

Mario Díaz · Anders P. Møller · Fernando J. Pulido

## Fruit abortion, developmental selection and developmental stability in *Quercus ilex*

Received: 29 March 2002 / Accepted: 28 January 2003 / Published online: 18 March 2003  
© Springer-Verlag 2003

**Abstract** Fruit abortion has been hypothesized to be a parental means of selective removal of propagules with low viability. In particular, aborted zygotes have been suggested to have developmentally deviant phenotypes, and surviving offspring may therefore give rise to adults with a developmentally stable phenotype. We tested predictions from this hypothesis using acorns of holm oaks *Quercus ilex* as a model system. Fecundity of oak trees was negatively related to mean fluctuating asymmetry of leaves, and abortion rates were positively related to leaf fluctuating asymmetry in at least one population. Aborted acorns were asymmetric in 83–99% of cases in three samples, while mature acorns were only asymmetric in 57–78% of cases. Acorn asymmetry was unrelated to germination probability and germination date, and had no significant effect on number of leaves, leaf mass, stem mass, seedling height or leaf area of seedlings. However, acorn asymmetry affected the trade-off between number and size of leaves in seedlings. Seedlings from asymmetric acorns showed a positive relationship between acorn size and number of leaves, but no relationships between acorn size and leaf area, while symmetric acorns showed the opposite. A positive relationship between acorn size and number of leaves in spring was found for naturally

emerged seedlings that died during their first summer, whereas the number of leaves produced by surviving seedlings did not depend on acorn size. These findings are consistent with the hypothesis that stressed trees selectively abort propagules of low viability, and that developmental selection acts on a measure of developmental instability of fruits.

**Keywords** Acorn · Fluctuating asymmetry · Leaf number · Leaf size · Maternal effect

### Introduction

The number of gametes and propagules produced by most organisms is exceedingly large compared to the number of successful survivors. Such excessive production allows parents to adjust the number of survivors to that which is optimal under current environmental conditions, and parental “optimism” thus seems to be an adaptation to fluctuating environments (Mock and Forbes 1995). Not only the number, but also the quality of gametes and zygotes can be adjusted during these selective events either as a means of bet-hedging or selective abortion (Kozłowski and Stearns 1989). The selection-arena hypothesis suggests that reproduction can be considered an integrated system of phenotypic screening favouring genotypes that are the most likely to contribute to parental fitness. Numerous studies have shown that aborted gametes and zygotes do not represent a random sample (review in Møller 1997). Irregular phenotypes of gametes and propagules often characterize those that are aborted, while successful propagules have more regular phenotypes. Møller (1997) proposed that developmental selection, i.e. parental favouritism of offspring of high genetic or phenotypic quality, often acts against gametes and zygotes with asymmetric or otherwise irregular phenotypes, because such phenotypes would characterize gametes and zygotes with low viability. Extensive evidence for both plant and animal species (review in Møller 1997) suggests that developmental selection

M. Díaz (✉)

Departamento de Ciencias Ambientales,  
Facultad de Ciencias del Medio Ambiente,  
Universidad de Castilla-La Mancha,  
45071 Toledo, Spain  
e-mail: mariod@amb-to.uclm.es  
Tel.: +34-925-268800  
Fax: +34-925-268840

A. P. Møller

Laboratoire d'Ecologie Evolutive Parasitaire, CNRS UMR 7103,  
Université Pierre et Marie Curie,  
Bâtiment A, 7ème étage, 7 quai St. Bernard, Case 237,  
75252 Cedex 05 Paris, France

F. J. Pulido

Departamento de Biología y Producción de los Vegetales,  
E.U.I.T. Forestal, Centro Universitario,  
Universidad de Extremadura,  
10600 Plasencia Cáceres, Spain

against developmentally unstable offspring appears to be a widespread phenomenon of general importance.

Studies of developmental selection at both the pre- and post-zygotic stages have shown that the intensity of selection is greater when environmental stress is more intense (e.g. Marshall and Ellstrand 1988; Searcy and McNair 1990, 1993; Polak et al. 2002). Since developmental instability tends to increase under adverse environmental conditions (reviews in Møller and Swaddle 1997; Leung et al. 2003), we can hypothesize that a larger fraction of propagules is asymmetric under adverse conditions, and that this is the reason for the higher levels of spontaneous abortion in such situations. Genetic (i.e. mutations, inbreeding) or entirely environmental (i.e. resource allocation to reproduction by parents, competition with sibs) causes of developmental instability may be invoked, but in either case parents may benefit from exercising developmental selection against propagules with abnormal phenotypes if such propagules will also have irregular phenotypes as adults, and therefore have low reproductive value (review in Møller 1997). Selective abortion at earlier stages of offspring development may also save energy and materials that would be invested in offspring of higher value. We can thus expect larger proportions of irregular phenotypes in aborted offspring as compared to offspring that reach maturity, as well as abnormal growth patterns and reduced fitness of the irregular offspring that would survive, if there is developmental selection against developmentally unstable offspring.

In addition to direct developmental selection, adults may through their choice of symmetric partners with regular phenotypes indirectly select against the production of abnormal gametes and offspring (Møller 1997). Numerous studies of sexual selection in relation to asymmetry have shown an average negative Pearson correlation coefficient between success and asymmetry adjusted for sample size of  $-0.130$  to  $-0.170$  (a small effect size, sensu Cohen 1988; reviews in Møller and Thornhill 1998; Møller and Cuervo 2003). Some of these studies have found that reduced success was mediated by higher abortion rates of the progeny derived from crosses involving asymmetric parents (Petrie et al. 1991; Petrie and Halliday 1994; Hasegawa 1995; Møller 1996, 1997). These findings suggest that there are cross-generational effects of developmental instability on reproductive performance, but also on the phenotype of offspring, so that we can expect a direct relationship between the developmental stability of parents and their offspring.

