packaging

Introduction
Plant morphology and physiology are ultimately regulated by the pattern of allocation to competing structures and functions (Mooney 1972; Tilman 1988, 1990). The way a plant allocates its resources is therefore a key factor for understanding its life-history strategies and as such it has received much attention (Fitter 1986; Lovett Doust 1989; Kozlowski 1992; Gleeson and Tilman 1994).

The allocation of plant resources between different types of reproductive material (e.g. seeds and fruit walls; hereafter allocation) and the partitioning of the same type of material for reproduction (e.g. seed number versus seed size; hereafter partitioning) define some fruit characteristics such as fruit size, seed to fruit wall ratio, seed size, seed number and, within loculate fruits, number and size of locules. All of these fruit traits have been reported to be important factors affecting predispersal seed predation by insects (Janzen 1971; Green and Palmbald 1975; Bradford and Smith 1977; Mitchell 1977; Herrera 1984;
Knight 1987). Insect seed predation can also exert a strong selective pressure promoting insect–plant coevolution (Fenner 1985; Ehrlich and Murphy 1988; but see Crawley 1983; Labeyrie 1987). Predispersal seed predation could therefore have an important influence on the results, in terms of fitness, of such allocation and partitioning strategies through the selection of different fruit features. The analysis of both reproductive allocation strategies and predation patterns must be considered simultaneously for an accurate assessment of plant reproduction performance (Janzen 1971; Zimmerman 1980; Louda 1982a, 1982b; Crawley 1992 and references therein).

Insects that attack fruits whose seeds are partitioned into two or more isolated cavities or locules (loculate fruits in a wide sense) have access only to those seeds found in the locule they penetrate. So they must bore the fruit wall several times in order to access sufficient resources (see e.g. Bradford and Smith 1977). Therefore, fruit profitability could decrease from the perspective of predators, and then insect predation could be reduced by an increase in the number of fruit locules. Yet increasing the number of locules also has its drawbacks for the plant. For example, this could increase the cost of packaging per seed, which may decrease the returns of reproductive maternal investment (Uma Shaanker et al. 1988; Willson et al. 1990).

In this work, we have analysed the patterns of resource allocation and partitioning in the loculate fruits of a woody perennial plant, as well as the parallel patterns of insect predation observed in these fruits, in relation to their consequences in terms of fitness.

Our approach is focused on the division of fruits into locules (compartmentation) and thus we have specifically assessed: (1) variation in the number of locules per fruit in the population and within individuals; (2) the relation between fruit compartmentation and other fruit and plant features (fruit size, seed number, cost of packaging-protective structures, fruit production and fruit predation); and (3) the influence of these fruit features upon seed losses due to predation by the larvae of a lepidopteran species (main predator in the study area). Our analysis takes into consideration both the susceptibility to predation of fruits with a different number of locules, and the observed outcome of such predation in terms of locule losses for each type of fruit.

Material and methods

Study area and species

The study area is located near the village of Tres Cantos, 20 km north of Madrid (central Spain). All fieldwork was carried out in a continuous natural patch of *Cistus ladanifer* L. (Cistaceae) which also contains some scattered trees of the Mediterranean holm oak *Quercus rotundifolia* Lam.

*C. ladanifer* fruits are globular lignified capsules with 7–10 internal valvae, which delimit the locules (Martín and Guinea 1949). This variability in locule number is a typical feature of this species, not shared by other species of the same genus, which almost invariably present fruits with five locules (Castroviejo et al. 1993). Each fruit contains many seeds (500–1000) which remain inside it during a period of several weeks before being released. The major seed predators (both in terms of number of individuals and biomass) in our study site are the larvae of the moth *Cleonymia (Serryvania) yvanii* (Duponchel) (Lepidoptera, Noctuidae). The larvae bore a hole in the external wall of the capsule to feed upon the seeds, using the same hole to leave the fruit. The number of holes in a fruit is thus a reliable measure of the number of larvae that have infested that fruit (Serrano et al.
Larvae pierce the fruit wall, eat all (or most) seeds inside the first locule and then drill through a valva to move on to the next locule. The number of locules pierced by the larvae provides a reliable measure of seed losses in a fruit, because even when this predator leaves some uneaten seeds inside these pierced locules, these are further consumed by secondary predators (mainly lygaeid bugs) or attacked by microbes (usually associated with seed predator Lepidoptera, see e.g. Knight 1987 and references therein).

