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Allometric allocation in fruit and seed packaging conditions the conflict among selective pressures on seed size

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Abstract Selective pressures on seed size could vary among the different stages of plant life cycles, so no simple relation could explain *a priori* its evolution. Here, we determined the relationships between seed size and two fitness components—seed dispersal and survival from predation—in a bird-dispersed tree, *Crataegus monogyna*. We interpret these relationships in relation to the patterns of mass allocation to fruit and seed components. Selection patterns were assessed at two levels (1) selection pressures on the parent tree; comparing seed dispersal efficiency among individual plants and (2) selection pressures at the individual seed level; comparing seed size variation (i) before and after dispersal, and (ii) before and after postdispersal seed predation. Dispersal efficiency (percentage of seed crop dispersed) was positively correlated with fruit mass and fruit width. Differences in crop size did not offset this effect, and larger seeds were overrepresented in the seed rain relative to the seed pool before dispersal. However, the advantage of larger seeds during the dispersal stage was cancelled later by an opposite selection pressure exerted by seed predators. As a result, smaller seeds had a higher probability of surviving postdispersal seed predation, establishing an evolutionary conflict imposed by the need for dispersal and the danger of being predated. Birds and rodents preferentially selected highly profitable fruits and seeds in terms of the relative proportion of their components. Larger fruits had a higher pulp to seed proportion than smaller ones, and all seeds had the same proportion of coat relative to the embryo-plus-endosperm fraction. Hence, although predator pressures were stronger than disperser ones, larger seeds invested proportionally less in structural defense than in dispersal.

Keywords Allometry · *Crataegus monogyna* · Phenotypic selection · Seed dispersal · Seed predation · Seed size

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Introduction

Seed size plays a main role in early plant fitness, determining the fate of seeds during processes such as dispersal and establishment (Westoby et al. 1996; Leishman et al. 2000; Moles and Westoby 2004). The role of seed size might differ for each stage, since there are different selection pressures involved throughout life history. Although earlier hypotheses concerning the evolution of seed size stressed the importance of multiple selection pressures in offspring fitness estimation, few empirical studies have pursued this idea. First attempts have showed that postdispersal seed predation may oppose (Alcántara and Rey 2003) and even cancel (Gómez 2004) the usual recruitment advantages of larger seeds in later stages (germination, early seedling survival, reviewed in Leishman et al. 2000). There is also evidence for a trade-off between between dispersal and establishment (Ganeshaiah and Uma Shaanker 1991; Hedge et al. 1991; Parciak 2002; Debain et al. 2003).

In fleshy fruited plants, seed dispersal directly depends on the frugivores activity and preferences. Frugivores may exert a double selection effect on plants, showing preference for certain plants, and then choosing certain fruits (and indirectly seeds) within each plant (Sallabanks 1993; Jordano 1995). It is important to consider this double effect in relation to seed size selection for two reasons: (1) individual seed size and mean seed size per plant (maternal seed size) might have a partly different genetic basis (Mojonnier 1998), and (2) selective pressures on individual and mean seed size per plant might not be coupled (Jordano 1995). Both experimental and observational studies found evidence for frugivores as selective agents on fruit and seed size, but often variable selection pressures on that trait have been found, with studies reporting selection of large (Sallabanks 1993; Wheelwright 1993) or small items (Jordano 1995; Alcántara and Rey 2003). However, this may reflect a more complex trait selection if the trait selected is not just the fruit but some of its components, i.e. the pulp or the seeds. In this sense, changes in total fruit size may be due to differences in the relative resource allocation among these components, and allometric relationships could exist. In fact, fruit ‘pulpiness’ seems to be an important cue for intra- and interspecific fruit selection by birds (Edwards 2005 and references therein). On the other hand, this selective effect of dispersers might not translate into net phenotypic selection if it is offset by factors operating later in the life cycle, such as postdispersal seed predation (Schupp 1995; Herrera 2002). Again, resource allocation patterns among seed components, such as seed coat, endosperm and embryo, are likely to be important on seed survival prospects. In fact, the texture, hardness or thickness of the seed coat, that is, a relative increase in the size of a seed component, may be the most important traits under postdispersal predators selection, seeds with higher allocation to protective components achieving enhanced survival independently of seed size (Smith 1970; Blate et al. 1998; Mazer 1998). Thus, both seed selection by frugivorous dispersers and seed predators may respond to the allocation in fruit and seed packaging rather than to actual fruit or seed size. Despite this fact, the potential of allometric relationships among fruit and seed components to condition the role of seed dispersal and seed predation as selective pressures on seed size remains largely unexplored.

Here we analysed the relationship between seed size and two components of fitness: dispersal success and survival from predators in *Crataegus monogyna*. This species is dispersed by frugivorous birds that exert selective pressures by preferentially consumption of larger fruits in a hierarchical process (Sallabanks 1992, 1993) and dispersed seeds that are preyed by rodents (García et al. 2005a). Then, selective pressures by dispersers and predators may work in the same direction or in opposite ways depending on size-related selection patterns. In this sense, we expected that the intensity of these selection pressures varies depending on the isometric or allometric nature of the relationship among fruit components, with higher interindividual differences in fitness in the allometric case. In fact, it could be argued that the selection gradient could turn in the case of a negative allometric relationship. We examined here the fitness additive outcome of these contrasting selective forces in relation to the patterns of mass allocation to fruit and seed components. Specifically, we address the following questions: (1) What is the nature of the iso- or allometric relationships between allocation to pulp, seed coat and endosperm plus embryo? (2) What is the pattern of phenotypic variation of fruit and seed size among individual plants? (3) Which is the sign and the magnitude of the selective pressures exerted by dispersers on seed size? (4) Are the phenotypic pressures acting on mean seed size (selection among fruit crops) and individual seeds (selection among individual fruits within a crop) coupled? and (5) Does a selective conflict among disperser and predator selection pressures acting on seed size exist? If it exists, is the pattern of selection on seed size exerted by dispersers reverted by predator pressures?