Maternal (and paternal) effects arise as a consequence of the maternal (and paternal) phenotype affecting the phenotype and the performance of offspring (Mousseau and Fox 1998). If asymmetric parents tend to produce offspring that also have an irregular phenotype, these effects can be interpreted as either genetic or maternal effects. Studies of the heritability of fluctuating asymmetry or developmental instability have shown small, but statistically significant heritabilities of approximately 5–10% (reviews in Møller and Thornhill 1997; Van Dongen

2000; Fuller and Houle 2003; see, however, Fuller and Houle 2002 for a case of lower heritability). Although these heritabilities are small, they may still have significant evolutionary consequences given the potentially large effects of irregular phenotypes on fitness. If the cross-generational effects of developmental instability are maternal effects, these could have important evolutionary consequences by changing the speed and the direction of evolutionary change (Wade 1998). Currently, there are only few studies quantifying the importance of maternal effects for developmental instability of offspring (e.g. Polak 1997; Cadée 2000; Polak and Starmer 2001). Polak and Starmer (2001) found that fluctuating asymmetry in the number of sternopleural bristles of *Drosophila falleni* has a low, non-significant heritability, whereas heritability of the positional fluctuating asymmetry of these bristles was significant and due to additive and dominance effects, with no additional significant influences of maternal or common environment effects. It is unknown whether this result can be considered as general for other traits or organisms.

The main objectives of the present study were to test the predictions of the developmental selection hypothesis, using our studies on abortion, developmental selection and developmental stability in holm oak, *Quercus ilex*, as a model system. Specifically, we tested:

1. Whether maternal plants that have experienced stress from herbivores during the previous year, and hence have more asymmetric leaves in the current year (Díaz et al. 2003), suffer a reduction in fecundity.
2. Whether the abortion rate of fruits is positively related to leaf asymmetry of the maternal plant in the current year.
3. Whether aborted acorns have a higher frequency of irregular phenotypes than mature fruits.
4. Whether rate of germination of acorns with irregular phenotypes is reduced and germination is delayed compared to acorns with regular phenotypes.
5. Whether the size and morphology of seedlings depends on the phenotype of acorns from which they originated.
6. Whether seedling viability is related to seedling morphology.

## Material and methods

### Study area

The study populations of holm oak are thoroughly analysed in Pulido et al. (2001). They were located in northern Extremadura (Cáceres province, central-western Spain;  $39^{\circ}45'N$ ,  $6^{\circ}00'W$ ; 350 m a.s.l. on average) in a flat area of Mediterranean climate (extreme mean temperatures of  $8^{\circ}C$  and  $26^{\circ}C$  and 623 mm of average annual rainfall). Holm oak woodlands are subjected to two distinct types of management. Forests are well-preserved tracts of the original Mediterranean vegetation that are composed of a tree layer with ca. 70 trees  $ha^{-1}$  and a dense understorey of tall shrubs. Dehesas have resulted from tree clearing and pruning and ground ploughing of the

former forests to enhance grass and acorn production for livestock. Oak trees are regularly distributed over an understorey composed of grasslands with sparse shrubs. We established a 150 m×150 m (2.25 ha) plot within the forest area and a 200 m×200 m (4.00 ha) plot within the dehesa area. The distance between the two plots was 9 km.

#### Tree fecundity and abortion rates

We randomly selected and marked 30 mature trees in each study plot. Fifteen trees in each plot were randomly assigned to a treatment with an insecticide during early spring 1997 and 15 were left as controls (see Díaz et al. 2003 for details). Tree fecundity was estimated by counting the number of female flowers (May 1997 and May 1998) and the number of full-grown acorns (September 1997 and September 1998) per shoot in four groups of 25 shoots per tree. Shoots were not marked, and were located in each sampling session in random positions (Skalski 1987) around the tree canopy at 3.5–4.5 m height.

Abortion rates of control trees in 1997 were measured by means of seed traps, which consisted of 0.12-m<sup>2</sup> plastic buckets, 25 cm deep, attached with wire to the lower canopy of trees in random positions. Eight traps were installed in each of the 15 dehesa trees and four traps in each of 15 forest trees, in such a way that ≈3% of the horizontal canopy projection of each tree was sampled. Traps were inspected each fortnight from the second half of May 1997 to the first half of January 1998. Propagules found in traps were classified as: (1) non-fertilized flowers; (2) aborted fruits, with incomplete development but no sign of damage; (3) drippy fruits, showing an abundant sap exudation in the junction point with the cap; (4) fruits infested by weevil or moth larvae; and (5) viable fruits. Abortion rates (i.e. fruit losses due to intrinsic causes) were estimated as the proportion of aborted fruits, drippy fruits and non-fertilized flowers relative to the total number of propagules found in all traps of each tree (see Pulido and Díaz 2002a, 2002b for details).

#### Developmental stability of leaves and fruits

We developed a composite index of fluctuating leaf asymmetry based on measurements of the width and number of spines on both the right and the left sides of each leaf, corrected by trait size and by the baseline values represented by leaves grown the previous year in the shoot from which current-year modules developed. Measurements were taken by the same person (M. D.) on 20 leaves per tree collected in mid-June 1997, just after the insecticide treatment, and in mid-June 1998. Mean tree values of leaf fluctuating asymmetry were reduced by the insecticide treatment and were related to herbivory rates with a delay of 1 year, and they were positively related to the current-year production of pistillate flowers. Mean leaf asymmetry can therefore be considered to be a reliable estimator of the intensity of the delayed physiological stress induced by insect herbivory (Díaz et al. 2003).

