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**Title:**

Variation in seed packaging of a fleshy-fruited conifer provides insights into the ecology and evolution of multi-seeded fruits

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**Abstract**

The study of intraspecific seed packaging (i.e. seed size/number strategy) variation across different populations may allow a better understanding of the ecological forces that drive seed evolution in plants. *Juniperus thurifera* (Cupressaceae) provides a good model to study this due to the existence of two subspecies differentiated by phenotypic traits, such as seed size and cone seediness (number of seeds inside a cone) across its range. The aim of this study was to analyse seed packaging (seed mass and cone seediness) variation at different scales (subspecies, populations and individuals) and the relationship between cone and seed traits in European and African *J. thurifera* populations. After opening more than 5,300 cones and measuring 3,600 seeds, we found that seed packaging traits followed different patterns of variation. Large-scale effects (region and population) significantly contributed to cone seediness variance, while most of the seed mass variance occurred within individuals. Regarding variation in cone seediness, we validated previous reports on the intraspecific

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differences in of *J. thurifera* seed packaging between the two sides of the Mediterranean Sea, with African cones bearing fewer but larger seeds than the European ones. However, no differences in seed mass were found between populations when taking into account cone seediness; thus, both subspecies followed the same seed size/number trade-off. Regarding variation in individual seed mass, larger cones contained more pulp and seeds and displayed a larger variation in seed mass, revealing that the positive relationship between seed and cone sizes may not be straightforward. We hypothesize that the large variation of seed size found within cones and individuals in *J. thurifera*, but as well in other fleshy-fruited species, could represent a bet-hedging strategy for dispersal.

## Introduction

Seed packaging, i.e. the seed size/number combination within fruits, can have important consequences for plant fitness since it will affect the number and quality of the seeds dispersed for each fruit (Schupp et al. 2010). There is a trade-off between the number of seeds that each fruit may contain (i.e. fruit seediness) and the minimum size that seeds should achieve to successfully germinate and establish after dispersal (Leishman 2001; Gómez 2004). On the one hand, large seeds benefit from size-related advantages to survive the hazards of establishment, such as burial depth, competition, shade, drought, defoliation, and nutrient shortage (Moles & Westoby 2004). On the other hand, small seeds are less prone to be found and eaten (Gómez 2004), can be more easily buried and persist in the soil (Peco et al. 2003), and are less likely to be regurgitated and, thus, retained longer in dispersers' guts (Jordano 1995; Obeso et al. 2011).

Seed traits are genetically, developmentally and physiologically linked to fruit traits constraining the potential evolutionary responses of seed packaging traits (Karlova et al. 2014). Fruit size, seed size and fruit seediness show significant levels of phenotypic

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integration, higher than other fruit traits such as nutritional or visual traits (Valido *et al.* 2011). Seed mass is usually positively correlated with fruit size (Sallabanks 1993; Jordano 1995; Alcantara & Rey 2003; Edwards 2005; Martinez *et al.* 2007; Chen *et al.* 2010), whereas negative covariation generally occurs between fruit size and pulp nutrients (Valido *et al.* 2011). In multi-seeded fruits, where several seeds are packed within the same structure, fruit seediness may mediate the relationship between seed and fruit size. Fruit seediness is commonly positively correlated with fruit size (Valido *et al.* 2011), while negatively with mean seed size (Eriksson & Ehrlen 1991; Méndez 1997). In addition, packaging costs, i.e. the resources allocated to fruit pulp per seed, usually decrease as fruit seediness increase (Herrera 1981; Obeso 2004a).

Increasing seediness comes at the expense of seed size, yet not all seeds in a multi-seeded fruit reduce their size homogeneously, and consequently large within-fruit variation in seed size has been observed (Méndez 1997, Obeso 2004a). Architectural effects may favour asymmetric resource competition between early and late ovules within the fruit, similarly to those described in angiosperm inflorescences (Diggle 1995, 2003), increasing within-fruit variation. Fruit and seed traits usually vary within individuals (Harper *et al.* 1970) indicating that several optimal seed size/number combinations could occur within a plant. Indeed, variation in seed mass within individual plants is a widespread phenomenon, frequently being the larger component of the total observed variance in seed mass, even larger than among individuals or populations (Michaels *et al.* 1988). Recently, it has been shown that within-individual variation in seed and fruit traits may have functional implications and fitness consequences for plants (Herrera 2009; Sobral *et al.* 2013, 2014; Shimada *et al.* 2015) and might influence the structure and functioning of ecological networks (González-Varo & Traveset 2016). However, whether the variation in seed size within fruits has any adaptive significance is still unclear (Bañuelos & Obeso 2003; Obeso 2004b).