Variation within individuals

The sampling work was carried out in late summer (September 1996), immediately before the fruit capsules release the seeds, which coincides with the end of the annual period of insect predation. To assess the pattern of fruit compartmentation at the individual plant level, 50 plants were randomly selected. All fruits per plant were removed and the number of locules per fruit was recorded. The heterogeneity in fruit compartmentation within each plant was estimated according to an “entropy equation” (equivalent to the Shannon index) as follows:

$$H = - \sum_{i=7}^{10} p_i \log_2 p_i$$

where $p_i$ is the proportion of fruits with $i$ locules in the fruit set of each individual plant. Since there are only four possible types of fruits (7, 8, 9, and 10 locules), entropy values can theoretically range from 0 (all fruits being equal) to 2 (equal proportions of each type of fruit). The observed frequency distribution of the entropy values in the sub-sample represents the range of variation in locule number within plants.

To compare this distribution with the one arising according to the null hypothesis of random production of fruits with different numbers of locules within individual plants, we obtained an expected frequency distribution using computer simulation. We used a computer algorithm that extracts fruits randomly from the observed set of fruits in the sub-sample and randomly assigns them to each simulated plant. Since plants with the same number of fruits ($j$) should have the same expected values, simulations were performed for each group of plants in the sub-sample with the same number of fruits. A total of 3000 runs per group were performed calculating entropy values per each individual simulated plant. The results of the simulation were arranged as a frequency distribution of entropy values for each group of plants with the same number of fruits ($fH_j$). The sum of these functions, each one multiplied by its probability of occurrence (i.e. the observed probability of plants in the sub-sample with the same number of fruits: $q_j$), is the expected frequency distribution function of entropy values in the sub-sample for the null hypothesis of a random production of fruits within plants:

$$\sum fH_j q_j$$

Expected and observed frequency distributions were compared by means of a $\chi^2$ test.

Variation in the population

Five hundred additional C. ladanifer plants were randomly selected within the study area. To compare fruit features in the plant population using fruits as the units of comparison, and in order to avoid pseudoreplication (Hurlbert 1984), only one fruit per plant was
randomly selected. All 550 fruits collected were classified into four categories according to the number of locules (7–10). Fruits within each category were externally examined to assess predation, and the number of holes per fruit was recorded. Since it was not possible to take reliable weight measurements on fruits damaged by seed predators, we recorded total seed dry weight and fruit wall dry weight only for intact fruits. Total fruit dry weight was calculated as the sum of both measurements. The costs of packaging per seed were estimated as the seed to fruit wall ratio, calculated as the total dry weight of seeds divided by total fruit wall dry weight. The volume of each non-predated fruit was estimated as the volume of an ellipsoid according to the following equation:

\[ V = \frac{4}{3} \pi \left( \frac{d}{2} \right)^2 h \]

where \( h \) is fruit height and \( d \) is the maximum diameter of the fruit measured in a plane perpendicular to fruit height.

All damaged fruits were opened by hand to count the number of damaged locules inside them. Seed losses in each fruit were estimated as the number of infested locules (all seeds eaten by insects) divided by the total number of locules.

All mass and volume measurements of fruits were compared using analyses of variance to assess whether fruits with different numbers of locules also differed in other features. The relationship between the number of locules in a fruit and its susceptibility to be damaged by larvae (‘‘external’’ predation) was analysed by means of contingency table analysis. Finally, quantitative seed losses in fruits with different numbers of locules (‘‘internal’’ predation) were compared by means of analysis of variance. To test for possible effects of the number of fruit locules on seed losses, the potential confounding effects of fruit volume or fruit mass were assessed by means of analysis of covariance (Zar 1984; Wilkinson 1987), using the number of locules as a classification variable and the other variables as covariates. Data were log-transformed in order to achieve normality and homogeneity of variances, except seed to fruit wall ratio and internal predation that were arcsine-transformed.