We measured the radial asymmetry of 50 holm oak fruits of a variety of sizes collected in the study area in September 1997. Each fruit (cupule removed) was set upright on a piece of plaster with the axis of radial symmetry perpendicular to the horizontal plane. Two radii were measured under a dissecting microscope (×20) from the fruit apex to the nearest and to the farthest edge with an ocular micrometer (precision 0.01 mm). Radial asymmetry was measured as the difference between maximum and minimum radii divided by the average radius. We repeated the measurements 3 times at 1-week intervals with no reference to previous values. Measurement errors estimated from these repeated measures (Yezerinac et al. 1992) were too high (89.99% for fruit asymmetry;  $F_{49,100}=1.33$ ,  $P=0.113$ ), so that we opted to classify the 50 fruits visually as either symmetric or asymmetric (see Møller et al. 1996 for a similar procedure). A fruit was considered to be symmetric if its apex was at or close to the centre of the fruit when it was seen from above with its axis parallel to the line of vision. Given the bullet shape of

acorns, this procedure was able to detect even slight deviations from perfect symmetry. All measurements and classifications were made by the same person (M. D.). Two persons unaware of the goals of our study were asked to classify the same 50 fruits as either symmetric or asymmetric, using the explicit criteria listed above, and all classifications matched closely (gamma test,  $Z=6.49$ , 5.92 and 5.27, respectively, for the comparison between the two independent observers' data and between those of each observer and M. D.;  $P<0.0001$ ,  $n=50$  acorns). Fruits classified as asymmetric had significantly larger values of radial asymmetry than fruits considered to be symmetric (Mann-Whitney test,  $U=131.00$ ,  $P=0.0008$ ; symmetric acorns, median=0.21, range=0.11–0.33,  $n=20$ ; asymmetric acorns, median=0.30, range=0.10–0.60,  $n=30$ ; test based on the average values for the three repeated measures of each acorn).

The developmental instability of fruits produced by each tree was measured as the proportion of asymmetric fruits in random samples taken from marked trees. In 1997 we only sampled dehesa trees because of the low fecundity of forest trees. Ten aborted fruits per tree were taken from the ground under the trees in early October and classified in the laboratory as either symmetric or asymmetric, whereas 100 mature acorns per tree were classified *in situ* in November to avoid interference with other studies at work (Pulido and Díaz 2002b). Sampling dates were adjusted to peak numbers of either aborted fruits or mature acorns on the ground. Inspection of variation in the estimated proportion of asymmetric acorns, since the number of acorns assessed varied from 1 to 100, revealed that 40–50 acorns were sufficient to estimate this proportion accurately, and even ten acorns sufficed if the proportion of asymmetric acorns was either very large (>80%) or very small (<20%). In 1998 we sample all trees with sufficient acorn production. The proportion of asymmetric aborted fruits was estimated from 50 aborted fruits per tree taken in late September, and the proportion of asymmetric acorns from 50 acorns taken in November. All acorn classifications were made by the same person (M. D.). All mature acorns taken from the dehesa trees in 1998 were collected and placed in labelled plastic bags and were given for classification to a person unaware of the experiment. Field estimates and estimates of the person unaware of the purpose of the study were positively correlated ( $r_s=0.425$ ;  $P=0.027$ ,  $n=27$  trees).

#### Germination, seedling morphology and seedling survival

We estimated the germination capacity and the characteristics of seedlings emerged from symmetric and asymmetric acorns by means of a common or garden field experiment. We sowed 20 acorns per tree taken from 11 dehesa trees not treated with insecticide in a 2.5 m×1-m vertebrate-proof enclosure constructed close to the tree population. The enclosure was covered with 2-mm plastic mesh that provided shade and protection against direct strong rain. Acorns were taken at random under tree canopies, discarding those affected by predispersal seed predators. The acorns from each mother tree were arranged at random in a grid of five rows by four columns, 7 cm apart, and they were weighed to the nearest 0.1 g and classified as either symmetric or asymmetric just prior to sowing. We buried them loose at a depth of 2 cm in the first week of December 1997, and emergence was monitored from February to June at biweekly intervals. The date of emergence was measured as the number of days between sowing and emergence.

All seedlings were collected by cutting them at ground level on 1st July 1998. After 2 weeks of air-drying, seedlings were dried in an oven with a forced air current for 3–5 days at 60°C. Dried seedlings were cleaned and their stems were separated from the leaves, taking a leaf from the mid-part of the stem for additional measurements. Each fraction was weighed separately to the nearest 0.001 g with an electronic balance. The number of leaves was counted, the length of the largest stem (seedling height) was measured to the nearest 0.01 mm with a vernier calliper, and the area of the mid-leaf (excluding the petiole) was measured to the nearest mm<sup>2</sup> on its digitized outline by means of image analysis software (NIH Image 1.60 f).