Despite this high within-individual variation, seed packaging can differ at large scales, i.e. among populations, displaying geographic patterns of seed traits. Abiotic conditions associated with resource availability may determine intra-specific phenotypic trends of seed packaging. Plant species tend to produce smaller fruits with more, smaller and less viable seeds at higher-elevation, higher-latitude or drier sites (García *et al.* 2000; Hampe 2003; Guo *et al.* 2010). Nevertheless, intraspecific geographic patterns for seed mass do not seem universal (Hampe & Bairlein 2000; Soper *et al.* 2016). For instance, trends for larger seeds in drier sites have been observed as a result of selection for surviving the hazards of establishment (Murray *et al.* 2004, Moles & Leishman 2008). Contrasting distribution patterns of seed predators and dispersers may also lead to large-scale differences in seed packaging. Some pre-dispersal predators, such as insects, may select both larger fruits with more seeds and lower pulp-to-seed ratio that facilitate the oviposition (Herrera 1984; Knight 1987, DeSoto *et al.* 2016) and larger seeds which produce larger predator offspring (Napela & Grissell 1993; Moegenburg 1996). Fruit and seed traits can be also mediated by dispersers that select for both larger fruits and higher pulp content, limited by their gape size, constraining as well the maximum seed size (Jordano 2000, Alcantara & Rey 2003). Finally, post-dispersal predation may also exert a selective pressure towards less frequently consumed smaller seeds (Gómez 2004). Therefore, fruit and seed traits may follow different patterns of variation revealing that seed packaging is controlled by a large assortment of ecological drivers acting at different levels.

Here, we assess seed packaging variation at different scales (subspecies, populations, and individuals) in the Spanish juniper, *Juniperus thurifera* L. This species represents an extraordinary model to explore the variation in seed packaging strategies. Previous taxonomic studies have described large intraspecific variation in cone and seed sizes and cone seediness (Boratyński *et al.* 2013). Cones are fleshy and dispersed by a diverse assemblage of animals

(Escribano-Avila *et al.* 2012) and seed packaging can be constrained by pre-dispersal seed predators (DeSoto *et al.* 2016). Thus, in this paper, we analysed seed packaging variation in *J. thurifera* using over 5,300 cones (1) to estimate the level – region, population, individual – at which seed packaging variation occurs; (2) to explore the relationship between cone size and seed packaging; and (3) to discuss the potential adaptive significance of within-individual and within-cone variation on seed packaging.

## Materials and Methods

### *Study species and sites*

*Juniperus thurifera* L. (Cupressaceae) is a dioecious long-lived (up to 600 years) conifer tree up to 20 m in height with a conical to rounded or irregular crown (DeSoto *et al.* 2012). It is endemic to continental areas of the western Mediterranean Basin at elevations ranging from 200 to 3,400 m.a.s.l. over a disjunct distribution in South Western Europe and North Africa, with the most abundant populations in Spain (200,000 ha) and the Medium and High Atlas Mountains in Morocco (30,000 ha; Gauquelin *et al.* 1999). Differences in leaf, cone and seed sizes, and cone seediness between the two sides of the Mediterranean Sea have been previously described. Particularly, European cones are larger and contain more but smaller seeds than do those in African populations (Boratyński *et al.* 2013). Cone dispersers are mainly birds such as thrushes (*Turdus* spp.), and mammals, mainly small carnivores (red foxes and stone martins), rabbits and sheep in the Iberian Peninsula (Escribano-Avila *et al.* 2012). In Moroccan populations, thrushes have been reported as *Juniperus* spp. dispersers (Ryall & Briggs 2006), although other effective dispersers such as livestock or small mammals cannot be discounted.

We selected three populations in Spain, Luna, Soria and Monegros Desert (Monegros) and two populations in the Moroccan High Atlas, Azzaden Ousseem (Ousseem) and Tizi Techt

(Techt, Fig. S1). In each population ca. 1,500 mature cones (150 cones from 10 haphazardly chosen female trees) were haphazardly collected all around the crown to avoid orientation effects. Sampling was done in October 2010 in Morocco and May-June 2011 in Spain when cones were completely ripened (DeSoto *et al.* 2016).

### *Sampling procedure*

Seed packaging evaluation considered both seed and cone traits, namely, individual seed mass (seed size), cone seediness (the number of viable seeds within a cone) and total seed mass (the sum of the masses of all seeds within a cone). To extract the seeds, cones were first embedded in a 1% sodium hypochlorite solution for two days to clean the waxes and resins (García-González *et al.* 2009). For each tree, we opened ca. 100 cones and counted the potential viable seeds and aborts inside. Since the frequency of aborted seeds was low (less than 4%) and they were too small (< 10mg) to demand resources and space within the cone, we used just the number of potential viable seeds for cone seediness. Seed size was measured as seed mass after weighing individually the seeds of 60 cones per tree. We only considered non-depredated cones for seed mass analysis since seed predation can exert a significant effect on the size of uneaten seeds within a depredated cone (DeSoto *et al.* 2016).

Before seed extraction, we weighed and then measured the largest and smallest diameters of 30 cones per tree; because these diameters were highly correlated ( $r = 0.91$ ,  $p < 0.001$ ,  $n = 1,526$ ), we used the largest diameter as a measure of cone size. We selected this characteristic because it will define the dispersers that can swallow the cones. We determined fresh pulp mass by subtracting the total seed mass from the cone mass, because we aim examining how the allocation of pulp mass, i.e. the packaging cost, varied with cone seediness and cone diameter. We used two measurements: i) pulp mass per individual seed, and ii) percentage of cone mass that corresponds to pulp (hereafter % cone mass to pulp).

