Reproductive ecology of *Thymelaea velutina* (Thymelaeaceae)-Factors contributing to the maintenance of heterocarpy

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Abstract. *Thymelaea velutina* (Thymelaeaceae) is a dioecious shrub that presents a unique type of heterocarpy which consists of the simultaneous production of dry and fleshy fruits. It is endemic to the Balearic Islands (Western Mediterranean) and is found both in dunes and mountain areas. The goal of this study was to identify which factors influence the production of both fruit types, examining the variation of their effects at a spatio-temporal scale (comparing two localities in different years). Specifically, we investigated (1) whether pollen limitation influences the type of fruit produced, and (2) the possible differences in seed size, mass, dispersal capacity, seed predation, germination patterns and seedling survival between fruit types. We also examined if the production of fleshy fruits was modified with the application of gibberellins to reproductive branches. Although fleshy fruits were consistently more abundant than dry ones at both populations, their proportion was significantly higher at the site with greater precipitation. The addition of either pollen or gibberellins did not affect the proportion of each fruit type. Seeds in fleshy fruits are consistently larger, heavier and more likely to be dispersed than seeds in dry fruits, but germinability, germination rate and seedling survival was similar among fruit types. Heterocarpy in this species is currently maintained as there is no apparent factor that exerts any strong selective pressure on either fruit type. The two fruit types might even have different 'functions', one serving especially for dispersal and population expansion and the other for producing a seed bank that ensures an eventual germination.

Key words: Balearic Islands, germination, heterocarpy, seed predation, seed dispersal, seedling survival, *Thymelaea velutina*, Thymelaeaceae.

Most plants possess a normal or skewed frequency distribution of seed size and shape but some produce two or more defined fruit types (heterocarpy or heterodiaspory) with different effects on dispersal, germination recruitment, and seedling survival. Such seed polymorphism is considered as a mechanism that plants have to increase fitness under different conditions (Mandák 1997 and references therein), and is frequent in Asteraceae, Chenopodiaceae, Poaceae and Brassicaceae (Harper et al. 1970).) This phenomenon is indeed typical of habitats such as desert, semi-desert environments or even human- disturbed habitats, in which the environmental conditions are highly variable in time (Stebbins 1974, Harper 1977) or in space (Ellner and Schmida 1984, Ungar 1987). In these habitats, the capacity of producing different types of diaspores with a different degree of dormancy and dispersability may be especially beneficial (Mandák 1997). Seed heteromorphism can also be

favoured by spatial heterogeneity in microsite availability if seedling success is microhabitat-specific (Lloyd 1984) and appears to be rather frequent in herbaceous Mediterranean plants, especially annuals (Mathez and Xena de Enrech 1990). Theoretical and empirical surveys suggest that natural selection should favour a negative correlation between seed dispersal and dormancy. Under unfavourable or stressful conditions, heterocarpic plants tend to invest a greater relative quantity of resources towards the production of diaspores with a low capacity of dispersal and germinability and with high levels of dormancy (Venable and Lawlor 1980, Venable and Brown 1988, Venable et al. 1995). Furthermore, recent studies have demonstrated that the biomass allocation to a particular type of fruit/seed and its ecological behaviour are more environmentally than genetically determined (Imbert et al. 1999, Mandák and Pysek 1999).

In the present study, we tried to elucidate the factors that influence the production of two fruit types in *Thymelaeae velutina* (Pourr. ex Cambess.) Endl. (Thymelaeaceae), examining the variation of their effects both at a spatial scale (comparing two different altitudes at which the species is found) and at a temporal scale (comparing different years). This species presents a unique type of heterocarpy that consists of the production of both fleshy and dry fruits (Tébar and Llorens 1993). As far as we know, this type of heterocarpy has not been previously reported for any other species in the world. Both types of fruits are found simultaneously and randomly distributed in the branches of individual females. Specifically, we tested the effect of pollen limitation (by means of hand-pollination experiments) and growth hormones (by applying gibberellins on reproductive branches) on the type of fruit produced. Moreover, by means of field experiments, we investigated whether seed dispersal, predation, germination and seedling survival in natural conditions may act as possible selective factors on fruit type.

Material and methods

Study species. *Thymelaea velutina* is a perennial shrub that can reach up to 1 m, endemic to the Balearic Islands, specifically to Mallorca and Menorca. It is found in two types of habitat: at sea level – usually in dunes- and in open shrublands at high altitudes in mountain areas (the latter only in Mallorca). Most of its populations in coastal habitats have disappeared due to human disturbance, mainly urbanization (Alomar et al. 1997). It is a dioecious species, although some individuals may bear the two sexes simultaneously (pers. obs.); this sexual lability, however, is much less frequent than in the congeneric *Thymelaea hirsuta* (El-Keblawy et al. 1996). The species is ambophilous, i.e. it is pollinated by both insects and wind (de la Bandera and Traveset, in press). The plant flowers during spring and early summer, producing a variable number of inflorescences each bearing 3–5 flowers. Male flowers are yellow, bear eight stamens and present a rudimentary ovary. It is possible that the species has evolved towards dioecy, as the existence of intermediate morphs seems to be the rule rather than the exception among species that have evolved dioecy from monoecy (Freeman et al. 1997). Female flowers are greenish and have a unilocular ovary. Two types of fruits are produced by the same individual plant: (1) dry fruits, achenes that are covered by the hypanthium and are dispersed by barochory, most of them remaining under the mother plant, and (2) fleshy fruits, oval, yellow and translucent drupes, which break the hypanthium when ripening and fall to the ground. Such fleshy fruits are often consumed by beetles and ants once on the ground. The seed produced in both types of fruit is dark brown and has a pyriform shape. Fruit and seed dimensions are shown in Table 1. A more detailed description of the species can be found in Pedrol (1997).

Study sites. We chose two localities representing the two types of habitats where the species is found: the dunes of Ca'n Picafort, Alcudia Bay, at the north of Mallorca, and the mountain site at Puig Major (named Sa Coma de N'Arbona, 1250 m) which is the highest peak (1450 m) of the mountain chain, Serra de Tramuntana, that expands from northwestern to southwestern Mallorca. These localities represent the two extremes of the altitudinal gradient where *T. velutina* lives. The dune system in Alcudia bay is currently the main population of this species, which has recently

Table 1. Means and standard error of size (mm) and fresh weight (mg) measurements for dry and fleshy fruits and seeds of *Thymelaea velutina* in the two study populations.

Variable	Dune		Mountain	
	Dry $N = 100$	Fleshy $N = 117$	Dry $N = 100$	Fleshy $N = 110$
Fruit mass	3.323 ± 0.058	14.963 ± 0.345	2.940 ± 0.061	12.658 ± 0.355
Fruit length	3.981 ± 0.039	4.286 ± 0.043	3.974 ± 0.040	4.078 ± 0.045
Fruit width	1.982 ± 0.020	2.511 ± 0.032	2.149 ± 0.021	2.458 ± 0.033
Mass without hypanthium	2.477 ± 0.051	–	1.830 ± 0.051	–
Pulp mass	–	9.840 ± 0.278	–	8.590 ± 0.291
Seed mass	1.937 ± 0.042	3.541 ± 0.052	1.4 ± 0.04	3.2 ± 0.1
Seed length	2.271 ± 0.027	2.430 ± 0.025	2.243 ± 0.024	2.356 ± 0.015
Seed width	1.375 ± 0.014	1.406 ± 0.009	1.364 ± 0.015	1.399 ± 0.008

disappeared from all sites in southern Mallorca. The predominant species in the dunes of Ca'n Picafort belong to families such as Ericaceae, Cistaceae, Lamiaceae and Poaceae, and *Juniperus oxycedrus* and *J. phoenicae* are the most important shrubs/trees at this site. *T. velutina* is relatively abundant here, its density 0.1 indiv/m². Mean annual temperature and precipitation here are 17°C and 628 mm, respectively (Guijarro 1986). The flowering period of *T. velutina* in this population ranges from late February to early May, the peak being at late March. Sex ratio is 1 female: 1.5 males ($N = 429$). Flowering synchrony between males and females is almost absolute, although some years the former may begin flowering several days earlier.