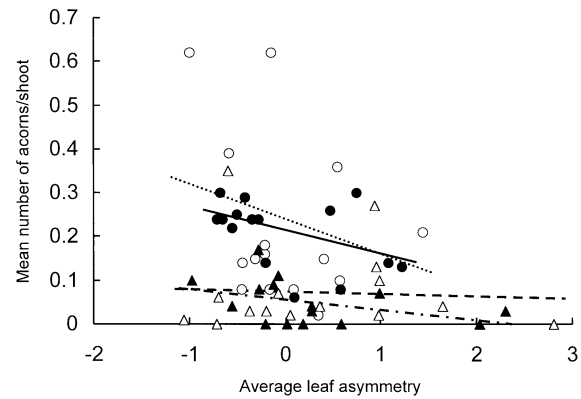
## Survival of naturally emerged seedlings

We studied seedling emergence and survivorship under field conditions of the 1998 cohort by means of regular and intensive sampling of both study plots (details in Pulido and Díaz 2002b). All emerged seedlings found were marked and their survival through their first drought season was monitored at monthly intervals until September 1998, since very few seedlings that survived their first summer died afterwards. For all seedlings we noted the microhabitat in which they had emerged (under *Quercus*, *Retama*, *Phillyrea* or *Cistus*), and the number of leaves and the length (to the nearest 0.1 mm) of the acorn from which they germinated measured with a vernier calliper, if still attached to the seedling.

## Results

### Leaf fluctuating asymmetry, fecundity and abortion rates

We found no significant effects of insecticide treatment on the number of acorns per shoot in either plot or year, and the interaction terms between treatment and year were also non-significant ( $F_{1,28}=0.01-3.00$ ,  $P=0.942-0.094$ ; two-way ANOVAs with treatment as a between-subjects factor, year as a within-subject, repeated-measures factor, and square-root-transformed average values for individual trees as the independent observations). The insecticide treatment had no direct effect on the production of mature acorns, either immediate or delayed, and mean fecundities were  $0.09 \pm 0.01$  and  $0.19 \pm 0.02$  acorns shoot<sup>-1</sup> for forest and dehesa trees, respectively (mean  $\pm$  SE,  $n=30$  trees per plot). Indirect effects of herbivory on acorn production were analysed by testing the relationships between average number of acorns per tree (square-root transformed) and average leaf fluctuating asymmetry, as this trait has been shown to reveal the delayed effect of herbivory on tree developmental stability (Díaz et al. 2003). We only used control trees for this analysis because the insecticide treatment was found to suppress the relationship between asymmetry and fecundity (Díaz et al. 2003). Mean number of acorns produced tended to be negatively correlated with fluctuating leaf asymmetry for all years and tree populations (Fig. 1). However, the relationships found did not reach statistical significance, a fact that could have been due to low sample size and low power (see Díaz et al. 2003 for a similar result, and Møller 1999 for a review). A meta-analysis combining the four independent tests supported this hypothesis, as the overall relationship was significant and there was no heterogeneity in the effect sizes among tests (Fig. 1).



**Fig. 1** Relationship between mean fecundity (number of acorns per shoot in September, square-root transformed) and mean leaf asymmetry of holm oak trees not treated with insecticide in 1997 ( $n=15$  trees per plot) according to study year and plot. Lines are linear regression lines. Correlation coefficients for these relationships were  $-0.308$ ,  $-0.467$ ,  $-0.060$  and  $-0.408$  (dehesa trees in 1997, dehesa trees in 1998, forest trees in 1997 and forest trees in 1998, respectively). None of them were statistically significant for  $n=15$  ( $P=0.079-0.832$ ), but their combination by means of a meta-analysis (Rosenthal 1991; Møller 1999) indicates an overall negative relationship ( $r_{60}=-0.318$ ,  $P=0.013$ ) which was homogeneous among tree populations ( $\chi^2_3 = 1.375$ ,  $P=0.711$ ). Dehesa trees in 1997 ( $\circ$ , -), dehesa trees in 1998 ( $\bullet$ , ---), forest trees in 1997 ( $\triangle$ , - -), forest trees in 1998 ( $\blacktriangle$ , ---)

Abortion rates during 1997, as measured from seed trap data from control trees, were significantly positively related to mean leaf fluctuating asymmetry in the dehesa but not in the forest population ( $r_{15}=0.525$ ,  $P=0.044$  and  $r_{15}=0.015$ ,  $P=0.958$ , respectively; linear regression with the arcsin-transformed proportion of fruits lost due to intrinsic factors as the dependent variable). Abortion rates derived from differences between the number of flowers per shoot in spring and the number of acorns per shoot in fall did not correlate with abortion rates derived from seed trap data ( $r_{28}=0.054$ ,  $P=0.778$ ), so we could not test whether the relationship between abortion rate and mean leaf asymmetry also applied to treated trees or to either treated or control trees in 1998.

### Fruit asymmetry and abortion

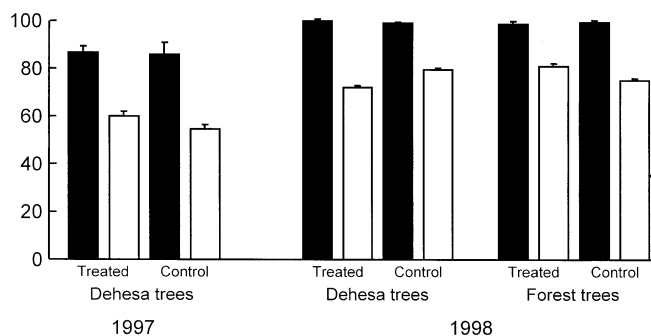
The proportion of asymmetric propagules was larger for aborted fruits than for mature acorns in both study years and tree populations (Table 1). Almost all aborted fruits