Results

Variation within individuals

The number of locules per fruit in our population ranged from 7 to 10 and the number of fruits per plant from 26 to 180. The frequency distribution of the individual plant entropy values (heterogeneity in fruit compartmentation) in the sub-sample differed significantly from that predicted by a null model of random production of fruits within individual plants (Figure 1). Most expected entropy values (approximately 95%) were very high, larger than 1.6, whereas most observed entropy values (around 70%) were lower than 1.6 (Figure 1). This indicates that, within individual plants, the predominance of one type of fruit is clearly more frequent than that expected at random.

The proportion of individual plants in which a single fruit type was predominant (i.e. observed frequency of occurrence \( \geq 0.5 \)) was around 70%. Moreover, the second more frequent fruit category within the same plant was always a consecutive one (e.g. fruits with seven and eight locules) and both of them constituted at least 70% of the total fruit production for 96% of the individual plants. These results suggest that individuals could be confidently divided into three categories: (1) those producing fruits with a small number of locules (7 and 8), (2) those producing fruits with an intermediate number of locules (8 and
9), and (3) those producing fruits with a large number of locules (9 and 10). In our sample, the proportion of each type of individual was clearly not random ($n=50$; df=3; $\chi^2=2.971$; $P<0.001$).

More individuals than expected produced fruits with a high number of locules (58%) whereas those producing fruits with few locules were quite rare (8%). These different types of plants did not significantly differ in terms of number of fruits per individual or in terms of proportion of the fruit yield saved from insect predation (Table I).

**Variation in the population**

In the population, the number of locules per fruit ranged from 7 to 10 with different proportions (Figure 2). Fruits with different numbers of locules did not differ in fruit volume, fruit wall mass, total seed mass, total fruit mass, or seed to fruit wall ratio

|                      | Mean ± SE            | Mean ± SE            | Mean ± SE            |
|----------------------|----------------------|----------------------|----------------------|
|                      | $F$ value            | Large ($n=30$)       | Intermediate ($n=16$)| Small ($n=4$)         |
| Total number of fruits | 0.219 (NS)           | 99.033 ± 14.921      | 110.688 ± 19.510     | 125.000 ± 68.393      |
| Proportion predated   | 0.205 (NS)           | 0.442 ± 0.024        | 0.468 ± 0.043        | 0.471 ± 0.041         |

NS, not significant.
These results suggest that partitioning of the fruit biomass in different number and sizes of locules is not influenced by the total amount of biomass to partition, and also that this compartmentation does not affect the way fruit biomass is allocated to seeds and packaging structures.

Fruits with different number of locules did not significantly differ in their proportions of external predation \((n=550; \text{df}=3; \chi^2=2.971; P>0.05)\). Approximately 45% of fruits of each category were predated. In addition to this, the proportion of each fruit destroyed by predators (approximately 60%) did not differ between the fruit categories either (Table II). Insect predation seems therefore not to be related to the number of fruit locules, both as an

![Figure 2. Frequency distribution of fruits with different numbers of locules (random sample of 550 fruits).](image)

| Table II. Results of the analyses of variance on fruit features and proportion of destroyed locules (internal predation) in the studied *Cistus ladanifer* plants. Comparisons were made for fruits with different numbers of locules. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | \(F\) value     | 7 locules       | 8 locules       | 9 locules       | 10 locules      |
| Total fruit mass (g) | 0.120 (NS) | 0.377 ± 0.015 | 0.387 ± 0.014 | 0.382 ± 0.012 | 0.381 ± 0.017 |
| Total seed mass (g)  | 0.434 (NS) | 0.143 ± 0.008 | 0.154 ± 0.008 | 0.147 ± 0.006 | 0.153 ± 0.008 |
| Fruit capsule mass (g) | 0.256 (NS) | 0.221 ± 0.011 | 0.235 ± 0.010 | 0.221 ± 0.008 | 0.227 ± 0.010 |
| Seed to fruit wall ratio | 0.073 (NS) | 0.743 ± 0.083 | 0.726 ± 0.077 | 0.723 ± 0.062 | 0.762 ± 0.105 |
| Fruit volume (cm\(^3\)) | 0.489 (NS) | 0.689 ± 0.026 | 0.726 ± 0.023 | 0.695 ± 0.019 | 0.709 ± 0.023 |
| Internal predation   | 0.069 (NS) | 0.629 ± 0.039 | 0.648 ± 0.034 | 0.633 ± 0.027 | 0.617 ± 0.023 |

NS, not significant.
observed probability for a fruit to be predated and for the proportion of the fruit being destroyed.