Cone diameter may represent the cue observed by the disperser, whereas pulp mass per seed and % cone mass to pulp were the fractions showing, respectively, the amount of resources invested in seeds and the final reward value for dispersers. Given that the ultimate objective was to use all the seeds in a germination experiment (see DeSoto *et al.* 2016), seeds and cones were weighed fresh and all results provided are fresh values. Although *Juniperus* cones usually contain much less water than other fleshy fruit pulps (Herrera 1981), results provided here are not directly comparable with dry mass estimations. Because cones were collected at different times in each population and would have different water content, the exploration of cone size and pulp mass variation was only assessed within each population. Nevertheless, cone diameter differences among European and African populations have been already described with larger cones (8 mm) in Europe than in Africa (7 mm; Boratyński *et al.* 2013).

#### *Statistical analysis*

To estimate the level – region, population, individual – at which seed packaging variation occurs, we calculated the variance of individual seed mass and cone seediness explained by each level with a hierarchical partitioning approach. In hierarchical partitioning, the variance independently explained by each predictor (level) to describe the response variable is calculated (Chevan & Sutherland 1991). A predictor variable may have a high correlation with the response variable explaining a high amount of its variance, but also part of this variance may be shared with other variables that are correlated in a similar way. Hierarchical partitioning allows the identification of those variables explaining variation of the response variable independently of the other variables (MacNally 2002). This method has been broadly used for identifying important predictor variables of species distributions (e.g. Gutiérrez Illán *et al.* 2010), but also for assessing the importance of highly correlated reproductive traits on plant reproductive success (e.g. Torices & Méndez 2011). We computed the hierarchical partitioning of the effect of region (Africa vs. Europe), population,



tree and cone on cone seediness and individual seed mass. MacNally's (2002) randomisation method was used, with 499 simulations, to assess the statistical significance of the independent contribution of each effect. The analyses were conducted using the hier.part R-package (MacNally & Walsh 2004; R Development Core Team 2013).

In order to determine whether populations differed in seed and cone traits we conducted generalized linear mixed models (GLMMs). GLMMs provide a flexible way to model traits that do not satisfy the assumptions of a standard linear modelling, while allowing the distinction between fixed and random factors in the model. First, we modelled cone seediness considering a Poisson error distribution with log link function (McCullagh & Nelder 1989). Second, to model individual seed mass and total seed mass we assumed a Gaussian error distribution with an identity link. Individual seed mass was analysed with two different models, either including or excluding cone seediness as a fixed factor. The former model allowed us to explore seed mass differences taking into account the effect of cones with different seediness. In addition, we estimated the within-cone coefficient of variation (CV) for individual seed mass and compared it with cone seediness in each region in order to determine if cones with more seeds displayed higher individual seed mass variation. This approach allowed testing whether resources were homogeneously allocated to individual seeds within a cone regardless of cone seediness (Obeso 2004a). In other words, the higher the CV, the higher the mass inequality among seeds within a cone. We modelled CV of individual seed mass with GLMM assuming a Gaussian error distribution with an identity link, and region, cone seediness and the interaction between them as fixed factors.

Finally, we explored the relationship between cone diameter and seed packaging within populations using two approaches. First, we analysed the relationship between cone diameter (fixed factor) and individual seed mass, pulp per seed and % cone mass to pulp (response variables) for each population fitting GLMMs. Second, we explored whether cone

diameter, individual seed mass, pulp per seed and % cone mass to pulp differed among cones with different cone seediness, modelling these traits (response variables) by means of GLMMs, where cone seediness was included as the fixed factor. In all GLMMs, tree was considered as a random factor. Cone nested within tree was also included as a random factor in those models in which individual seed mass was the response variable. Differences between least-squares means were tested pairwise through multiple comparisons. We fitted all GLMMs using the GLIMMIX procedure of SAS (SAS Statistical package 9.2).

## Results

### *Variation in seed packaging*

We found significant independent effects of all predictors, region, population, tree and cone, on seed packaging traits, with cone being the major contributor to the variance (Fig. S2). For cone seediness, the variance was mainly explained by cone (43 %), but was similarly distributed between the three remaining studied levels: tree (21 %), population (18%), and region (18%). For individual seed mass, 68 % of the variance occurred among cones within individual trees, and 24 % of the variance was found among trees within the populations, while the effects of region and population were lower (4 % each).

### *Variation in seed packaging between regions and populations*

Cone seediness ranged from 1 to 9 for the European populations and from 1 to 5 for the African populations with clearly different frequency distributions (Fig. 1a). However, individual seed mass was lower in European populations than in the African ones (Table 1 and Table S1 for GLMMs results). Luna, Soria and Monegros had on average between three or four seeds per cone and a mean individual seed mass ( $\pm$  SE) of  $41.4 \pm 4.5$  mg, whereas Oussem and Techt had on average only one seed per cone and a mean individual seed mass

of  $63.4 \pm 4.4$  mg (Table 1). Total seed mass per cone was larger in the European populations ( $135.3 \pm 11.1$  mg) than in the African ones ( $72.6 \pm 10.8$  mg, Table 1 and Table S1). However, we did not find differences in individual seed mass among sites when cone seediness was considered as a covariate (Table S1).