At the mountain locality of Puig Major, the main vegetation consists of *Ampelodesmos mauritanica*, *Hypericum balearicum*, *Teucrium marum* and *Rosmarinus officinalis*. The density of *T. velutina* shrubs here is lower than in the dunes, c. 0.06 indiv/m². Mean annual temperature and precipitation are 9°C and 1238 mm, respectively (Guijarro 1986). The flowering period extends from early May through late June, with a peak at late May. Sex ratio is about the same as in the dune, 1 female: 1.6 males ($N = 105$), and both sexes also flower synchronously here. Other mountain localities in Mallorca have small populations of *T. velutina* and are of much more difficult access.

Production of fleshy and dry fruits. In each population we marked ten randomly chosen female individuals, selecting nine branches with a similar number of flowering buds from each female. We compared the proportion of fruits produced that were fleshy among three different treatments: (1) addition of pollen, (2) pollen transport exclusively by wind (anemogamy), and (3) open pollination, which served as control. Three branches per female were used for each treatment. In the first group, flowers were manually pollinated, by means of forceps due to the reduced flower size, with pollen from a minimum of five males. These flowers were bagged with muslin to avoid any insect removing the added pollen. The second group was bagged with cloth bags, with a mesh of 1 mm that allowed pollen but not insect passage through them. In both cases, bags were placed before flower anthesis. Flowers of all treatments were monitored every three-four days (as this is the lifetime of a flower), recording the number of buds, open, and withered flowers in each branch. Pollination experiments were performed during March and April in 2000, and from February to April in 2001 in the dune, and between May and June in 2000 and 2001 in the mountain. Once the flowering period was over, we bagged all marked branches with a cloth that allowed the passage of light and air but not fruits. This was done to avoid losing fruits that could be removed by ants or dropped by the plant and lost. We monitored plants weekly during the entire fruiting period, recording in each visit the number of dry and fleshy fruits in each branch.

The female plants used in the pollination experiments were the same the two years of the study, except for three individuals of Ca'n Picafort that died during the summer of 2000 and that had to be replaced for others in 2001.

Experiment with gibberellins. Gibberellins are plant hormones, synthesized mainly in the apex of stems and roots, in expanding leaves and in developing fruits and seeds. Reproductive tissues

usually contain high levels of such hormones compared to other tissues (Barceló et al. 2001). Gibberellic acid GA_3 is one of the most commercially employed hormones, usually used to increase fresh weight of cultivated fruits such as grapes (Salisbury and Ross 1994). It is especially effective with species with one or a few ovules (Barceló et al. 2001). In *T. velutina*, thus, we wanted to test if the addition of GA_3 could alter the ratio of fleshy: dry fruits. In 2001, we marked nine branches of similar size in five females in one population; we used the mountain one as the production of fleshy fruits here was found to be more abundant than in the dune during 2000. Three of the branches were sprayed with a solution of ethanol, GA_3 (100μg/ml), and tween (a substance that prevents the branch leaking after the spraying); the other branches were used for two type of controls: half were sprayed with a solution of ethanol and tween (0.1%), and the last three were not sprayed at all. The spraying was performed twice, during the flowering and fruiting period. All branches were bagged after the spraying to avoid losing any fruit.

Fruit/Seed removal. Rate of fruit removal was studied in the two populations during the springs 2001–2003. The experiment consisted in placing groups of four Petri dishes under fruiting adults, two of them with dry fruits and two with fleshy fruits. Half of the dishes in each pair were covered by a wire mesh (1 cm light) to prevent access to vertebrate seed predators while the other half were opened to all predators (and thus acted as the controls). Five replicates were used and each dish initially bore five fruits. Fruit removal was recorded a week after the experiment was set. On that date, five new fruits were placed in the Petri dishes, and removal was recorded a week later. In 2001, fruit removal at the mountain could be recorded only once due to the intensive rainfall in this population that year.

To have an estimate of the relative abundance of each species removing fruits/seeds of *T. velutina* in the field, we buried 50 vials - 7 cm deep and 5.5 cm wide, containing soapy water- at the ground level, regularly distributing them all over the study areas. After a month, we removed them and recorded the number of invertebrates of each taxon.

Germination and seedling survival in common garden conditions. Dry and fleshy fruits were gathered during the fruiting periods in 2000 and 2001 from the marked individuals. Seeds were individually weighed with a precision balance (Salter model HX-100) and stored in paper bags at room temperature. Seed length and diameter were also measured (only in 2000) with a digital caliper to the nearest 0.1 mm. Each seed was individually sown, at a depth of 0.5 cm, in a germination tray filled with standard commercial soil mixture during the autumn (November - December of 2000 and 2001, respectively) and trays were placed in a common garden near the laboratory. They were kept moist as needed and monitored daily for germination. For each germinated seed, we recorded the time to germination (T_0) and periodically (every month) monitored seedling survival.

Laboratory germination trials. Due to the low levels of seed germination found in this species, we tested the effect of light and temperature on this process. During the spring of 2002, a total of 800 seeds gathered from fleshy fruits were set to germinate under controlled conditions. Half of those seeds had been collected in 2000 (all at Puig Major) whereas the other half was collected in 2001 (200 at Puig Major and 200 at Ca'n Picafort). Half of each group of seeds were stratified during 45 days in moist sand at 2–3°C of temperature. All seeds were placed in Petri dishes (25 seeds/ dish) on filter paper that was periodically wetted with distilled water. Petri dishes were placed in a germination chamber at 16°C and with a photoperiod of 12 hours. Half of the dishes were covered with aluminium foil to simulate darkness. They were inspected daily during three months. Each germinated seed was recorded and removed from the dish.

During the spring of 2003, we additionally evaluated the effect of seed age on germination, by testing 200 seeds from fleshy fruits coming from Puig Major (100 collected in 2000 and 100 in 2002). These seeds were placed in Petri dishes and left to germinate in the dark in the germination chamber at 16°C. They were monitored daily during four months.

Seedling emergence and survival in the field. In autumn 2002, seeds from fleshy and dry fruits of *T. velutina* were sown in their natural habitat. We established ten sowing 'stations' at Ca'n Picafort and eight at Puig Major in each of three microhabitats: (1) open interspaces (with no vegetation

cover), (2) under female conspecifics, and (3) under other shrub species. In each sowing unit, we planted -in an alternate way- 10 seeds from fleshy fruits and 10 coming from dry fruits, at a distance of about 5 cm from each other, and at a depth of 0.5 cm. Seedling emergence and mortality were monitored once a month during an entire year. Some sowing units were lost due to vandalism and animal disturbance. In the dune population, we additionally performed a transplant experiment, planting 100 seeds (in ten randomly chosen sowing stations) from dry fruits gathered at Puig Major to evaluate their performance in this different habitat. The opposite was not possible because of scarce seed availability from Ca'n Picafort.