Materials and methods

Study area and study species

The study was conducted at Teixeu site, Sierra de Peña Mayor (43°17′ N, 5°30′ W, 1,000 m a.s.l.; Asturias province, NW Spain) during 2003 and 2004. The study site covers an area of ca. 2 ha, including pastures and forest stands of hawthorn *Crataegus monogyna* and other fleshy-fruited trees such as holly *Ilex aquifolium* L. and yew *Taxus baccata* L.

Crataegus monogyna Jacq. (Rosaceae; *Crataegus* hereafter) is a deciduous small tree distributed throughout western Europe. Fruits are single-seeded drupes, red when ripe, whose seeds are typically bird-dispersed (Snow and Snow 1998). Fruit components were denoted hereafter as follows: ‘‘pulp’’ is the soft tissue that enclosed the seed, ‘‘coat’’ is the woody endocarp and ‘‘EEF’’ is the embryo-plus-endosperm fraction. Chemical analysis of the pulp reported by Herrera (1987) identified 2.5% protein, 4.3% ash, 2.3% lipids, 20.5% fiber and 72.4% nonstructural carbohydrate. Predispersal fruit losses by fruit-pulp eating insect larvae (38–57% in Manzur and Courtney 1984; and in Guitián and Fuentes 1992) and passerine birds (bullfinch *Pyrrhula pyrrhula*, chaffinch *Fringilla coelebs* and chiffchaff *Phylloscopus collybita* in Guitián and Fuentes 1992 and robin *Erithacus rubecula*, authors’ personal observation) have been recorded.

In the Cantabrian range (north-western Spain) hawthorn is widely spread. Fruits ripen in early September and are mainly dispersed by thrushes, *Turdus* spp., mostly by blackbird *T. merula*, redwing *T. iliacus* and fieldfare *T. pilaris* (authors’ unpublished data). After dispersal, seeds are consumed by the almost exclusive predators woodmouse *Apodemus sylvaticus* and yellow-necked mouse *A. flavicolis* (García et al. 2005a). These rodents gnaw the seed at a single side, making a hole from which they obtain EEF (embryo-plus-endosperm fraction) and leaving a single husk, or break the seeds into two halves. Husk remains are usually accumulated at the entrance of their burrows, located at the bottom of trees.

Fruit and seed traits

To measure resource allocation to fruit and seed components, and to characterize the relationships among them, we collected 30 fruits per tree in a total of 33 trees located in a partly isolated patch (~250 m apart), in late September 2003. At this time, most *Crataegus* fruits had ripened but had not been yet removed from the trees by frugivorous birds. Within each plant, we randomly selected the fruits from several clusters situated in branches distributed along the canopy perimeter, and took them immediately to the laboratory, where they were stored at 2°C and processed within the next 48 h. We determined the fresh mass of the fruits ($n = 20$ fruits/tree) with an accuracy of 0.1 mg, and measured the diameter (estimated as the maximum cross width of the fruit) to the nearest 0.1 mm using a digital caliper. Then, all samples were oven dried at 60°C for five days and fruit dry mass was determined. We manually removed the pulp of the fruit, and determined the dry mass of the clean seed, including the coat and the EEF (embryo-plus-endosperm fraction). After that, each seed was cracked, and the EEF was weighed. Pulp dry mass was calculated by subtracting the seed dry mass from the whole fruit dry mass, and the coat dry mass was calculated by subtracting the EEF dry mass from the seed dry mass.

Plant characteristics and seed dispersal

We measured canopy height, width and length for 20 marked trees (to the nearest 0.1 m) and estimated canopy volume assuming an ellipsoidal shape. These trees were selected with an homogeneous surrounding environment to minimize inter-individual differences due to the spatial context (Sallabanks 1992; Jordano 1995). The total number of fruits per plant (i.e. the crop size, T hereafter) was estimated during the last week of September (T_0), before the massive fruit consumption by migrant frugivores started, and again during the first week of January (T_1), when we considered that the dispersal season had finished. Because of the magnitude of T (200–20,000 fruits per plant), we assumed that any effect of sampling 30 fruits was negligible. In both samplings, we recorded the number of fruits on ten branches per tree located at different canopy orientations, and we counted the number of branches with fruits for the whole tree. T was calculated by multiplying the number of branches with fruits by the mean number of fruits per branch, or just counting all fruits per plant in trees with small crop size ($T_i < 700$).

"Seed dispersal efficiency" (SDE hereafter) was defined as the number of fruits removed by seed dispersers relative to the total crop size. In September 2003, we established three permanent $0.5 \times 0.5\ \mathrm{m}^2$ quadrats regulary spaced beneath each individual tree. During the dispersal season (October to January) we monthly collected all fruits and dispersed seeds found in these plots. Since thrushes were never seen eating fruits on the ground and mammal faeces containing hawthorn seeds were rare in the study site (authors' unpublished data), we considered that the removal of fallen fruits by frugivores from the plots was negligible. Samples were transported to the laboratory to be oven dried at 60°C for 24 h and assigned to one of the following categories: (1) *Crataegus* dispersed seeds, defecated or regurgitated by seed dispersers; (2) *Crataegus* preyed seeds, those showing signs of mice attack: empty coat with teeth marks. Rodents almost exclusively eat dispersed seeds, and seeds of fallen fruits or fruits on the tree are rarely consumed (authors' personal observation); and (3) nondispersed seeds, that included intact fruits fallen beneath the mother plant; beak-marked fruits, i.e. fruits selected by frugivorous birds but then rejected or dropped as a consequence of handling failures (Sallabanks 1992; Obeso 1998; García et al. 1999); fruits dropped by pulp consumers, recognized by the pecks of pulp fragments detached; damaged ripe fruits, with signs of insect damage (small holes) or fungi attack (discoloured spots); and unripe (green coloured) and aborted (abnormally small and shrivelled) fruits. We calculated the number of fallen fruits per plant (F_i, fruits dropped beneath the plant and thus, not consumed by seed dispersers) extrapolating the sum of the cumulative density of nondispersed seeds in the sampled area (3) to the total canopy projected area. The absolute number of fruits consumed (C_i) was estimated as follows: $C_i = T_0 - F_i + T_1$). Finally, seed dispersal efficiency (SDE) was expressed as C_i/T_0 (Jordano 1995).