**Table 1** Results of two-way ANOVAs testing for differences in the proportion of asymmetric fruits (arcsine transformed) according to fruit type (aborted or mature) and to whether trees were treated with

insecticide in 1997. Separate analyses were made for dehesa trees in 1997 and for forest and dehesa trees in 1998

Source of variation	Dehesa trees 1997			Dehesa trees 1998			Forest trees 1998		
	df	F	P	df	F	P	df	F	P
Fruit type	1, 27	65.62	<0.0001	1, 24	389.91	<0.0001	1, 12	94.21	<0.0001
Insecticide treatment	1, 27	0.44	0.513	1, 24	0.57	0.459	1, 12	0.22	0.650
Fruit type $\times$ Insecticide	1, 27	0.28	0.602	1, 24	9.13	0.006	1, 12	1.78	0.207



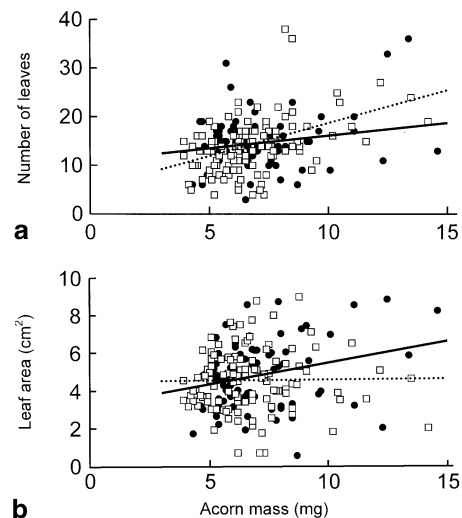


**Fig. 2** Mean (+SD) proportion of asymmetric fruits (*filled bars* aborted fruits, *open bars* mature acorns) for the 29 dehesa trees in 1997 and the 27 dehesa trees and 17 forest trees in 1998 that had sufficient flower and acorn production to estimate these proportions ( $n=10\text{--}100$  propagules per tree; see text for details). Values for trees treated with insecticide in 1997 and controls are shown separately for each year and tree population

were asymmetric (83–99%), whereas the proportion of asymmetric acorns was much lower (57–78%; Fig. 2). These differences were unaffected by insecticide treatment either in the same or in the previous year (Table 1). The significant fruit type  $\times$  treatment interaction in the dehesa population in 1998 merely indicated that the proportion of mature fruits that were asymmetric was larger in control trees than in the trees treated with insecticide in 1997, while the proportions of aborted fruits that were asymmetric did not vary according to insecticide treatment (Fig. 2).

#### Acorn asymmetry and seedling traits

Germination rates of acorns were very high ( $82.3\% \pm 9.3$  (mean  $\pm$  SD),  $n=11$  trees; see also Pulido and Díaz 2002b), and they were unaffected by acorn mass (logistic regression, Wald's statistic=0.67,  $df=1$ ,  $P=0.415$ ), acorn asymmetry (Wald's statistic=0.25,  $df=1$ ,  $P=0.620$ ) or mean leaf fluctuating asymmetry of mother trees (Wald's statistic=3.18,  $df=1$ ,  $P=0.075$ ). Mean emergence dates differed significantly among mother trees after correcting for the negative effect of acorn mass on emergence date (larger acorns emerged earlier;  $r_{169}=-0.189$ ,  $P=0.013$ ), whereas acorn asymmetry was not significantly related to emergence date or the relationship between acorn mass and emergence date (Table 2). There were also strong between-tree differences in seedling size and number of leaves after correcting for the strong effect of acorn mass on these variables (Table 3). Acorn asymmetry was not significantly related to the corrected seedling size (Table 3). However, the relationship between acorn size and the number of leaves and the leaf area of the seedlings depended on acorn asymmetry, whereas there was no significant between-tree difference in these relationships (Table 3). The number of leaves increased with acorn size for seedlings produced by asymmetric acorns, whereas leaf size remained constant. The opposite trend was found



**Fig. 3** Relationship between acorn mass and number of leaves (**a**) or area of the mid-leaf (**b**) for seedlings grown from acorns planted in a common or garden dehesa plot in December 1997 and harvested in July 1998 according to the asymmetry of these acorns ( $\bullet$ , – symmetric acorns;  $\square$ , ... asymmetric acorns). Lines are back-transformed linear regression lines. Acorn mass vs. number of leaves,  $r_{114}=0.430$ ,  $P<0.0001$  (asymmetric acorns),  $r_{76}=0.193$ ,  $P=0.091$  (symmetric acorns); acorn mass vs. leaf area,  $r_{114}=0.014$ ,  $P=0.877$  (asymmetric acorns),  $r_{76}=0.268$ ,  $P=0.018$  (symmetric acorns)

**Table 2** Results of mixed analysis of covariance (ANCOVA) testing for the effects of mother tree (random), acorn asymmetry (fixed) and acorn mass (covariate, square-root transformed) on date of emergence (days after sowing, log-transformed) of seedlings grown from acorns planted in a common or garden dehesa plot in December 1997 and harvested in July 1998

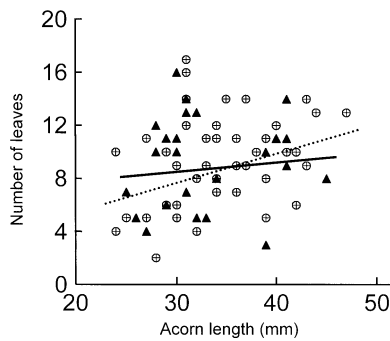
Source of variation	<i>F</i>	<i>df</i>	<i>P</i>
Mother tree	4.35	11, 169	$<<0.0001$
Acorn asymmetry	1.12	1, 11	0.312
Tree $\times$ Acorn asymmetry	0.60	11, 169	0.826
Covariate (Acorn mass)	6.24	1, 169	0.013
Covariate $\times$ Acorn asymmetry	0.44	1, 171	0.509

in seedlings derived from symmetric acorns, as leaf size increased with acorn size whereas the number of leaves remained constant (Fig. 3). Hence, seedlings from asymmetric acorns may have traded leaf number against leaf size, whereas seedlings from symmetric acorns showed the opposite pattern.