During 2001 and 2002, we also recorded natural seedling emergence at both localities. For this, we intensively searched through the entire study area for emerging seedlings. We marked them and evaluated their survival during 2002 and 2003.

Statistical analysis. Differences in the number of fleshy fruit relative to the total number of fruits produced (hereafter, proportion of fleshy fruit) were tested between populations by means of a nested analysis of variance, using population and individual nested within population as the independent variables. Data from 2000 and 2001 were tested separately because individuals were not the same the two years of the study. The effect of pollination treatment and year on the proportion of fleshy fruits was also tested with a general linear model (GENMOD) for each population separately. As all treatments were applied to each individual, we considered a repeated measures design with individual as subjects and treatment and year as fixed factors. In this case we only used the repeated individuals the two years of study. The dependent variable was normalized by using the angular transformation.

The effect of hormone addition on the proportion of fleshy fruits was tested with a Generalized Linear Mixed Model (GLIMMIX procedure in SAS) using a binomial error distribution and a logit link function. Treatment and individual were included as fixed and random effects, respectively. This model was used due to the impossibility of normalizing the dependent variable.

Fruit removal data were analyzed using a repeated-measures ANOVA where the dependent variable was the proportion of fruits removed, normalized with the angular transformation, and

the independent variables were treatment, type of fruit, population and year. Differences in seed mass (logarithmically transformed to reach normality) and dimensions (length and width) were tested by means of nested ANOVAs, with type of fruit, population, individual within population and year as independent variables.

Seed germinability was tested with another GLIMMIX using a binomial error distribution and a logit link function. In this case, type of fruit, population, and year were included as fixed effects, individual as a random effect, and seed mass as a covariate. Differences in germination and survival rates in the common garden were tested with a failure time analysis, by fitting a Cox proportional hazards regression model to the number of days between sowing and seedling emergence for each individual seed. Type of fruit and year were introduced as categorical fixed effects and seed mass as a continuous variable. Such analysis could be performed only with data from the dune population, due to the low germination levels of the mountain seeds. The incidence of different treatments (light/dark and cold/no cold) on germination was tested by means of a one-way ANOVA for each population and year separately, as we could not apply all treatments for each case due to insufficient number of seeds available one year. The effect of seed age on total number of germinated seeds (normalized with the square root transformation) was analysed with a t-test. Finally, seedling emergence in the field was tested with a GLIMMIX, using a binomial error distribution and a logit link function. Microhabitat and type of fruit were the fixed effects while station was the random effect. Seedling survival in those natural conditions was tested with a Chi-Square test. The statistical packages employed were STATISTICA 6.0 (GLM), SAS 8.02 (GENMOD and GLIMMIX), and S-Plus 2000 (failure-time analysis).

Results

Fleshy fruit production. Fleshy fruits were produced more frequently than dry fruits in the two populations, but in greater proportions in the mountain than in the dune, and this was consistent between years (2000: $F_{1,17} = 9.96$, $P < 0.01$; 2001: $F_{1,18} = 34.72$, $P < 0.001$; Fig. 1). Differences among treatments were
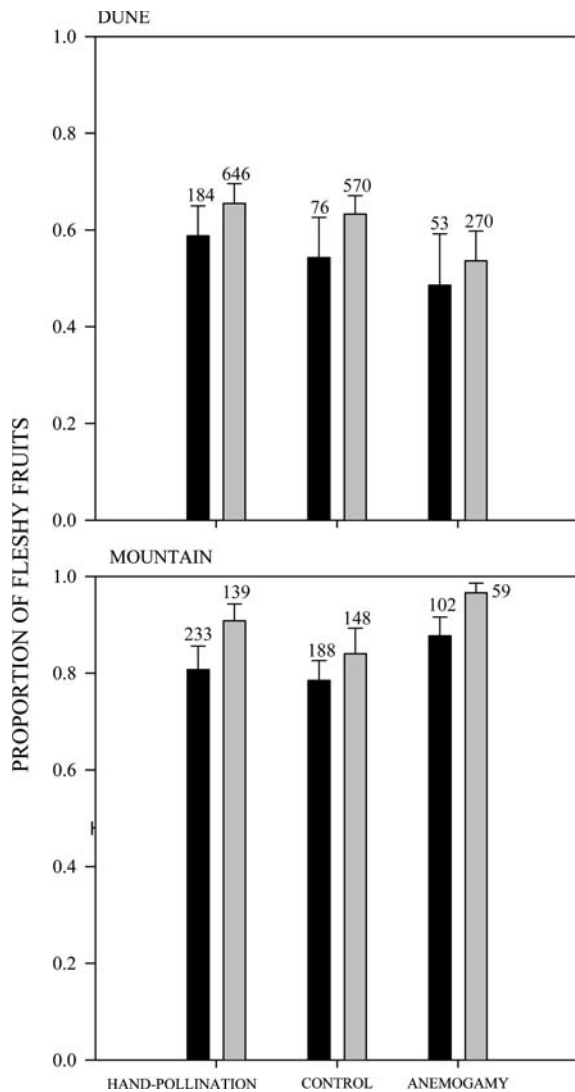
Fig. 1. Proportion of fleshy fruits of *Thymelaea velutina* produced in the different treatments in 2000 (black bars) and 2001 (grey bars) in each population. One standard error bars are shown. Numbers above bars represent sample sizes

found in the mountain site ($F_{20,20} = 2.166$, $P = 0.05$) but not in the dune ($F_{14,14} = 0.86$, $P = 0.61$). In the former, flowers pollinated by wind produced a slightly higher proportion of fleshy fruits than hand-pollinated or control flowers (Tukey's test) (Fig. 1). There were also significant differences between years in the mountain ($F_{10,20} = 3.41$, $P = 0.009$); the proportion of fleshy fruits was greater in 2001 than in 2000 and four individuals actually produced only this

fruit type (Fig. 2). In contrast, no differences in time were found in the dune ($F_{7,14} = 1.50$, $P = 0.24$). The interaction between treatment and year was significant in the dune ($F_{14,60} = 2.15$, $P = 0.02$) but not in the mountain ($F_{20,123} = 0.95$, $P = 0.53$) (Fig. 1). Figure 2 shows how the proportion of fleshy fruits can vary within any given individual between years, indicating that such trait is not only genetically determined but that is affected by the environmental conditions prevailing in a particular year.

The addition of gibberellins to reproductive branches did not have any effect on the production of production of fleshy fruits ($\chi^2 = 2.94$, $P = 0.23$).

Fruit removal. Fleshy fruits were removed by animals much more frequently than dry fruits ($F_{1,69} = 51.67$, $P < 0.001$). Differences between populations and years were also significant ($F_{1,69} = 9.36$, $P = 0.003$; and $F_{2,69} = 8.87$, $P < 0.001$, respectively). In the dune, dry fruits were not removed at all, either in 2002 or 2003 (Fig. 3). No differences in removal were observed between treatments (excluded from vertebrates vs controls) ($F_{1,69} = 2.19$, $P = 0.14$), as different species insects appeared to be responsible for such removal. In the dune, the beetles *Pimelia criba* and *Tentyria schwamii* were the main species consuming fruits and the most frequently trapped (we collected 225 and 173 specimens, respectively, in the insect traps placed at this site), although the ant *Cremato-gaster scutellaris* was also commonly observed consuming fruit pulp (we collected only three specimens in the vials, but ant abundance is usually underestimated with pitfall traps; M. Palmer, pers. com.). At the mountain site, in contrast, the only species we observed removing fleshy fruits was the ant *Tapinoma nigerrimum*. Based on our direct observations and on the few specimens collected in the containers, this species was actually not very abundant in the area.