Seed size distributions

Seed size distributions were assessed for the seed population before dispersal, from fruit samples described above, and for the seed population after dispersal, from pooled seed dispersal samples of the quadrats beneath the trees (intact dispersed seeds). We added to this sample *Crataegus* dispersed seeds collected beneath the canopies of ten additional hollies and several additional hawthorns and assumed that all dispersed seeds proceeded from the population under study. Because no significant correlation was found between mean fruit or seed size per plant and crop size (Table 1), no crop size adjustment was necessary to calculate seed size distribution before dispersal. The high frequency of sampling, the relatively low hawthorn predation rate at the dispersal season and the unusual secondary dispersal guarantee a negligible seed removal effect on sampling plots (as validated by the use of seed traps, García et al. 2005a). The size distribution of preyed seeds was inferred from seed remains collected in rodent trash heaps at the bottom of beech, holly, yew,

Table 1 Spearman rank correlation coefficients (above diagonal) of *Crataegus monogyna* fruit and seed traits, $n = 20$ trees

	Pulp dry (ms)	Seed dry (ms)	EEF dry (ms)	Coat dry (ms)	Fruit fresh (wd)	Fruit fresh (ms)	Canopy (vol)	Crop size	SDE	RF
Fruit dry (ms)	***0.737***	***0.848***	0.383	***0.726***	***0.829***	***0.789***	–0.080	–0.202	***0.602***	0.006
Pulp dry (ms)	–	0.414	0.059	0.289	***0.690***	***0.639***	–0.205	–0.326	0.420	–0.215
Seed dry (ms)	–	–	**0.504**	***0.949***	***0.686***	***0.692***	–0.020	0.081	**0.495**	0.259
EEF dry (ms)	–	–	–	**0.456**	0.102	0.116	–0.143	–0.183	0.159	–0.003
Coat dry (ms)	–	–	–	–	***0.632***	***0.642***	0.015	0.143	0.408	0.280
Fruit fresh (wd)	–	–	–	–	–	***0.973***	–0.084	–0.122	***0.583***	0.053
Fruit fresh (ms)	–	–	–	–	–	–	–0.053	–0.071	**0.504**	0.062
Canopy (vol)	–	–	–	–	–	–	–	0.394	–0.030	0.120
Crop size	–	–	–	–	–	–	–	–	–0.218	***0.794***
SDE	–	–	–	–	–	–	–	–	-	0.266

ms Mass (mg); *wd* width (mm); *vol* volume; *SDE* percent of seeds removed relative to fruit crop size; *RF* percent of seeds removed relative to the population mean. Boldface figures, $P < 0.05$; boldface italic figures, $P < 0.001$

hazel and hawthorn trees, and rocks ($n = 10$ heaps/microhabitat, García et al. 2005a). We used seed transversal diameter as a surrogate for seed size (*seed mass* = 3.12 + 20.07 *seed width*, $R^2 = 0.60$). Only husks larger than a half were measured to avoid double seed counts (see Study area and study species).

Postdispersal seed removal experiment

Seed size preferences by predatory rodents were also studied by using choice experiments in the field, in which we recorded seed removal of large and small seeds offered simultaneously to predators in seed trials. We considered seed size as a categorical variable and seeds were classified into two seed size categories: ‘‘large’’ seeds, i.e. those seeds heavier than the third-quartile seed mass, and ‘‘small’’ seeds, i.e. those lighter than the first-quartile seed mass (quartiles were calculated from the seed mass distribution of 1,000 fresh pooled seeds collected in the study site, for similar methods see Celis-Diez et al. 2004). The biological meaning of this quartile-based classification is justified based on a previous tentative experiment with captured rodents (unpublished data). The time that rodents spent in open by gnawing hawthorn seeds was substantially higher for large seeds (greater than 3rd quartile) in comparison with small seeds (always more that 2 min and mean time of 1.38 min, respectively, for large and small seeds, $n = 10$ seeds). The differences found in handling time between the two seed size categories gave us a biological meaning to the quartile-based classification employed in the postdispersal seed removal experiment. According to this classification we detached 500 seeds for each size class from fresh seeds collected from the seed rain in the study site. We assumed that most of *Crataegus* seeds were viable (García et al. 2005a). Each trial consisted of ten seeds (five of each size class) alternatively glued with a low odour, rain-proof thermoplastic glue, in a 12×6 cm^2 plastic mesh rectangle (1.5 mm pore). In spring 2004, we established 3 trials at each of 33 marked trees ($n = 99$ trials) by nailing them to the ground (see Alcántara et al. 2000; García et al. 2005a, b for similar methods). After 30 days, we surveyed the experiment and recorded the number of intact seeds in each trial. We repeated the survey again after 60 days and removed the trials. We considered a seed as preyed by a rodent if it was missing from the plastic mesh or if it was still on the mesh but gnawed and empty (García et al. 2005a, b). Because seeds were solidly glued on the plastic rectangles, seed disappearance due to abiotic factors (wind, rain) was considered negligible. However, the experiment suffered some losses due to animal trampling (three trials located in different trees). These seed depots were not considered in the statistical analysis. Thus, only 90 trials were included in the analysis (30 of the initial 33 trees).