Overall, we found 133 naturally emerged seedlings, most of them (75%) growing under oaks and shrubs in the forest plot and the remaining growing almost exclusively under oaks in the dehesa plot (see Pulido and Díaz 2002b for details). Out of these 133 seedlings, 70 had the acorn still attached when located, but four had to be removed from the database since they had been partially eaten by an herbivore. Out of the remaining 66, 24 survived until September, whereas the remaining 42 died. There were no significant differences in survivorship between seedlings with the acorn still attached or not (logistic regression,

**Table 3** Results of a mixed multiple ANCOVA testing for the effects of mother tree (random), acorn asymmetry (fixed) and acorn mass (covariate, square-root transformed) on the number of leaves (square-root transformed), leaf area, mass of leaves, mass of stems and stem height of seedlings grown from acorns planted in a common or garden dehesa plot in December 1997 and harvested in July 1998

Source of variation	Wilks' $\lambda$	df	P
Mother tree	0.45	55, 767	<<0.0001
Acorn asymmetry	0.65	5, 7	0.610
Tree $\times$ Acorn asymmetry	0.71	55, 767	0.307
Covariate (Acorn mass)	0.74	5, 180	<<0.0001
Covariate $\times$ Mother tree (multivariate)	0.66	55, 716	0.129
Covariate $\times$ Acorn asymmetry (multivariate)	0.91	5, 164	0.007
	F	df	P
Covariate $\times$ Acorn asymmetry(No. leaves)	5.06	1, 168	0.026
Covariate $\times$ Acorn asymmetry(Leaf area)	5.48	1, 168	0.020
Covariate $\times$ Acorn asymmetry(Mass of leaves)	0.03	1, 168	0.859
Covariate $\times$ Acorn asymmetry(Mass of stems)	0.16	1, 168	0.686
Covariate $\times$ Acorn asymmetry(Seedling height)	0.65	1, 168	0.421



**Fig. 4** Relationship between acorn length and number of leaves for seedlings that emerged naturally in the two study plots during spring 1998 and that were either alive ( $\blacktriangle$ , —;  $r_{23}=0.110$ ,  $P=0.609$ ) or dead ( $\circ$ , ...;  $r_{42}=0.361$ ,  $P=0.019$ ) in September 1998. Lines are back-transformed linear regression lines

Wald's statistic=0.71,  $df=1$ ,  $P=0.399$ ) or between seedlings with different number of leaves (Wald's statistic=1.03,  $df=1$ ,  $P=0.311$ ). Seedlings that died during their first summer showed a significant positive relationship between acorn size (log-transformed) and number of leaves (square-root transformed), whereas the seedlings that survived did not show such a relationship (Fig. 4).

## Discussion

We studied the relationship between developmental instability of leaves of a tree and abortion rates, fruit abortion with respect to fruit phenotype, fruit phenotype in relation to seedling phenotype, and the relationship between seedling morphology and survival prospects. The realized fecundity of holm oak trees was related to their developmental instability. More developmentally unstable trees produced less flowers and aborted a larger proportion of their initial flower production, thus producing fewer mature fruits. Developmentally unstable acorns gave rise to seedlings with different resource allocation strategies between leaf number and leaf size than did more developmentally stable fruits, and we found evidence suggesting that these strategies were directly related to survival prospects during the first drought

season. We are unaware of any other study of these phenomena of developmental selection being related to developmental instability, although circumstantial and anecdotal evidence from other organisms is consistent with such an interpretation (see review in Møller 1997).

The production of full-grown acorns by holm oaks tended to be inversely related to fluctuating asymmetry of oak leaves in both study years and tree populations. Lack of statistical significance of these relationships could be attributed to low power, as their strength was close to the average recorded for 21 studies of 14 species reviewed by Møller (1999) on the relationship between developmental instability and fecundity, and its combination by means of a meta-analysis revealed a consistent and significant negative association between fluctuating leaf asymmetry and acorn production. This pattern closely resembled that found when analysing flower production as related to leaf asymmetry and insect herbivory (Díaz et al. 2003). Therefore, part of this result could be attributed to a lower overall reproductive effort of the developmentally more unstable trees. However, we also found that loss of propagules due to fertilization failure and abortion were directly related to leaf fluctuating asymmetry, at least for one tree population. This result is consistent with the stronger developmental selection expected under more stressful environmental conditions (Searcy and McNair 1990, 1993). Møller (1996) found a similar relationship between abortion rate and flower asymmetry of maternal plants in fireweed *Epilobium angustifolium*, a result that was attributed to developmental selection against developmentally unstable seeds. However, the level of developmental instability of aborted seeds was not assessed, neither was the phenotype of aborted seeds compared to that of seeds that reached maturity. Therefore, the present study provides an important, but hitherto missing piece of information on the effects of developmental selection by directly quantifying the relationship between asymmetry of maternal plants, seed abortion and seed asymmetry.