Seed mass and germination. Fleshy fruits had heavier seeds than dry fruits ($F_{1,12} = 174.21$, $P < 0.001$). Moreover, seeds were heavier in the dune than in the mountain population ($F_{1,12} = 5.85$, $P = 0.032$), and this
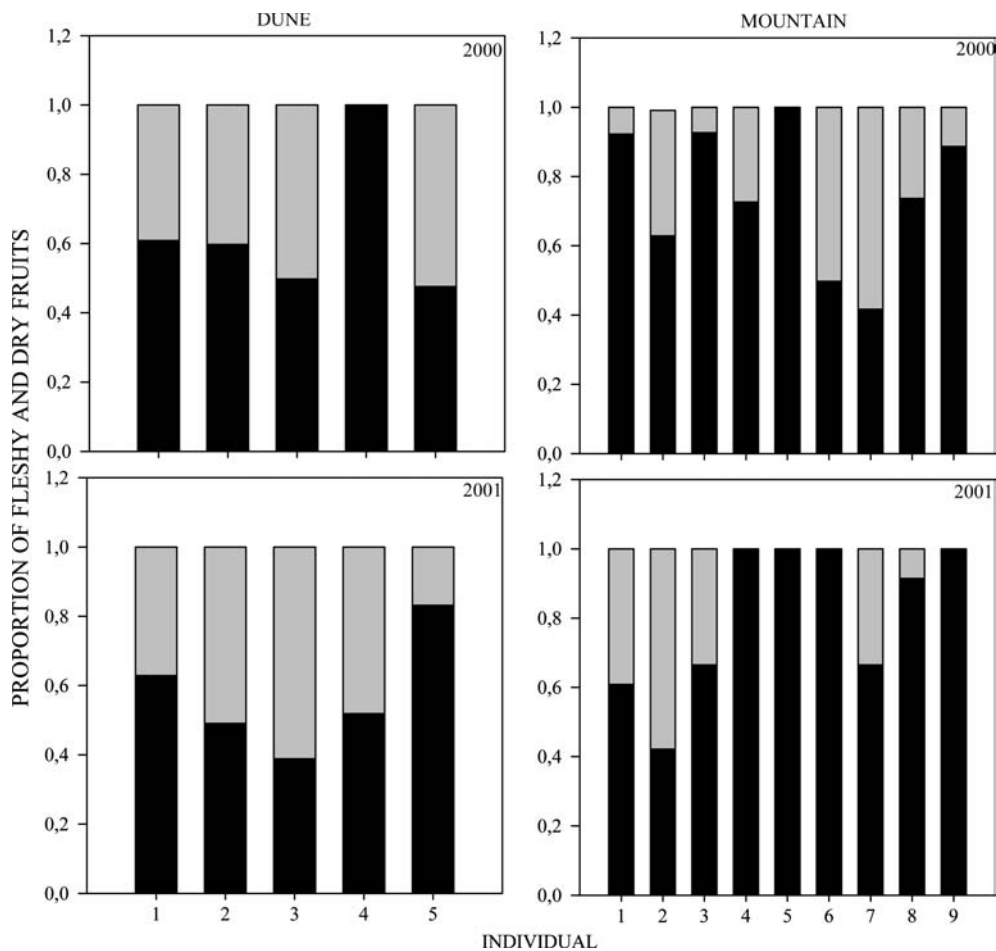
Fig. 2. Proportion of each fruit type (black: fleshy, grey: dry) produced in individuals of *Thymelaea velutina* during the two study years at each population

was consistent in time ($F_{1,12} = 1.73$, $P >> 0.05$). Differences in seed mass among years were greater in the mountain than in the dune, and that is why the interaction between population and year was significant ($F_{1,12} = 10.04$, $P = 0.008$). Differences in seed dimensions were also significant between fruit types ($F_{1,17} = 101.51$, $P < 0.001$ and $F_{1,17} = 7.62$, $P = 0.013$ for seed length and width, respectively; Table 1), although not between populations ($F_{1,17} = 0.31$ and $F_{1,17} = 0.25$, $P >> 0.05$, respectively).

After controlling for seed mass, seed germination behavior in the common garden was similar between fruit types ($\chi^2 = 0.70$, $P > 0.05$). Differences existed, however, between populations ($\chi^2 = 11.50$, $P < 0.01$), and this was consistent the two years of study ($\chi^2 = 0.62$, $P > 0.05$). Germination was several times greater for dune than for mountain seeds (Fig. 4). Only a small proportion of seeds germinated from the mountain population the two years; in 2001, most of those germinated seeds came from dry fruits whereas all germinated seeds in 2002 were from fleshy fruits, although differences were not significant either between types of fruit ($\chi^2 = 0.01$, $P = 0.93$) or between years ($\chi^2 = 0.63$, $P = 0.43$). The interaction population x fruit type x year was only marginally significant ($\chi^2 = 3.12$, $P = 0.08$).
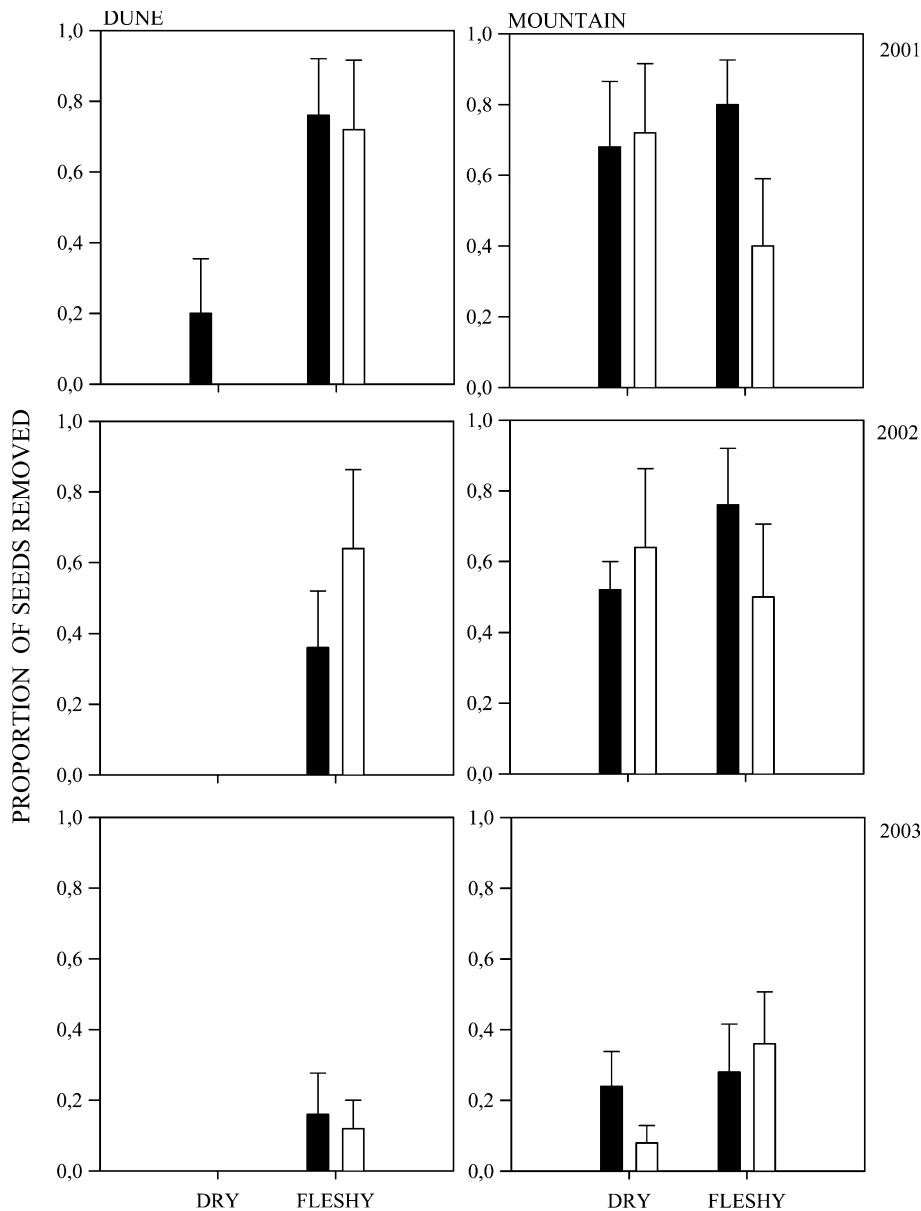
Fig. 3. Proportion of fruits of each type removed in the two populations and from the two treatments: excluded from vertebrates (black bars) and controls (white bars) during three different years. One standard error bars are shown