Statistical analysis

Allometric relationships

The allometric equation is generally stated as $\log(\boldsymbol{y}) = \log(a) + b\,[\log(\boldsymbol{x})]$, where $\boldsymbol{x}$ and $\boldsymbol{y}$ are the two variables being compared; a is the value of $\boldsymbol{y}$ where it intercepts the vertical axis; and b is the slope (the allometric exponent). This relation is defined as isometric when b takes a value near unity and as an allometric relationship otherwise (positive or negative allometry when $b > 1$ and $b < 1$, respectively). Major axis regression, model II (MAR) and least squares regression, model I (LSR) were used to estimate the linear relationship between pulp and seed mass, and coat and EEF (embryo-plus-endosperm fraction) mass (log-transformed variables). MAR method was used to calculate the regression slope because it considers that both variables ***x*** and ***y*** are measured with error and does not distinguish between predictor and criterion variables (Herrera 1992; Legendre and Legendre 1998). Thus, the regression slope b_{MAR} was calculated as the LSR regression slope divided by r, i.e. the correlation coefficient. We checked if the relationship was allometric by testing significance of b_{MAR} with a t test (H_0: $b_{\mathrm{MAR}} = 1$). We used for this the LSR standard error, since the estimate of this parameter is the same in both methods (Legendre and Legendre 1998).

Relationship among plant fitness and fruit traits

A correlation matrix (Spearman rank correlation coefficient with untransformed data) was built to determine the relationships among plant traits and fitness components. We considered the following variables: (1) *Fruit traits*: fruit dry mass, pulp dry mass, seed dry mass, EEF (embryo-plus-endosperm fraction) dry mass, coat dry mass, fruit fresh width, fruit fresh mass; (2) *Plant traits*: canopy volume, crop size and (3) *Plant fitness parameters*: seed dispersal efficiency (SDE) and relative fitness (RF), defined as the number of dispersed seeds per plant relative to the average number of dispersed seeds in the population ($\mathrm{RF} = C_i/[\Sigma\ C_i/n]$, where n is the number of trees in the population).

Phenotypic selection

Janzen and Stern (1998) proposed the adoption of logistic regression as a method for estimating selection coefficients when fitness is dichotomous (i.e. 0 vs. 1). This framework enables the estimation of the relative importance of different selective pressures. According to this methodology, W denotes the selection outcome (fitness) for an individual, with $W = 0$ indicating death and $W = 1$ indicating survival. In the same way, the fitness component at the dispersal stage can be considered as a dichotomous variable too, with $W = 0$ indicating absence of dispersal of an individual seed and $W = 1$ actual dispersal. This consideration is based on the common assumption that dispersed seeds are more likely to have higher fitness than undispersed seeds i.e. dispersed seeds may escape from the parental environment; its germination should be enhanced after passage throughout the animal gut; and also because they are more likely to land in unoccupied sites. However, the actual fitness of undispersed seeds is not null because some of them could survive to seedling and later. The logistic regression model relates the individual survival probability $W(z)$ (or expected fitness) depending on the value of the phenotypic trait z, and it is calculated as follows:

$$W(z) = \frac{\exp(\alpha_0 + \alpha_1 z)}{1 + \exp(\alpha_0 + \alpha_1 z)} \qquad (1)$$

where α_1 is the logistic regression slope and α_0 is the intercept.

When there is a sequence of selective episodes, total fitness $W_{\mathrm{t}}(z)$ can be calculated by multiplying the partial fitness components, $W_{\mathrm{t}}(z) = \Pi W_{\mathrm{k}}(z)$ (Campbell 1991).

In this study we used the logistic regression to check for phenotypic selection acting on seed size through two sequential stages; (1) seed dispersal, comparing dispersed seeds (scored as 1, $n = 260$) and seeds before dispersal (control, scored as 0, $n = 330$), and (2) postdispersal seed predation, comparing preyed seeds (scored as 0, $n = 366$) and nonpreyed seeds (scored as 1, $n = 351$). Thus, $W_d(z)$ indicates the probability of being dispersed, and $W_p(z)$ indicates the chance of surviving to postdispersal seed predation, where z is seed mass. With this consideration we are being quite restrictive in our analysis since some of the control seeds in (1)—seeds before dispersal—could be dispersed and may cause parameter underestimation. In any case, the same reasoning was applied to the predation stage since some of the control seeds (2)—dispersed seeds—could be preyed. Unfortunately, we did not know if the error concerning the two processes is the same, and we lack the appropriate data (actual nondispersed and nonpreyed seeds recollected at the end of each process) to contrast this hypothesis. For each seed, we calculated the survival probability at the dispersal and postdispersal stage using equation (1). Then, fitness functions were estimated fitting these survival probabilities to seed mass. As in Gómez (2004), total fitness $W_t(z)$ was estimated projecting $W_d(z)$ and $W_p(z)$ in the initial pool of seeds before dispersal, and then applying equation $W_t(z) = W_d(z) W_p(z)$.

We transformed the logistic regression slope using the technique proposed by Janzen and Stern (1998) to calculate the average selection gradient $\beta_{avggrad}$ (see a worked example at http://www.public.iastate.edu/~fjanzen/homepage.html). With this method, the logistic coefficient was standardized by multiplying it by the standard deviation σ_z, and then rescaled to the relative fitness multiplying it by the constant ($Ag/\overline{W}$), where Ag is the probability that the event happens at random (estimated by the frequency of observed successes) and $\overline{W}$ is the average fitness.