Exploring seed packaging strategies in each region, we found a significant negative relationship between individual seed mass and cone seediness in all populations ( $F_{6,935} = 12.77$ ,  $P < 0.001$ ; Fig. 1b), namely, larger seeds were found in cones with fewer seeds. For each category of cones with the same seediness, no differences in individual seed mass were found between populations ( $F_{4,65} = 0.28$ ,  $P = 0.89$ ; Fig. 1b). Within-cone variation in individual seed mass, measured as coefficient of variation (CV), was positively correlated with cone seediness. Cones with more seeds showed a higher CV for seed mass and this relationship was similar in both regions (Fig. 1c, Table S2).

#### *Relationship between cone size and seed packaging*

Considering the most frequent cone seediness categories (3- and 4-seeded cones for European and 1-seeded cones for African populations) individual seed mass was positively correlated with cone diameter in all populations (Table 2). In contrast, individual seed mass decreased as cone seediness increased in all populations (Table S3 and Fig. 3), whereas total seed mass per cone increased with cone seediness (Table S3). Cone diameter increased with cone seediness in Soria, Monegros and Oussem but not in Luna and Techt (Fig. 2, Table S3).

Pulp mass per seed decreased with cone seediness in all populations (Fig. 3ab, Table S3), but it increased significantly with cone diameter in Soria and in both African populations (Table 2). The % cone mass to pulp significantly increased with cone seediness in European populations and marginally in African ones (Fig. 3cd, Table S3). In addition, % cone mass to

pulp significantly increased with cone diameter in Soria and Monegros populations, while decreased for larger cones of Oussem (Table 2).

## Discussion

### *Seed packaging variation between regions*

Seed packaging traits followed different patterns of variation. While large-scale effects (within regions and populations) significantly contributed to cone seediness variance, most of the individual seed mass variance occurred among cones (within individuals). These different patterns indicate that different ecological drivers acting at different levels might be shaping seed packaging traits.

Seed packaging strategies differed between Europe and Africa in the studied *J. thurifera* populations where cones from African populations had fewer but heavier seeds (single-large-seeded cones) than those from European populations (multi-small-seeded cones). This difference at a large scale agrees with the subdivision of *J. thurifera* into two subspecies, subsp. *thurifera* (European) and subsp. *africana* (African), that have been described based on differences in leaf shape, cone and seed sizes, and seediness (Boratyński *et al.* 2013; but see Farjon 2005). Among these traits, cone seediness rather than individual seed mass might be more useful to discriminate between European and African populations, since the effect of population and region was important in determining cone seediness variation but seed mass variation was largely found within cones and trees. Furthermore, no differences in individual seed mass between populations were found when comparing cones with the same seediness. This result suggests that both strategies, multi- and single-seeded cones, may display the same seed size/number trade-off in both regions (and subspecies) but still differ in cone seediness. Hence, understanding the causes of the variation in cone seediness between African and European populations of *J. thurifera*, mediated by the seed

size/number trade-off and the covariation with other traits at cone (fruit) level, may provide insights on the large-scale drivers of the evolution of seed packaging traits in fleshy-fruited species.

We hypothesize that in Africa, single-large-seeded cones could have evolved as a response to abiotic conditions. Climatic conditions could exert selection on cone and seed traits in at least two different processes: resource allocation to cones and seed establishment. Drier environments may affect fruit traits associated with carbon and water availability limiting fruit size and pulp allocation. A large-scale study of fruit trait variation of vertebrate-dispersed plants showed that fruit size and fresh mass decline towards the drier sites, which could be a consequence of water availability (Hampe 2003). In *J. thurifera*, small cones in dry African populations (Boratyński *et al.* 2013) might be a consequence of reduced water availability. European populations occur in a colder and more humid climate (except in Monegros Desert, Fig. S1; DeSoto *et al.* 2012), while African populations, mainly located in the High Atlas Mountains, cope with a drier and warmer climate with a longer summer drought (Fig. S1; Romo & Boratyński 2005; DeSoto *et al.* 2014).

In addition, smaller cones in *J. thurifera* bear only one (or few) large seeds. Single-large-seeded cones produced in African populations could be favoured because larger seeds benefit from size-related advantages to survive the hazards of establishment in hot dry environments (Parciak 2002; see Moles & Leishman 2008 for a review). Within the same species larger seeds are required to overcome metabolic costs associated with higher temperatures (Murray *et al.* 2004) and usually have a higher germination success (Alcántara & Rey 2003; Gómez 2004; Seltnann *et al.* 2006). Higher germination rates of large seeds have also been observed in *J. thurifera* (Escribano-Ávila *et al.* 2013; DeSoto *et al.* 2016). Therefore, drought and temperature could act as selection agents towards single-large-seeded and smaller cones through resources availability to cones and seedling performance in the

Atlas Mountains (Africa). However, single-large-seeded, small cones may be also an evolutionary response to other ecological pressures. For instance, despite the similar climate observed in African sites and in Monegros, cones in the latter were large and multi-small-seeded. Given the lack of information on dispersal in the Atlas Mountains, it cannot be discounted that small dispersers, limited by their gape size, might be selecting for small cones and, thus, constraining seed packaging (Jordano 2000, Alcantara & Rey 2003).