The light and temperature treatments carried out in the laboratory showed different effects on seed germination, especially for the mountain population ($F_{3,12} = 10.49$, $P = 0.001$) and marginally for the dune ($F_{3,12} = 2.88$, $P = 0.08$). Table 2 shows the proportion of germinated seeds for each treatment at each site. Germination was highest when seeds were left in the dark with no cold treatment. When comparing seeds collected in different years (2000 and 2002) from the mountain, we additionally found that older seeds had a higher germination percentage than younger ones (unpubl. data).

The rate of germination could be analyzed only for the dune population due to the low germination of mountain seeds. Both
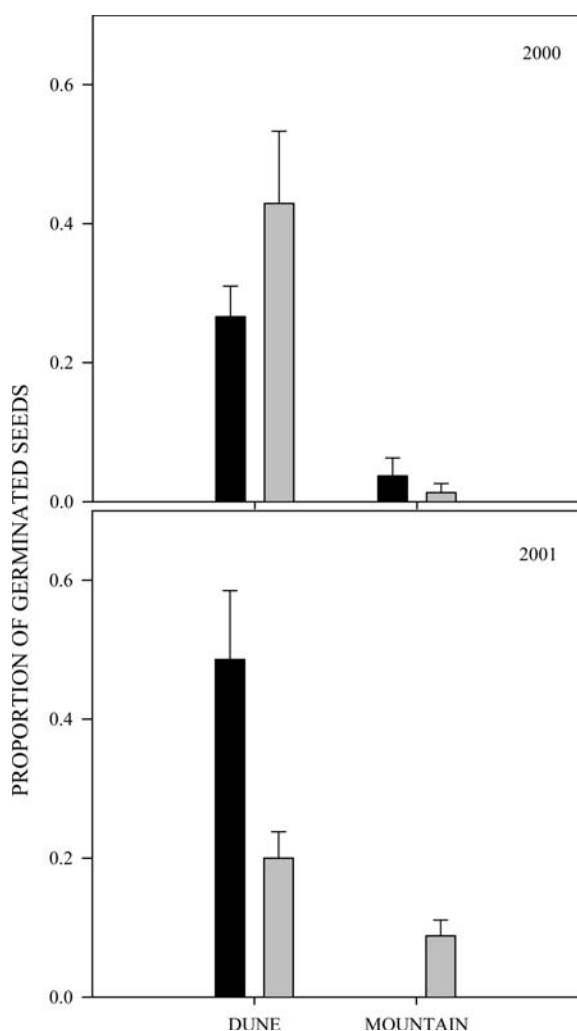
Fig. 4. Proportion of germinated seeds for dry (black bars) and fleshy (grey bars) fruits of *Thymelaea velutina* in the two populations and the two years. One standard error bars are shown

types of fruits germinated at a similar rate ($Z_4 = 1.4$, $P = 0.16$) and differences were observed only between years ($Z_4 = -3.33$, $P < 0.001$), germination being faster in 2000 than in 2001.

In the field, percentages of germination were very low: 1.66% in the dune ($N = 600$) and 2.50% in the mountain ($N = 480$). In both populations, differences in seedling emergence were significant among microhabitats ($\chi_1^2 = 28.56$, $P < 0.001$ and $\chi_2^2 = 10.87$, $P = 0.004$ for the dune and mountain, respectively) but

not between fruit types. There was no interaction microhabitat x fruit type ($\chi_1^2 = 1.23$ and $\chi^2 = 6.17$, all $P >> 0.5$). In the dune, the greatest emergence took place under female conspecifics whereas in the mountain it was under other shrub species. At both sites, the lowest seedling emergence occurred in open interspaces (Fig. 5). None of the seeds from Puig Major germinated in the dune population.

Seedling survival. From the seeds planted under controlled conditions, we found no significant differences between fruit types in seedling survival ($Z_4 = -0.758$, $P = 0.45$). Seedling survival was higher in 2000 than in 2001 ($Z_4 = 4.21$, $P < 0.001$).

In the field, natural recruitment was very low at both populations during 2001, although it was greater at the dune than at the mountain site ($\chi^2 = 35.29$, $P < 0.01$), mostly because of the larger plant sizes and fruit crops in the former (De la Bandera and Traveset, in press). Seedling survival after a year, however, was much greater at the mountain (64%, $N = 28$) than at the dune (7%; $N = 68$). In 2002, we recorded 118 new seedlings in the dune but we did not find any seedling at the mountain site. Seedling survival this year was again rather low (11.86%). Both years, the greatest mortality occurred during the summer (Fig. 6).

Discussion

Maintenance of heterocarpy. Although the proportion of different morphs in heterocarpic species is usually genetically determined (Venable and Búrquez 1989, Mathez and Xena de Enrech 1990, Imbert 2001), it can also be influenced by the prevailing environmental conditions (Ungar 1987, Mandák and Pyšek 1999). In the Chenopodiaceae and in the Asteraceae families, for instance, changes in allocation to particular fruit types under various environmental conditions have produced contradictory results (Ungar 1987, Mandák and Pyšek 1999).

In the case of *T. velutina*, heterocarpy has been considered to be regulated by environ-

Table 2. Mean and standard error of the germination percentage in laboratory trials of fleshy fruits seeds of *Thymelaea velutina* from the two populations. $N = 100$

Treatment	Dune (2001)	Mountain (2000)	Mountain (2001)
Cold / Darkness	9.0 ± 2.0	38.7 ± 4.8	–
Cold/ Light	7.2 ± 2.6	23.3 ± 5.5	10.0 ± 2.6
No cold / Darkness	22.2 ± 4.7	85.0 ± 1.6	–
No cold / Light	11.0 ± 3.4	51.0 ± 1.1	23.0 ± 6.4

mental factors, specifically water resources and temperature conditions (Tébar and Llorens 1993). These authors argued that the genus *Thymelaea* might actually have evolved from taxa related to the genus *Daphne*, which produces only fleshy fruits; *Daphne* belongs to the Tertiary flora, and was thus adapted to the warm and humid conditions that existed in the Mediterranean region before the glaciations. It is possible that the precursor forms of *Thymelaea* might have responded to the increased xeric conditions, prevailing when the Mediterranean climate appeared, by modifying the type of fruit. Thus, *T. velutina* might be considered as a step between both genera:

Daphne (producing fleshy fruits) and *Thymelaea* (producing dry fruits). In the present work, by means of observations and experiments, we confirm that environmental conditions notably influence the proportion of fleshy fruits. We found both spatial and temporal variation in the relative production of such fruits. On the one hand, although fleshy fruits are more abundant than dry ones in both habitats, a larger proportion of the former is found in the mountain, which we attribute to the greater water availability at this site. On the other hand, the production of fleshy fruits was greater in 2001 than in 2000 in both populations, coinciding with an increase in
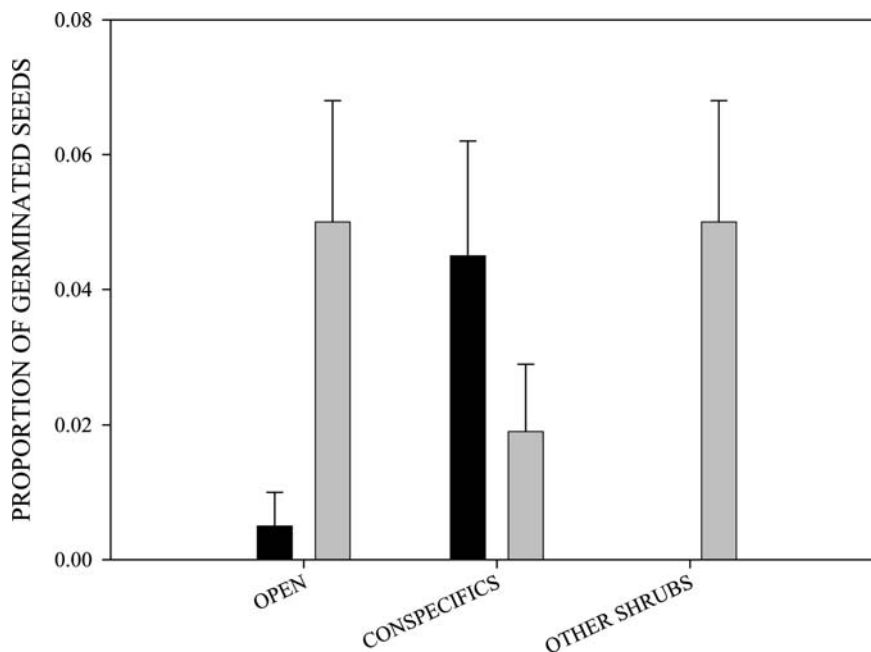
Fig. 5. Proportion of germinated seeds of *Thymelaea velutina* in three microhabitats (open interspaces, under female conspecifics and under other shrub species) in the dune (black bars) and the mountain (grey bars) population. One standard error bars are shown
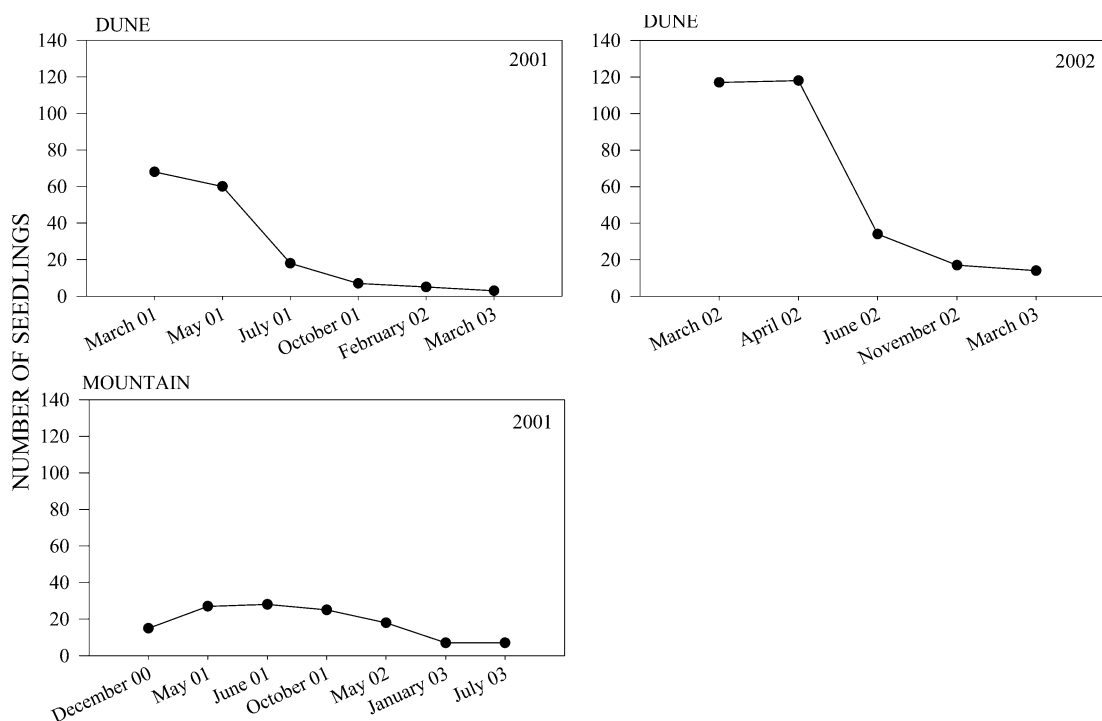
Fig. 6. Number of seedlings of *Thymelaea velutina* found in each population during 2001 and 2002. No seedlings were detected at the mountain site in 2002

total precipitation at the two sites (according to data from the National Institute of Meteorology: at Ca'n Picafort (Muro s'Albufera station): 530.0 mm vs 419.3 mm, respectively, and at Puig Major (Escorca Son Torrella station): 1808.3 mm vs 851.4 mm, respectively). Therefore, water availability appears to be an important (probably the main) factor involved in the production of fleshy fruits, the pulp of which bears a high water content (approx. 78%). Our experiments on pollen and hormone (gibberellins) additions, in contrast, showed that they do not influence the production of fleshy fruits. The finding that pollen transported by wind in the mountain population tended to favour the production of fleshy fruits the two years of study could suggest that pollen source might also influence to some extent whether a fruit is going to have pulp or not. However, we have no explanation for such result. In the dune, wind-transported pollen actually tended to produce a lower proportion of fleshy fruits.

In heterocarpic species, particular types of diaspores differ in their dispersal ability (Sorenson 1978, Payne and Maun 1981, Baker and O'Dowd 1982). In *T. velutina*, fruits fall under mother plants as they are released. Fleshy fruits are easily dropped once ripe, and they are rapidly removed by consumers such as ants and beetles, but dry fruits remain under the mother canopy. It is probably for this reason that in the previous study on this species, Tébar and Llorens (1993) argued that the production of fleshy fruits was low; they also reported that fleshy fruits were more abundant in the dunes, which is the opposite of what is reported here.

When examining whether one type of fruit is more likely to be dispersed than the other, we did find a strong consistent selection for fleshy fruits, both in the dune and in the mountain sites. In the former, the abundant beetles are important consumers of fleshy fruits of *T. velutina* (they were observed eating the pulp of five fruits in 10 min, pers. obs.), although they

are not very effective dispersers as they usually feed on the pulp and leave the seed below or near the mother plant. Ants, in contrast, seem to be better dispersers of this plant as they often carry the seeds to their nests. The fruiting period of *T. velutina* is spring and early summer, when the dispersal activity of ants is more intense (Bas 2001). In the mountain, the dispersal of *T. velutina* is exclusively performed by ants of the species *Tapinoma nigerrimum*, which was frequently observed carrying the seeds to their nests, as it has been found for other fleshy-fruited species (e.g. Pizo and Oliveira 1999, Bas 2001). The explanation for the greater fruit removal in the mountain, thus, is probably the greater ant activity at the time mature fruits are available at this site.