Postdispersal seed removal experiment

We tested the effects of seed size (fixed factor) together with those of tree (random factor) and trial (random factor, nested within tree) on the proportion of preyed seeds in seed trials. We used a generalized linear mixed model (GLMM) because of the binomial distributions in the dependent variable and a logit link function (Proc GLIMMIX, SAS Institute 2004). The variance-mean scaling was altered to avoid over-dispersion, and quasi-likelihood methods were used in parameter estimation (McCullagh 1983). Significance of the fixed factor was evaluated with a F-test in the full model and a Satterthwaite approximation of the degrees of freedom. Random terms significance was assessed using likelihood ratio tests (LRT) (SAS Institute 2004).

Results

Fruit and seed traits

Dry fruit and dry seed mass averaged, respectively, 134.7 mg (SE = 0.9, $n = 978$ fruits) and 68.8 mg (SE = 0.5, $n = 978$ seeds). Average fruit water content was 17.25% (SE = 0.7, $n = 33$ trees). Fresh fruit mass and fruit width averaged, respectively, 308.3 mg (SE = 0.003, $n = 660$ fruits) and 8.1 mm (SE = 0.03, $n = 660$ fruits). Most of the fruits were single seeded (1.11% were two-seeded fruits). Dry seed mass distribution fitted a normal curve ($P > 0.05$, KS test). Seed and fruit dry mass differed significantly among plants ($F_{(32,945)} = 33.93$, $P < 0.001$ and $F_{(32,945)} = 31.34$, $P < 0.001$ respectively, model II ANOVA). The percentage of variance accounted for by differences among plants was 53.47 and 51.49% for seed and fruit dry mass, respectively.

Fruit allometry

Strong positive correlations were observed between pulp mass and seed mass, and coat mass and embryo plus endosperm fraction (EEF) mass (Table 1). MAR slope between pulp mass and seed mass showed a positive allometric relationship because it was significantly higher than 1 ($b_{\mathrm{MAR}} = 1.107 \pm 0.026$, $n = 978$, $P < 0.001$). Thus, pulp mass increased proportionally faster than seed mass with increasing fruit size and as a result, larger fruits had a higher proportion of pulp relative to seed than smaller ones (Fig. 1). By contrast, MAR slope between coat and EEF was positive but not significantly different of 1 ($b_{\mathrm{MAR}} = 0.995 \pm 0.048$, $n = 314$, $P = 0.541$). In this case, the relationship was isometric and consequently, all seeds produced equivalent ratios of coat to EEF mass (Fig. 1).

Phenotypic selection on maternal seed size

Many of the fruit and seed traits estimated at the tree level were correlated with one another (Table 1). Seed dispersal efficiency (SDE) was positively correlated with both fresh and dry fruit mass and fruit width mean per plant (Table 1); plants with larger fruits showed a higher percentage of seeds removed. By contrast, relative fitness (RF) was strongly and positively correlated with crop size (Table 1). Thus, plants with larger crop size dispersed a larger number of seeds in spite of their SDE. On the other hand, the correlation between crop size and mean fruit size per plant was not significant (Table 1), indicating no trade-off between these traits among individual trees.

Phenotypic selection on individual seed size

Seed size differed significantly between dispersed versus predispersed seeds and preyed versus prepreyed seeds ($F_{(1,589)} = 15.87$, $P < 0.001$ and $F_{(1,716)} = 48.38$, $P < 0.001$, respectively, model I ANOVA, Fig. 2). There was a significant positive selection on seed size for the fitness component related to the dispersal probability ($\beta_{\mathrm{avggrad}} = 0.154$, $P < 0.001$, $R^2 = 0.025$). The function relating $W_{\mathrm{d}}(z)$ and seed mass was a positive exponential (Fig. 3); larger seeds had a higher probability of being dispersed than smaller ones. By contrast, phenotypic selection acting on seed size was significantly negative for the fitness component related to the probability of surviving postdispersal seed predation ($\beta_{\mathrm{avggrad}} = -0.249$, $P < 0.001$, $R^2 = 0.085$). In this case, larger seeds had a much higher probability of being predated than smaller seeds as indicated the sharp negative exponential relationship between $W_{\mathrm{p}}(z)$ and seed mass (Fig. 3). The extent of the positive selection gradient through the dispersal stage was lower than the extent of the negative selection gradient on seed size during seed predation. As a consequence, the total fitness function reflects that smaller