Under less stressful abiotic conditions, the combined effect of dispersers and pre-dispersal predators could favour multi-seeded cones in European populations. In fleshy-fruited species, seed packaging can have important consequences for plant fitness since it will affect the number and quality of the seeds dispersed for each eaten fruit (Schupp *et al.* 2010). In multi-seeded fruits, the number of dispersed seeds per eaten cone is higher, and the relative cost per seed is usually lower than in single-seeded fruit because of the negative relationship between fruit seediness and pulp-to-seed ratio (Leishman 2001; Obeso 2004a). In *J. thurifera*, the amount of pulp per seed decreased with cone seediness, although the relative allocation of pulp mass per cone increased with cone seediness. Previous studies have evidenced that the proportion of pulp mass usually decreases with fruit seediness (Herrera 1981; Hedge *et al.* 1991; Méndez 1997). This unexpected pattern might be also a response to disperser's selection for larger cones and to high pre-dispersal seed predation regimens in these populations. First, an effective selection for larger multi-seeded fruits by birds may be favoured by an increase in the relative pulp allocation (Jordano 2000). The percentage of pulp mass increased with cone seediness and diameter in *J. thurifera* European populations. Hence, by selecting larger multi-seeded cones, thrushes may feed on more pulp, receiving an honest signalling from the plant. Second, it is assumed that large fruits with a low pulp-to-seed ratio could facilitate the oviposition of insects (Herrera 1984; Knight 1987). Thus, this increase in the relative allocation of pulp with cone seediness could be an adaptive response

against high levels of pre-dispersal predators. Understanding if a larger % cone mass to pulp of *J. thurifera* cones is effectively limiting the efficiency of oviposition requires quantitative examination.

Nevertheless, the advantage of multi-seeded cones vs. single-seeded cones under pre-dispersal seed predation seems clear (DeSoto *et al.* 2016). Seed predators of *J. thurifera* usually do not eat all seeds within a cone, leaving at least some uneaten seeds in multi-seeded depredated cones while single-seeded cones have no seeds after a seed predator attack, thus, paying a higher cost when they are depredated. Consistently, we observed greater levels of pre-dispersal predation in European *J. thurifera* populations with multi-small-seeded cones than in African populations with single-large-seeded cones (DeSoto *et al.* 2016). All these evidence suggest that pre-dispersal seed predators might have been one of the main drivers maintaining multi-seeded cones in European populations in spite of the germination advantage of larger seeds produced in cones with fewer seeds.

#### *Relationship between cone size and seed packaging*

The positive relationship between seed and fruit sizes may not be straightforward in a multi-seeded fruit species. In general, larger fruits contain larger seeds (Sallabanks 1993; Jordano *et al.* 1995; Alcantara & Rey 2003; Edwards 2005; Martinez *et al.* 2007; Chen *et al.* 2010), and we consistently found a positive relationship between cone diameter and average seed mass in *J. thurifera*. However, in multi-seeded fruits the relationships may become more complex because (1) fruit size is positively related not only to seed size but also to fruit seediness (Valido *et al.* 2011), (2) seed size decreases with fruit seediness following the size/number trade-off (Méndez 1997; Chen *et al.* 2010) and (3) fruit seediness presents a positive correlation with seed size variation (Méndez 1997; Obeso 2004a). In *J. thurifera*, cones with higher seediness contained smaller seeds with larger variation in seed mass and

were frequently packed in larger cones. Thus, smaller seeds can be found in both small and large cones but large seeds are very unlikely to occur in small cones (see Fig. S3). This result may have implications for understanding fruit selection by seed dispersers. Previous studies have assumed a direct correlation between seed size and fruit size for *J. thurifera* making conclusions about the size of cones consumed by different dispersers based on the size of seeds obtained from disperser's droppings (Escribano-Ávila *et al.* 2013). Given the non-straightforward relationship between seed and cone sizes shown in this study, the extrapolation of fruit selection from the size of seeds found in droppings should be avoided.

*An adaptive hypothesis for within-fruit and within-individual variation in seed size*

It is unknown whether the large within-cone and within-individual variation on seed mass observed in *J. thurifera* could have an adaptive significance. Sequential developmental patterns within *Juniperus* cones (Schulz *et al.* 2003) could lead to architectural effects similar to those described in angiosperm inflorescences (Diggle 1995, 2003) and could result in asymmetric resource competition between first and late ovules within the cone. Besides architectural effects, other factors such as multiple paternity (Temme 1986, but see Pélabon *et al.* 2015), sibling rivalry (Uma Shaanker *et al.* 1988), seed predation (Herrera 1984; DeSoto *et al.* 2016) or maternal effects (Halpern 2005) may also influence seed size variation.

Beyond the proximal causes responsible for seed size variation within a fruit, an important question remains unanswered: is this seed size variation adaptive? Theoretical models predict the opposite: one optimal parental-investment strategy leading to equal investment in each offspring (Smith & Fretwell 1974, McGinley *et al.* 1987, but see Geritz 1995). For instance, under parent-offspring conflicts it has been argued that a lower seed size variation may be beneficial for plants because homogeneous progeny might reduce



asymmetric competition between siblings (Uma Shaanker *et al.* 1988; Bañuelos & Obeso 2003; Obeso 2004a; Obeso 2004b). Nevertheless, multi-seeded fleshy fruits do not match the expectations of these models. As we observed in *J. thurifera* trees, most of seed size variation in multi-seeded species occurs within fruits, e.g. *Tamus communis* (Obeso 2004a), *Asphodelus albus* (Obeso 1993), or *Arum italicum* (Méndez 1997).