Most plant species show a continuous intra-individual variation in seed mass and/or seed morphology (Harper et al. 1970, Fenner 1985). In heteromorphic species, this variation is even more accused (Imbert 2002) and dispersal abilities of diaspores have been found to be closely associated with germinability: germination tends to be rapid and dormancy low in far-dispersed diaspores and vice versa (Venable and Levin 1985, Mandák and Pyšek 2001, Kigel 1992). In *T. velutina*, seeds of fleshy fruits have a slightly larger mass and size than those of dry fruits. However, we found no differences in seed germination patterns between them, and thus we exclude the possibility that a germination advantage selects for the production of fleshy fruits. In the germination chamber, we did find differences in germination rates between fleshy and dry fruits, although they were not consistent in time and no clear pattern emerged. Seed germination behavior has actually been found to vary significantly between controlled and natural conditions in a number of species (e.g. Traveset et al. 2001a, b). Germination in the chamber was also found to increase with the darkness treatment, as observed in a number of species (Navarro and Guitián 2003 and references therein). Thus, the transport of *T. velutina* seeds by ants to their nests is probably beneficial for germination. In fact, several studies have reported greater plant recruitment inside ant nests (Culver and Beattie 1980; Horvitz 1981; Hughes and Westoby 1992a, 1992b).

Seed mass was greater in the dune than in the mountain populations. A decrease in seed mass with elevation has been found in several studies that have examined patterns of seed mass variation across different altitudes (Baker 1972, Lord 1994). Moreover, resource limitation in the mountain might also cause a lower seed mass; the low winter temperatures, the strong winds that promote stomata closure, and the low depth and stonyness of the soil at the mountain site may result into a lower photosynthetic rate and, ultimately, into a lower seed mass compared to plants at sea level, where winter (period in which most plants in Mediterranean climate accumulate reserves that will be used later during flowering and fruiting) is milder. In fact, individuals are much larger at the dune than at the mountain (De la Bandera and Traveset, in press). Differences in seed mass between sites were congruent with differences in germination under controlled conditions: seeds from the dune germinated much more than those from the mountain. Germination in the field, however, was very low at both localities ($< 3\%$), and was somewhat greater in the mountain probably because of the greater water availability at this site. The transplanted seeds from the mountain to the dune had nil germination, which suggests that the environment rather than the genotype plays a more important role for germination.

Seed dormancy. When the probability of seedling establishment varies unpredictably, the evolutionary stable germination strategy favours a proportion of dormant seeds among the progeny of each mother (Zammit and Zedler 1990 and references therein). On the other hand, sibling competition can also favor mothers that produce a mixture of dormant and immediately germinable seeds in absence of environmental uncertainty (Ellner 1986, 1987). Two year old seeds took less time to germinate than six-month old seeds. Similar findings were obtained by Tan (1980) in *Thymelaea passerina*. As in that case,

T. velutina presents dormancy probably because of the exotegmen, which is the most characteristic feature of the family. Such exotegmen is always palisade-like, thick-walled and lignified and provides the seed with a high degree of impermeability, producing a delayed germination of seeds in several species (Tan 1980). Such dormancy might be beneficial especially to seeds of dry fruits, which remain in the seed bank under the mother plant, and would be germinating when conditions were suitable, as found for other species (e.g. Bastida and Talavera 2002).

Germination in the field was lower than in the chamber probably due to the scarcity of precipitations the two years of the study. Water stress appears to be more important than availability of microsites in either population. Similar results have been found for other species in Mallorca island, such as the endemic *Rhamnus ludovici-salvatoris* (Traveset et al. 2003). As in other Mediterranean species, germination was highest in covered microsites, either under conspecifics (in the dune, probably because of the larger fruit crops here; De la Bandera and Traveset, in press) or under other shrub species (in the mountain, probably related to the greater seed dispersal by ants at this site). The reason for such greater germination at covered sites might be that canopy-filtered light can trigger germination, as seen in several ruderal species such as *Amaranthus retroflexus*, *Potentilla norvegica* (Taylorson and Borthwick 1969) and *Atriplex sagittata* (Mandák and Pyšek 1998). Hence, we hypothesize that the function of seeds from dry fruits -which remain undispersed under the mother plant – is the maintenance of germination, whereas the advantage of fleshy fruits is seed dispersal and population expansion, as it has been suggested for the heterocarpic *Fedia pallescens* and *Fedia cornucopiae* (Mathez and Xena de Enrech 1990), both dispersed by ants, or for *Atriplex sagittata* (Mandák and Pyšek 2001). The two types of fruits in *T. velutina*, therefore, might be maintained because they have different 'functions' for the plant.

The highest seedling mortality of *T. velutina* in the field occurs when both temperature increases and precipitation is almost absent. Such circumstances are more pronounced in the dune population, where higher temperatures are reached and water availability is lower. For this reason, we think that the loss of natural habitats due to human disturbance represents a serious threat to this unique species which presents already a very low seedling recruitment.

As already mentioned, the production of two or more types of fruits is expected more in species that develop in habitats such as deserts (Zohary 1962), disturbed sites (Harper 1965), and also in the environmental conditions prevailing in the Mediterranean region, where rainfall is highly unpredictable (Ellner and Schmida 1981, Petit 1990). Heterocarpy in *T. velutina* may have evolved as a response to increasing xeric conditions in Mediterranean climate, but it appears to be maintained nowadays as there are no apparent strong selective forces that favor one fruit type more than the other. Moreover, it seems likely that the two types of seeds have different 'functions', one serving especially for dispersal and population expansion and the other for producing a seed bank that ensures an eventual germination.

References

Alomar G., Mus M., Roselló J. A. (1997) Flora endèmica de les Balears. Consell Insular de Mallorca, FODESMA, Palma.

Baker G. A., O'Dowd D. J. (1982) Effect of parent plant density on the production of achene types in the annual *Hypochoeris glabra*. J. Ecol. 70: 201–215.

Baker H. G. (1972) Seed weight in relation to environmental conditions in California. Ecology 53: 997–1010.

Barceló J., Nicolás G., Sabater B., Sánchez R. (2001) Fisiología vegetal. Ediciones Pirámide, Madrid.

Bas J. M. (2001) Frugivoria, dispersió i depredació de llavors en l'aladern (*Rhamnus alaternus* L.): paper dels ocells, rosegadors i formigues. PhD thesis, Universitat de les Illes Balears, Palma.

Bastida F., Talavera S. (2002) Temporal and spatial patterns of seed dispersal in two *Cistus* species (Cisteaceae). Ann. Bot. 89: 427–434.

Culver D. C., Beattie A. J. (1980) The fate of *Viola* seeds dispersed by ants. Amer. J. Bot. 67: 710–714.

De la Bandera, M. C. and Traveset, A. (2005) Breeding system and spatial variation in the pollination biology of the heterocarpic *Thymmelaea velutina* (Thymelaeaceae). Pl. Syst. Evol. (in press).

El-Keblawy A., Lovett-Doust J., Lovett-Doust L. (1996) Gender variation and the evolution of dioecy in *Thymelaea hirsuta* (Thymelaeaceae). Canad. J. Bot. 74:1596–1601.

Ellner S. (1986) Germination dimorphism and parent-offspring conflict in seed germination. J. Theor. Biol. 123: 173–185.