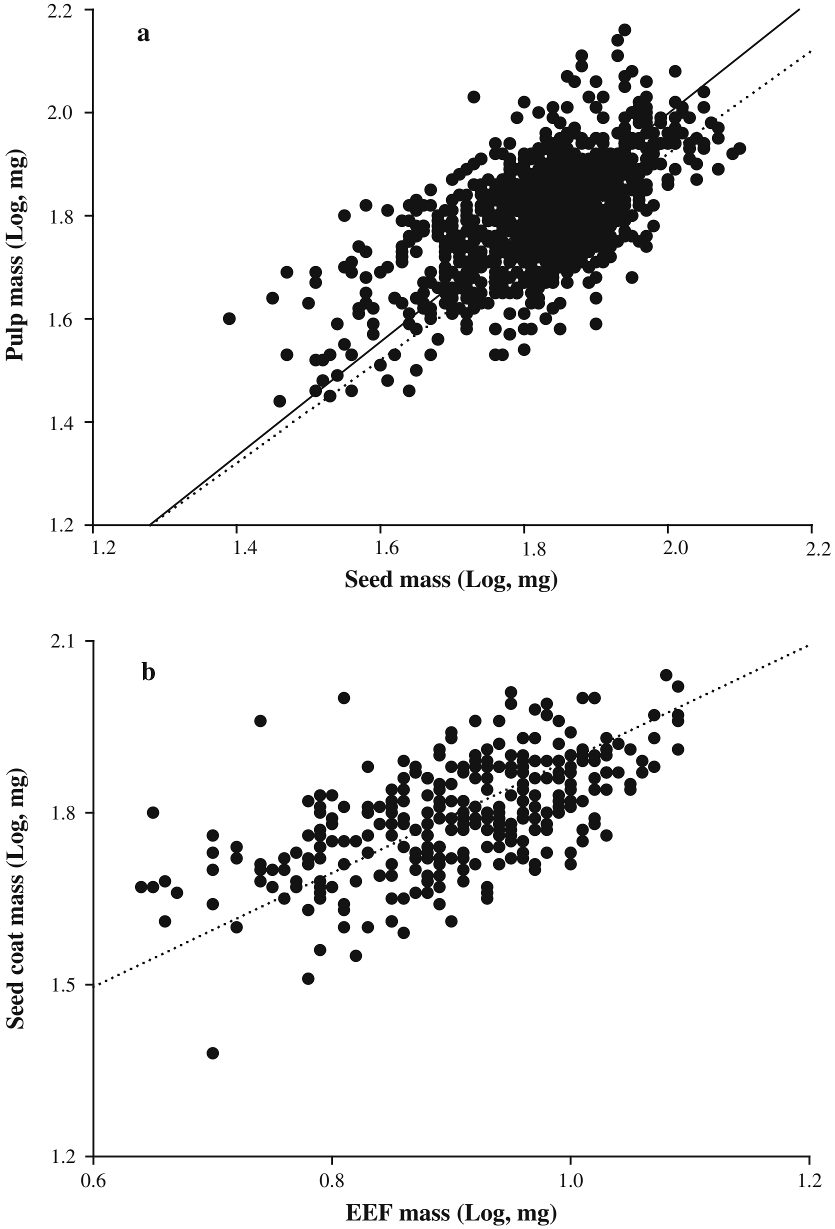

Fig. 1 Relationship between (**a**) pulp and seed dry mass, and (**b**) coat and EEF (embryo-plus-endosperm fraction) dry mass (log-transformed variables, $n = 978$ fruits and $n = 314$ seeds) together with the lines of the MAR slope (*thick line*) and slope 1 (*discontinuous lines*). Major axis regression (MAR) slope significance (*t* test with $n–2$ *df*, H_0: $b_{\mathrm{MAR}} = 1$): (**a**) $y = 0.62 + 1.12\ x$, $P < 0.001$; (**b**) $y = 1.26 + 0.99\ x$, $P > 0.05$

seeds had a higher probability of surviving than larger ones immediately after postdispersal seed predation operated, according to the negative exponential relationship between $W_t(z)$ and seed size (Fig. 3).

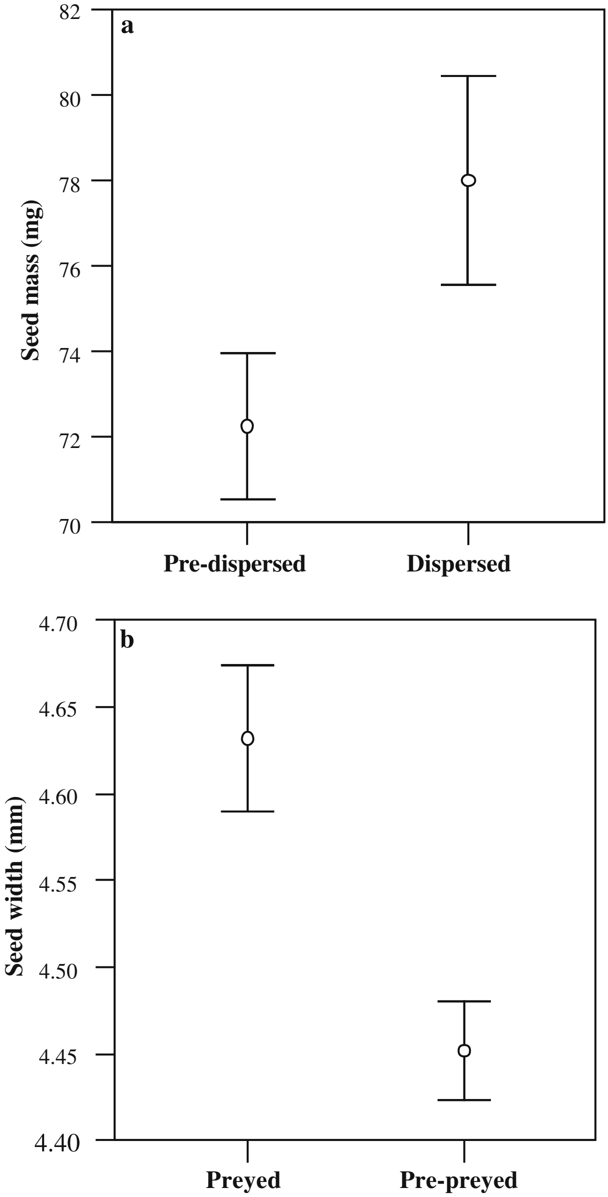

Fig. 2 Seed size differences between (**a**) dispersed seeds versus predispersed seeds (seeds from fruits on the tree), $n = 260$ and $n = 330$, respectively and (**b**) preyed (seeds from heaps) versus prepreyed seeds (sample of dispersed seeds), $n = 366$ and $n = 351$, respectively (mean ± 2 SE). ANOVA model I: (**a**) $F_{(1,589)} = 15.87$, $P < 0.001$; and (**b**) $F_{(1,716)} = 48.38$, $P < 0.001$