In spite of these results, fruits with different numbers of locules did differ in the way insect predation was distributed within fruits. The spread of data in the frequency distributions of fruit proportion destroyed in each fruit category diminished with increasing number of locules per fruit, as shown by sample variance values (Figure 3). This indicates that fruits with more locules suffer a relatively more similar predation than fruits with fewer locules, and therefore implies a wider variation in the proportion of saved seeds in the latter.

Discussion

In the studied population, individuals differed in the number of locules per fruit, with individuals producing fruits with a small, intermediate, or large number of locules. This result suggests that the number of locules per fruit presents a genetic basis, as has been reported in other species (Barrero and Tanksley 2004).

Biomass investment per *C. ladanifer* fruit did not change with the number of locules and the cost of packaging per seed was also equivalent. The reproductive partitioning pattern observed in a fruit (which gives rise to different configurations of number and size of locules) seems therefore to be independent of other allocation patterns taking place at the same level.

The existence of any such dependence between allocation/partitioning patterns and fruit configuration would have the potential for selective processes that could be related to seed production or to predispersal seed predation. In our case, however, the variability in the number of locules of fruits reported in the studied *C. ladanifer* population was not associated with differences in the total amount of seeds produced and was consequently unrelated to any benefits in terms of seed production. Furthermore, the observed pattern of seed production is not differently affected by predispersal seed predation by insects. In other words, *C. ladanifer* fruits with different numbers of locules seemed to be equally

![Figure 3](image-url)

**Figure 3.** Sample variances and frequency distribution functions of proportion of the fruit destroyed by insect predators for different types of fruits (with 7–10 locules). Variance decreases when increasing locule number (Gamma correlation: $\gamma = -2.04; P<0.05$). Least squares fitting line is shown to facilitate visualization.
profitable, producing (and releasing after predispersal seed predation) the same weight of seeds at the same cost.

We found no differences in the proportion predated among fruits with different numbers of locules, which suggests that predators are not exerting a differential selective pressure on this fruit trait. That phytophagous insects are weak selective agents has been argued elsewhere and has even found empirical support in some studies on specialist seed-eating insects (e.g. Courtney 1981; Labeyrie and Hossaert 1985). The great variability of predation in space and time, its dependence on microclimate, and future plant reproductive events that compensate for large but temporally restricted seed losses, have been reported as causes of the weakness of the selective pressure exerted by insects on plants (Crawley 1983; Labeyrie 1987; Jordano et al. 1990). The plant–insect relationship found in this study could also be interpreted in this way, with insects not selecting on locule number, nor favouring the variability of this fruit feature, which could have evolved under different selective pressures.

Regardless of the lack of difference in the average proportion of locules predated, the distribution of locule losses varied across fruits with different numbers of locules. In fruits with a large number of locules most fruits had about half of their locules predated (40–60%), whereas in fruits with fewer locules the distribution of locule loss was less uniform.

In the studied population, each individual plant produces fruits with different numbers of locules, but there is a predominance of fruits with a large, medium, or small number of locules in each plant. From these results it is plausible to assume that predation patterns found for the different groups of fruits generally correspond to whole plants because they act in fact as groups of fruits with similar numbers of locules. Different genotypes (represented by different individual plants), whatever the type of fruits they have (small to large number of locules) do not differ in the seed mass per fruit produced, nor in the average fruit proportion saved after predispersal seed predation by insects. Yet the significance of a predated fruit differs strongly in the different types of individual plants. Most predated fruits in an individual plant producing fruits with a large number of locules are similarly affected by predation, whereas predated fruits in a plant producing fruits with a small number of locules may equally lose most fruit seed production or nearly nothing. This different degree of variation of predation pressure among predated fruits in different individuals could also have relevant selective consequences. Differential effects of selection by insect predation are, therefore, more likely to occur within individual genotypes producing fruits with a small number of locules, because these show a wider variety of proportions predated in their fruits. This implies that the phenotypic selection pressure of insect predation will give rise to more variation in the saved seed output from different fruits in this type of individual plant.

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