Here, we propose that within-fruit and within-individual variation in seed size in fleshy-fruited plants might bring the opportunity to plants to diversify the dispersal of seeds from the same fruit (or same individual plant). Within-individual seed mass variation is high in fleshy-fruited species (Obeso & Herrera 1994, Michaels *et al.* 1988) and could be as high as in dry-fruited species. As it has been described for many dry-fruited plants (Venable 1985; Venable *et al.* 1998; Imbert 2002; Dubois & Cheptou 2012), fleshy-fruited species could benefit from producing seeds of different sizes. Seed size influences whether seeds are regurgitated or passed through the digestive track and, also affects the time of gut retention in birds (Jordano 1995, Hernández 2009). It could therefore favour that seeds from the same fruit or individual might be dropped at different locations, allowing the progeny derived from the same mother to sample a range of different environments for establishment and reducing sibling competition. Although this bet-hedging strategy may increase the potential seedling mortality associated with the sampling process, it could be evolutionary advantageous via the increase of long-term fitness (Gadgil 1971; Venable & Brown 1993; Childs *et al.* 2010) and deserves further investigation.

## **Conclusion**

Two seed packaging strategies with the same seed size/number trade-off exist within *J. thurifera*: African single-large-seeded cones vs. European multi-small-seeded cones. Although this has been previously described, our study highlights important consequences for

ecological studies of *J. thurifera*, and potentially other multi-seeded fruit species. First, there is high within-individual variation in cone and seed traits. Second, despite this high intra-individual variation, cone seediness is clearly distinct between regions. Third, while European multi-small-seeded cones may be maintained by biotic selective pressures, such as predators, and by a diverse assemblage of dispersers, African single-large-seeded cones might have evolved as a better strategy to overcome limiting climatic conditions for seedling establishment. Fourth, because *J. thurifera* multi-seeded larger cones contain more seeds but have smaller and more variable sizes, the positive relationship between seed and cone sizes may not be straightforward for multi-seeded species. Finally, the high seed size variation observed in multi-seeded cones and within individuals might allow individuals to diversify dispersal representing a bet-hedging strategy.

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## Figures captions

**Figure 1. Seed size/number strategies of each region.** Percentage of cones (a), model-adjusted least-square means  $\pm$  SE of individual seed mass (b), and CV of individual log-transformed seed mass (c) for each category of cone seediness for European and African populations. Significant differences of least-squares means ( $P < 0.05$ ) are indicated with different letters; uppercase for European, lowercase for African populations. Results are according to the GLMMs in Supporting Information Tables S1 and S2 (Percentages of cones with low representativeness were not included: European cones with eight seeds = 0.68 %, and nine seeds = 0.12 %; African cones with four seeds = 0.15 %, and five seeds = 0.10 %).

**Figure 2. Relationship between cone seediness and either individual seed mass or cone diameter.** Least-square means  $\pm$  SE of log-transformed cone diameter (left, closed circles) and log-transformed individual seed mass (right, open circles) for distinct cone seediness categories for European (Luna, Soria and Monegros) and African (Oussems and Techt) populations. The figures are based on the Type III test in Supporting Information Table S3. Significant differences of least-squares means are indicated with different letters ( $P < 0.05$ ; sample size are indicated in parenthesis).

**Figure 3. Seed packaging cost and cone seediness.** Least-square means  $\pm$  95% Confidence Intervals of mass of pulp per each seed (mg, log-transformed) (a,c) and % cone mass to pulp (b,d) both for distinct cone seediness categories for European, Luna (grey dots), Soria (black dots) and Monegros (white dots), and African Oussems (grey dots), and Techt (black dots). Significant differences of least-squares means ( $P < 0.05$ ) are indicated with different letters. Results are according to GLMMs in Supporting Information Table S3.

**Table 1. Cone and seed traits.** Cone largest diameter (mm), cone seediness, individual seed mass and total seed mass (mg) for the studied populations.

Population	No. Trees	No. Cones	Cone diameter (mm)		Cone seediness			Individual seed mass (mg)			Total seed mass (mg)			
			Mean	range	Mean*	CI*		No. Cones	Mean*	CI*	Mean*	CI*		
<b>Europe</b>														
Luna	10	211	9.2	7.6 - 11.2	3.3	2.9 - 3.7	a	177	43.4	34.0 - 52.8	a	136.2	114.1 - 158.2	a
Soria	10	467	9.0	7.2 - 11.8	3.3	3.0 - 3.6	a	290	40.1	30.9 - 49.2	a	133.1	111.6 - 154.7	a
Monegros	10	631	9.4	7.6 - 12.0	3.3	3.0 - 3.6	a	309	40.7	31.5 - 49.9	a	136.6	115.0 - 158.1	a
<b>Africa</b>														
Oussem	12	802	8.3	7.1 - 10.4	1.2	1.0 - 1.3	b	446	61	52.6 - 69.4	b	71.1	51.5 - 90.8	b
Techt	9	638	8.6	7.0 - 10.2	1.1	1.0 - 1.2	b	405	65.8	56.1 - 75.5	b	74.1	51.4 - 96.7	b

\*Mean, model-adjusted back-transformed least-square means; CI, 95% confident intervals, based on the Type III test in Table S1 in Supplementary Information. Significant differences of least-square are indicated with different letters ( $p < 0.05$ ). Cone diameters were not compared among populations because they were collected at different time likely displaying different size due to changes in water content.