Because our approach may have resulted in the underestimation of the selection gradient in the dispersal stage, we performed a simple simulation to examine this. We first calculated the difference between the probability density function (pdf) of

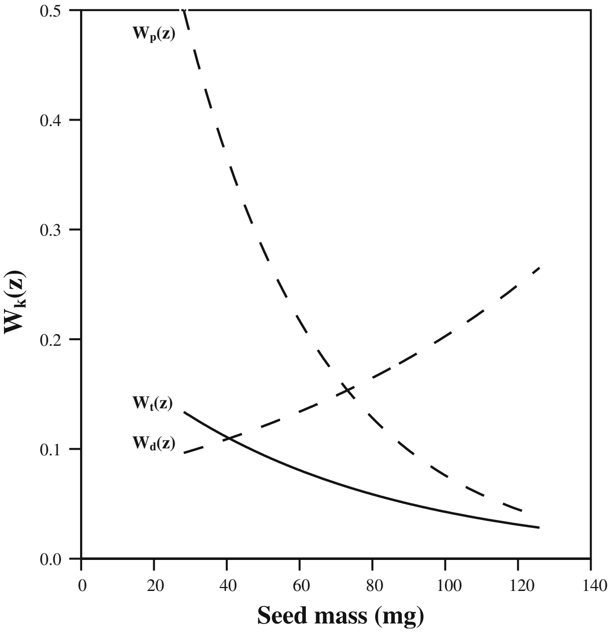

Fig. 3 Predicted fitness functions relating the individual survival probability $W(z)$ depending on seed mass, z, at the different fitness components studied: probability of dispersal, $W_{\mathrm{d}}(z)$, and probability of escape from predation, $W_{\mathrm{p}}(z)$ (*discontinuous lines*); and total fitness from dispersal to postdispersal seed predation ($W_{\mathrm{t}}(z)$, *thick line*) estimated using equation $W_{\mathrm{t}}(z) = W_{\mathrm{d}}(z)\ W_{\mathrm{p}}(z)$ (see Materials and methods). Fitness functions at each stage: $W_{\mathrm{d}}(z) = 0.2e^{10.36(\text{seed mass})}$, $\mathrm{W}_{\mathrm{p}}(z) = 2.91e^{-26.26(\text{seed mass})}$, and $W_{\mathrm{t}}(z) = 0.58e^{-15.9(\text{seed mass})}$, $R^2 > 0.95$ in all cases

the predispersed and dispersed seed sizes. Then, we obtained the pdf of nondispersed seeds by subtracting this difference from the predispersed seed size distribution. Mean and variance of this distribution were used to simulate 100 nondispersed seeds. The average selection gradient obtained was $\beta_{\mathrm{avggrad}} = 0.169$, that is, as expected, larger than that obtained using predispersed seeds as control ($\beta_{\mathrm{avggrad}} = 0.154$), but lower than the absolute value of the average selection gradient for the predation stage, $\beta_{\mathrm{avggrad}} = -0.249$. This simulated experiment supports the main conclusion of our study i.e. that the positive selection on seed size exerted by seed dispersers is reversed by an opposite and stronger selection exerted by seed predators (even with an underestimated selection gradient for the predation stage).

Postdispersal seed removal experiment

The respective proportions of preyed seeds in seed trials showed that large seeds were significantly more predated than small ones ($F_{(1,89)} = 4.16$, $P = 0.044$), despite this effect was also affected by tree identity (change in deviance = 149.96, χ^2; $df = 1$, $P < 0.0001$) and trial within tree (change in deviance = 44.91, χ^2; $df = 1$, $P < 0.0001$). Thus, the chance experiment results matched that of the selection gradient (above) based on the comparison of dispersed and preyed seeds found in seed quadrats and rodent trash heaps.

Discussion

In the population of *Crataegus monogyna* examined in this study frugivorous birds exerted a consistent pattern of selection on maternal and individual seed phenotypes, larger seeds being favoured in terms of realized dispersal. Nevertheless, the advantage of larger seeds during the dispersal stage was later reversed by opposite selection on this trait exerted by seed predators. Therefore, a trait (large seed size) which is advantageous in an early life stage turns to be detrimental in a subsequent one, establishing a conflict in selection pressures. This opposite effect was explainable in terms of the mass allocation patterns among fruit and seed components, since birds and rodents preferentially selected highly profitable items according to the relative proportion of their components.

Seed size and seed dispersal efficiency

Frugivorous birds tended to forage preferentially on larger *Crataegus* fruits (as in Sallabanks 1993). These fruits had a higher pulp to seed mass proportion than smaller ones. Hence, with the selection of large fruits, birds obtained a higher relative amount of pulp per unit of fruit mass processed. This foraging preference was translated in a differential dispersal success among plants; plants with larger fruits dispersed a higher proportion of seeds, therefore maximizing their seed dispersal efficiency (SDE). Although plants producing more fruits dispersed a larger number of seeds and thus contributed more to the population pool of seeds, differences in crop size did not cancel this effect. As a consequence, immediately after the dispersal event and before later factors of the cycle operated, seed mass distribution reflected the pattern of the frugivores selection; larger seeds were over-represented in the seed rain in comparison to the seed mass distribution initially available in the trees.