We had no measures of developmental instability of pollen donors or on the heritability of fluctuating asymmetry in holm oaks, so we could not ascertain whether increased abortion rates of developmentally unstable trees were due to a larger production of genetically stressed fruits or to a lower maternal invest-

ment in fruit development. On the other hand, we have direct estimates of the developmental instability of propagules that either aborted or reached maturity. The proportion of aborted acorns that were asymmetric was much larger than the proportion of asymmetric fruits in general. This result is the first direct evidence of developmental selection against developmentally unstable offspring. The only previous study supporting this mechanism is based on indirect evidence showing that a measure of developmental instability of experimental populations of fruit flies *Drosophila melanogaster* decreased rather than increased when they were reared under more severe conditions of sublethal toxicity, a result that was attributed to the selective mortality of developmentally unstable larvae (Polak et al. 2002).

As aborted acorns are much smaller than acorns that reach maturity (around 80–90% smaller in holm oaks), developmental selection against developmentally unstable fruits may have further benefited adult trees in terms of resource investment in reproduction if developmentally unstable acorns had lower fitness prospects than developmentally stable ones. We had no direct estimates of seedling survival in relation to acorn asymmetry, although seedling morphology has been found to be a predictor of seedling viability in some oak species (Rice et al. 1993). Seedlings derived from asymmetric acorns showed a different resource allocation strategy than seedlings produced by symmetric acorns as regards the distribution of cotyledonary resources to photosynthetic tissues in terms of number and size of leaves. Seedlings produced by asymmetric acorns produced a larger number of leaves, whereas seedlings produced by symmetric acorns had an increased leaf size compared to those produced by asymmetric acorns. This novel finding is consistent with the hypothesis that seedlings growing in stressful conditions tend to produce more leaves of smaller size than under more benign conditions (Sabaté et al. 1999).

Differential allocation of photosynthetic tissue by naturally emerged seedlings of holm oaks to a larger number of leaves was associated with subsequent death during the summer drought in our study population (survival during the first summer is a critical stage for recruitment of Mediterranean oaks; see Pulido and Díaz 2002a, 2002b). The mechanism underlying this association is at present unclear, as nearly no work has been devoted to the analysis of the optimal trade-off between size and number of leaves as compared to the size-number trade-offs in other organisms (review in Sakai and Harada 2001). Nevertheless, our empirical finding implies that the difference in resource allocation of seedlings produced by symmetric and asymmetric acorns was directly related to the survival prospects of seedlings. Hence, selective abortion of developmentally unstable fruits during development could have enhanced the fitness prospects of mother trees by reducing the loss of seedlings from developmentally unstable acorns. Such losses would otherwise have been additive to fruit losses due to fertilization failure or abortion (see Traveset 1993, Traveset et al. 1995, García et al. 1999 for other

mechanisms of reduction of additive losses of propagules during the regeneration cycle of woody plants).

The findings presented here raise a number of questions that need to be addressed in future studies. First, it remains to be determined whether the cross-generation effects of developmental instability identified here derive from maternal effects, additive genetic effects, or a combination of effects (Polak and Starmer 2001), i.e. whether increased developmental instability and abortion were due to genetic or purely environmental causes acting during fruit development. This will be a daunting task using trees as a model system. A more tractable study concerns the relationship between acorn asymmetry and acorn quality measured in terms of resource quality and quantity, and how this relates to fruit abortion, germination and subsequent survival. Such analyses will provide important information about the possible mechanisms involved. Finally, given that developmental selection against developmentally unstable offspring appears to be common (Møller 1997), detailed studies of other model systems are needed to determine the generality of our findings.

**Acknowledgements** We are very grateful to Hilda Fernández de Córdova (†) and Carlos Marcos for permission to work within their estates. Chema Torres helped us during field work, J. Barandica, F. López and J. V. Zorrilla with leaf measurements and J. Clobert with statistical tests. Comments of M. Polak, G. Moreno and two anonymous referees on earlier drafts were very helpful. F.J.P. was funded by a F.P.I. grant from the Consejería de Educación y Juventud of the Junta de Extremadura while carrying out the field work, and M. D. received a grant for research of the Spanish CICYT while writing the manuscript. This study is a contribution to the project PAC-02-008 of the Junta de Comunidades de Castilla-La Mancha.

## References

- Cadée N (2000) Parent barn swallow fluctuating asymmetry and offspring quality. *J Avian Biol* 31:495–503
- Cohen J (1988) Statistical power analysis for the behavioral sciences, 2nd edn. Erlbaum, Hillsdale, N.J.
- Díaz M, Pulido FJ, Møller AP (2003) Herbivore effects on developmental stability and fecundity of holm oaks *Quercus ilex*. Manuscript submitted to *Oikos*
- Fuller R, Houle D (2002) Detecting genetic variation in developmental instability by artificial selection on fluctuating asymmetry. *J Evol Biol* 15:954–960
- Fuller R, Houle D (2003) Inheritance of developmental instability. In: Polak M (ed) *Developmental instability: causes and consequences*. Oxford University Press, New York
- García D, Zamora R, Gómez JM, Hódar JA (1999) Bird rejection of unhealthy fruits reinforces the mutualism between juniper and its avian dispersers. *Oikos* 85:536–544
- Hasegawa M (1995) Sexual selection among peafowl — fluctuating asymmetry and parasite resistance. *Bull Assoc Nat Sci Senshu Univ* 26:19–26
- Kozłowski J, Stearns SC (1989) Hypotheses for the production of excess zygotes: models of bet-hedging and selective abortion. *Evolution* 43:1369–1377
- Leung B, Knopper L, Mineau P (2003) A critical assessment of the utility of fluctuating asymmetry as a biomarker of anthropogenic stress. In: Polak M (ed) *Developmental instability: causes and consequences*. Oxford University Press, New York