**Table 2. Relationship between cone diameter and seed packaging.** Type III test conducted on the generalized mixed model to test the effect of cone diameter (modelled as fixed factor) on cone seediness, individual seed mass, pulp mass per seed, and % cone mass to pulp for each population. For individual seed mass two models considering (I) all cone seediness categories or (II) more frequent cone seediness categories (3- and 4-seeded cones for European populations and 1-seeded cones for African populations) were used. Tree and cone (only for seed mass analysis) were also included in the model as random factors and both displayed significant effects (n = 9-12 trees per population). df, degrees of freedom. Estimate  $\pm$  SE, estimator and standard error of the slope coefficient.

Population	N	df	F	P	Estimate $\pm$ SE
<b>Pulp mass per seed</b>					
Luna	100	1,63.2	2.44	0.124	
Soria	188	1,183.9	13.83	< 0.001	0.14 $\pm$ 0.04
Monegros	238	1,233.3	0.64	0.422	
Oussems	344	1,341.9	17.64	< 0.001	0.18 $\pm$ 0.04
Techt	239	1,107.2	17.9	< 0.001	0.22 $\pm$ 0.05
<b>Cone mass pulp (%)</b>					
Luna	100	1,30.6	0.34	0.565	
Soria	188	1,185.0	4.71	0.031	0.06 $\pm$ 0.03
Monegros	238	1,228.6	4.64	0.032	0.05 $\pm$ 0.02
Oussems	344	1,341.4	10.42	0.001	-0.10 $\pm$ 0.03
Techt	239	1,94.3	0.94	0.336	
<b>Individual seed mass I</b>					
Luna	341	1,240	12.93	< 0.001	1.35 $\pm$ 0.38
Soria	652	1,463	33.52	< 0.001	1.60 $\pm$ 0.28
Monegros	813	1,574	16.19	< 0.001	0.88 $\pm$ 0.22
Oussems	436	1,91	0.55	0.459	
Techt	279	1,39	19.02	< 0.001	2.00 $\pm$ 0.46
<b>Individual seed mass II</b>					
Luna	252	1,180	19.44	< 0.001	1.58 $\pm$ 0.35
Soria	453	1,326	47.22	< 0.001	1.99 $\pm$ 0.29
Monegros	577	1,409	32.4	< 0.001	1.43 $\pm$ 0.25
Oussems	259	1,246	7.17	0.008	0.74 $\pm$ 0.28
Techt	201	1,191	18.81	< 0.001	1.77 $\pm$ 0.41