Seed size and the risk of predation

Our experimental work showed that rodents preferred larger *Crataegus* seeds. This selectivity might be due to the higher profitability of larger seeds because of their higher energetic reward and nutrient content (more embryo-plus-endosperm fraction mass, Kerley and Erasmus 1991). Accordingly, both intra- and interspecific studies have found that rodents preferred larger seeds (Van der Wall 1994; Hulme 1998; but Alcántara and Rey 2003; Kollmann et al. 1998). Additional evidence of this pattern of selection was found when we compared the size distribution of dispersed seeds and seed remains collected in rodent trash heaps. Large husk remains were overrepresented in rodent trash heaps in comparison to the seed size distribution available in the seed rain. This pattern was reflected in the significant negative selection gradient obtained for seed size at this stage. However, as suggested by our experimental results, the spatial context of the seeds (i.e. the microhabitat where they were deposited) also seemed to play an important role in their survival prospects (as in Rey and Alcántara 2000; García et al. 2005a).

Is it good to be a large seed?

Seed size increased the chance of dispersal but decreased survival probability during postdispersal seed predation (Fig. 3). The intense pressures on seed size exerted by seed predators cancelled the favourable effect previously exerted by frugivorous birds. As a result, size distribution of nonpreyed seeds was skewed towards smaller seeds. Consequently, total fitness decreased with seed size (Fig. 3). Unfortunately, we did not know the direction and the magnitude of the selective pressures on seed size in later stages as germination, seedling emergence and survival (most likely to be positive, Leishman et al. 2000), or other possible previous selective forces such as predispersal pulp or seed predation and pollination.

The existence of a trade-off between sequential plant developmental stages has long been recognized. For both wind-dispersed (Ganeshaiah and Uma Shaanker 1991; Debain et al. 2003) and animal-dispersed (Hedge et al. 1991; Alcántara and Rey 2003) plants, an increase in seed size results in reduced seed dispersal efficiency (contrary to what we found here), but in an enhanced success in seedling establishment. Also, another trade-off may occur because predatory pressures on seed size can oppose and counter or not the usual advantage of larger seed size during seedling establishment (Gómez 2004; Alcántara and Rey 2003 respectively). In this study we found a trade-off between two biotic pressures operating on seed size, firstly exerted by mutualistic avian frugivores (seed dispersers), and later by antagonistic predatory rodents. In southern Spain, Alcántara and Rey (2003) also detected these opposite forces acting on *Olea europaea* seeds, but operating in the opposite direction than we found here; larger seeds had lower dispersal efficiency but higher survival probability during postdispersal stages. The strong gape-limitation generated by the large *Olea* fruits and seeds, and the characteristic assemblage of avian seed dispersers in the Mediterranean shrubland (mainly dominated by small species, Herrera 1984; Rey et al. 1997), might underpin the differences with the *Crataegus* population. In our system, fruit size was small relative to the gape size of the main frugivores, and birds fed preferentially on larger fruits that, in fact were the most profitable as judged from the positive allometric relationship between pulp and seed mass. We found that pulp reward increase proportionally faster than seed mass with fruit size, but seed defensive tissue increase isometrically with seed mass (as found Moles et al. 2003 for interspecific comparisons). Hence, although predator pressures were stronger than disperser ones, larger seeds (those better provisioned and hence, probably advantageous in germination and early seedling survival) are shaped to favour dispersal but not to prevent rodent predation (but see Obeso 1998). In this sense, the allometric relation between pulp mass and seed mass might actually have been shaped by both, the frugivores and seed predators. Due to the strong selection against large seeds by rodent predation, but selection for large fruits by frugivores, there may be selection for large fruits with proportionally small seeds. On the other hand, this pattern of rodent seed selection within species, exclusively driven by seed size, contrasts with the pattern of selection among seed species exerted by rodents in the same area, in which the predation ranking runs inverse to the gradient of coat to EEF (embryo-plus-endosperm fraction) proportion: *C. monogyna* had much lower predation than species with a lower proportion of coat to EEF mass such as *I. aquifolium* and *T. baccata* (García et al. 2005a).

It is important to note the limited spatial and temporal extent of this study. Natural selection fluctuates among years and sites, even in a different way in every one of a sequence of several selective events, as in the life cycle of a plant. Consequently, the net selection effect on any trait can show a complex pattern of variation (Endler 1986). The lack of consistent selective forces on seed size might explain why there is still so much variance in this trait, and there might even be selection for seed size variation within trees. Most of the variance in hawthorn seed and fruit size was due to variation among individuals rather than within crops (as in Obeso and Herrera 1994, where differences among plants explained 71.5% of variance in hawthorn seed mass). Consequently, differential dispersal and/or predation by seed size might cause differential reproductive success (and therefore, natural selection) among individual plants. However, evolutionary significance of natural selection on any trait necessarily needs inheritable variation in that trait. This is a controversial issue, since there are studies showing opposite results in the heritability of seed size, with a mismatch among some empirical evidences and artificial selection (Biere 1991; Lynch and Walsh 1998; Mojonnier 1998 and references therein).

Comparisons within tropical rainforest species in the patterns of mass allocation between pulp and seed demonstrated no single relationship (Edwards 2005), suggesting species-specific patterns of mass allocation. Species-specific variation in fruit and seed traits may be due to phylogenetic effects (Herrera 1992; Mazer and Wheelwright 1993) and/or selection for those traits that enhance the likelihood of offspring survival. Nevertheless, few studies had investigated the relationship between selective pressures and within species allometry. Our results suggest that the ratio between fruit and seed components conditions the outcome of the potential conflict between selective pressures exerted sequentially by frugivorous seed dispersers and seed predators by, first, affecting the foraging decisions of both types of interacting animals, and second, by constraining the variability of seed size as a function of seed packaging. Additional studies examining intra- and interspecific scaling relationships and covering several plant fitness components are still needed to understand how natural selection actually operates on fruit and seed characteristics.

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