- Marshall DL, Ellstrand NC (1988) Effective mate choice in wild radish: evidence for selective abortion and its mechanism. *Am Nat* 131:739–756
- Mock DW, Forbes LS (1995) The evolution of parental optimism. *Trends Ecol Evol* 10:130–134
- Møller AP (1996) Developmental stability of flowers, embryo abortion, and developmental selection in plants. *Proc R Soc Lond B* 263:53–56
- Møller AP (1997) Developmental selection against developmentally unstable offspring and sexual selection. *J Theor Biol* 185:415–422
- Møller AP (1999) Asymmetry as a predictor of growth, fecundity and survival. *Ecol Lett* 2:149–156
- Møller AP, Cuervo JJ (2003) Asymmetry, size and sexual selection: factors affecting heterogeneity in relationships between asymmetry and sexual selection. In: Polak M (ed) *Developmental instability: causes and consequences*. Oxford University Press, New York
- Møller AP, Swaddle JP (1997) *Asymmetry, developmental stability, and evolution*. Oxford University Press, Oxford
- Møller AP, Thornhill R (1997) A meta-analysis of the heritability of developmental stability. *J Evol Biol* 10:1–16
- Møller AP, Thornhill R (1998) Bilateral symmetry and sexual selection: a meta-analysis. *Am Nat* 151:174–192
- Møller AP, Cuervo JJ, Soler JJ, Zamora-Muñoz C (1996) Horn asymmetry and fitness in gemsbok, *Oryx g. gazella*. *Behav Ecol* 7:247–253
- Mousseau TA, Fox CW (eds) (1998) *Maternal effects*. Oxford University Press, New York
- Petrie M, Halliday T (1994) Experimental and natural changes in the peacock's (*Pavo cristatus*) train can affect mating success. *Behav Ecol Sociobiol* 35:213–217
- Petrie M, Halliday T, Sanders C (1991) Peahens prefer peacocks with elaborate trains. *Anim Behav* 41:323–331
- Polak M (1997) Ectoparasitism in mothers causes higher positional asymmetry in their sons: implications for sexual selection. *Am Nat* 149:955–974
- Polak M, Starmer WT (2001) The quantitative genetics of fluctuating asymmetry. *Evolution* 55:498–511
- Polak M, Opoka R, Cartwright IL (2002) Response of fluctuating asymmetry to arsenic toxicity: support for the developmental selection hypothesis. *Environ Pollut* 18:19–28
- Pulido FJ, Díaz M (2002a) Dinámica de la regeneración natural del arbolado de encina y alcornoque. In: FJ Pulido, P Campos, G Montero (eds) *La gestión forestal de las dehesas*. IPROCOR, Mérida, Spain, pp 39–62
- Pulido FJ, Díaz M (2002b) Recruitment of holm oaks: disparate determinants of regeneration in Mediterranean forests and dehesas. Manuscript submitted to *Ecological Monographs*
- Pulido FJ, Díaz M, Hidalgo SJ (2001) Size-structure and regeneration of holm oak (*Quercus ilex*) forests and dehesas: effects of agroforestry use on their long-term sustainability. *For Ecol Manage* 146:1–13
- Rice KJ, Gordon DR, Hardison JL, Welker JM (1993) Phenotypic variation in seedlings of a keystone tree species (*Quercus douglasii*): the interactive effects of acorn source and competitive environment. *Oecologia* 96:537–547
- Rosenthal R (1991) *Meta-analytic procedures for social research*. Sage, New York
- Sabaté S, Sala A, Gracià CA (1999) Leaf traits and canopy organization. In: Rodà F, Retana J, Gracià CA, Bellot J (eds) *Ecology of Mediterranean evergreen oak forests*. Springer, Berlin Heidelberg New York, pp 122–133
- Sakai S, Harada Y (2001) Sink-limitation and the size-number trade-off of organs: production of organs using a fixed amount of reserves. *Evolution* 55:467–476
- Searcy KB, McNair MR (1990) Differential seed production in *Mimulus guttatus* in response to increasing concentrations of copper in the pistil by pollen from copper tolerant and sensitive sources. *Evolution* 44:1424–1435
- Searcy KB, McNair MR (1993) Developmental selection in response to environmental conditions of the maternal parent in *Mimulus guttatus*. *Evolution* 47:13–24
- Skalski JR (1987) Selecting a random sample of points in circular field plots. *Ecology* 68:749
- Traveset A (1993) Deceptive fruits reduce seed predation by insects in *Pistacia terebinthus* L. (Anacardiaceae). *Evol Ecol* 7:357–361
- Traveset A, Willson MF, Gaither JC (1995) Avoidance by birds of insect-infested fruits of *Vaccinium ovalifolium*. *Oikos* 73:381–386
- Van Dongen S (2000) The heritability of fluctuating asymmetry: a Bayesian hierarchical model. *Acta Zool Fenn* 37:15–24
- Wade MJ (1998) The evolutionary genetics of maternal effects. In: Mousseau TA, Fox CW (eds) *Maternal effects*. Oxford University Press, New York, pp 5–21
- Yezerinac SM, Loughheed SC, Handford P (1992) Measurement error and morphometric studies: statistical power and observer experience. *Syst Biol* 41:471–482