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Figure1

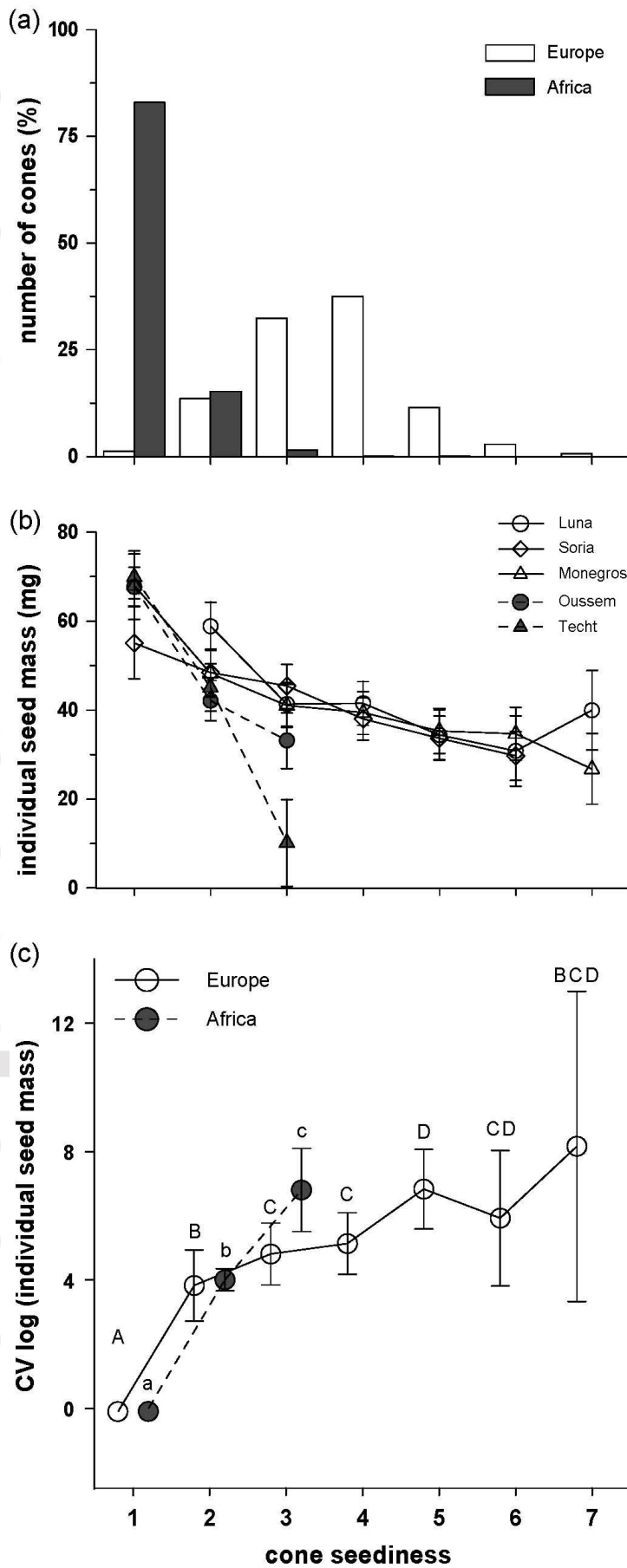


Figure 2

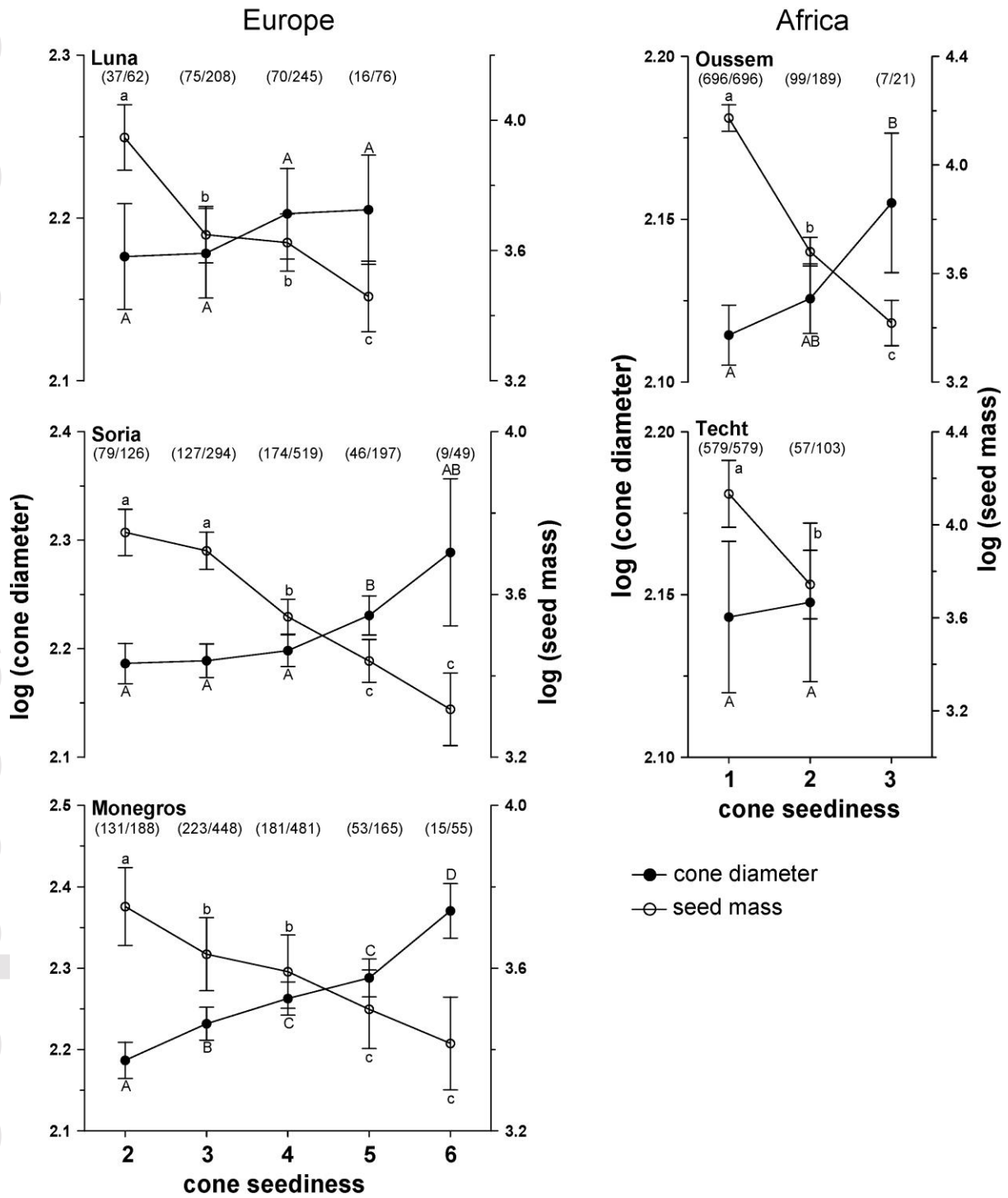




Figure 3