Ellner S. (1987) Competition and dormancy: a reanalysis and review. Amer. Naturalist 130: 798–803.

Ellner S., Schmida A. (1981) Why are adaptations for long-range seed dispersal rare in desert plants? J. Theor. Biol. 123: 173–185.

Ellner S., Schmida A. (1984) Seed dispersal in relation to habitat in the genus *Picris* (Compositae) in Mediterranean and arid regions. Israel J. Bot. 33: 25–39.

Fenner M. (1985) Seed ecology. Chapman and Hall, London.

Freeman D. C., Lovett-Doust J., El-Keblawy A., McArthur E.D. (1997) Sexual selection and inbreeding avoidance in the evolution of dioecy. Bot. Rev. 63:65–92.

Guijarro J. A. (1986) Contribución a la bioclimatología de Baleares. PhD thesis, Universitat de les Illes Balears, Palma.

Harper J. L. (1965) Establishment, aggression, and cohabitation in weedy species. In: Baker H. G., Stebbins G. L. (eds.) The genetics of colonizing species. Academic Press, London, pp. 243–268.

Harper J. L. (1977) Population biology of plants. Academic Press, London.

Harper J. L., Lovell P. H., Moore K. G. (1970) The shapes and sizes of seeds. Annual Rev. Ecol. Syst. 1: 327–356.

Horvitz C. C. (1981) Analysis of how ant behaviour affects germination in a tropical myrmecochore *Calathea microcephala* (P. & E.) Koernicke (Marantaceae): microsite selection and aril removal by neotropical ants *Odontomachus*, *Pachycondyla*, and *Solenopsis* (Formicidae). Oecologia 51: 47–52.

Hughes L., Westoby M. (1992a) Fate of seeds adapted for dispersal by ants in Australian sclerophyll vegetation. Ecology 73: 1285–1299.

Hughes L., Westoby M. (1992b) Effects of diaspore characteristics on removal of seeds adapted for dispersal by ants. Ecology 73: 1300–1312.

Imbert E. (2001) Capitulum characters in the seed heteromorphic species *Crepis sancta* (Asteraceae), variance partitioning and inference for the evolution of dispersal rate. Heredity 86: 78–86.

Imbert E. (2002) Ecological consequences and ontogeny of seed heteromorphism. Persp. Plant Ecol. Evol. Syst. 5: 13–36.

Imbert E., Escarré J., Lepart J. (1999) Local adaptation and non-genetic maternal effects among three populations of *Crepis sancta* (Asteraceae). Ecoscience 6: 223–229.

Kigel J. (1992) Diaspore heteromorphism and germination in populations of ephemeral *Hedypnois rhagadioloides* (L.) F.W. Schmidt (Asteraceae) inhabiting a geographic range of increasing aridity. Acta Oecol. 13: 45–53.

Lord J. M. (1994) Variation in *Festuca novae-zelandiae* (Hack.) Cockayne germination behaviour with altitude of seed source. New Zealand J. Bot. 32: 227–235.

Lloyd D.G. (1984) Variation strategies of plants in heterogenous environments. Biol. J. Linn. Soc. 21: 357–385.

Mandák B. (1997) Seed heteromorphism and the life cycle of plants, a literature review. Preslia. Praha 69: 129–159.

Mandák B., Pyšek P. (1998) History of spread and habitat preferences of *Atriplex sagittata* (Chenopodiaceae) in the Czech Republic. In: Starfinger U., Edwards K., Kowarik I., Williamson M. (eds.) Plant invasions: ecological mechanisms and human response. Backhuys Publishers, Leiden, pp. 209–224.

Mandák B., Pyšek P. (1999) Effects of plant density and nutrient levels on fruit polymorphism in *Atriplex sagittata*. Oecologia 119: 63–72.

Mandák B., Pyšek P. (2001) Fruit dispersal and seed banks in *Atriplex sagittata*: the role of heterocarpy. J. Ecol. 89: 159–165.

Mathez J., Xena de Enrech N. (1990) Etude comparative des syndromes de pollinisation et de dissemination chez deux espèces du genre *Fedia* (Valerianaceae). Naturalia Monspeliensia 55: 5–14.

Navarro L., Guitián J. (2003) Seed germination and seedling survival of two threatened endemic species of the northwest Iberian Peninsula. Biol. Conserv. 109: 313–320.

Payne A. M., Maun M. A. (1981) Dispersal and floating ability of dimorphic fruits segments of *Cakile edentula* var. *lacustris*. Canad. J. Bot. 59: 2595–2602.

Pedrol J. (1997) *Thymelaea* Mill. (nom. cons) In: Castroviejo S., Aedo C., Benedí C. M., Muñoz Garmendia F., Nieto Feliner G., Paiva J. (eds.) Flora Ibérica: plantas vasculares de la Península Ibérica e Islas Baleares, vol. 8. Real Jardín Botánico, CSIC, Madrid, pp. 50–51.

Petit D. P. (1990) Contribution à l'etude de l'évolution des Carduées et Lactucées (Composées). PhD thesis, University of Montpellier, Montpellier.

Pizo M. A., Oliveira P. S. (1999) Removal of seeds from vertebrate faeces by ants: effect of seed species and deposition site. Can. J. Zool. 77: 1595–1602.

Salisbury F. B., Ross C. W. (1994) Fisiología vegetal. Ediciones Iberoamericana, México.

Sorenson A. E. (1978) Somatic polymorphism and seed dispersal. Nature 276: 174–176.

Stebbins G. L. (1974) Flowering plants. Evolution above the species level. Arnold, London.

Tan K. (1980) Studies in the Thymelaeaceae I: Germination, seedlings, fruits and seeds. Notes Roy. Bot. Gard. Edinbourgh 38: 149–164.

Taylorson R. B., Borthwick H. A. (1969) Light filtration by foliar canopies: the significance for light controlled weed seed germination. Weed Sci. 17: 48–51.

Tébar F. J, Llorens L. (1993) Heterocarpy in *Thymelaea velutina* (Poiret ex Camb.) Endl.: a case of phenotypic adaptation to Mediterranean selective pressures. Bot. J. Linn. Soc. 111: 295–300.

Traveset A., Riera N., Mas R. E. (2001a) Passage through bird guts causes interspecific differences in seed germination characteristics. Funct. Ecol. 15: 669–675.

Traveset A., Riera N., Mas R. E. (2001b) Ecology of fruit-colour polymorphism in *Myrtus communis* and differential effects of birds and mammals on seed germination and seedling growth. J. Ecol. 89: 749–760.

Traveset A., Gulías J., Riera N., Mus M. (2003) Transition probabilities from pollination to establishment in a rare dioecious shrub species (*Rhamnus ludovici-salvatoris*) in two habitats. J. Ecol. 91: 427–437.

Ungar I. A. (1987) Population ecology of halophyte seeds. Bot. Rev. 53: 301–334.

Venable D. L. (1985) The evolutionary ecology of seed heteromorphism. Am. Nat. 126: 577–595.

Venable D. L., Brown J. S. (1988) The selective interactions of dispersal, dormancy and seed size as adaptations for reducing risk in variable environments. Amer. Naturalist 131: 360–384.

Venable D. L., Búrquez A. M. (1989) Quantitative genetics of size, shape, life-history and fruit characteristics of the seed heteromorphic composite *Heterosperma pinnatum*. I. Variation within and among populations. Evolution 43: 113–124.

Venable D. L., Dyreson E., Morales E. (1995) Population dynamic consequences and evolution of seed traits of *Heterosperma pinnatum* (Asteraceae). Amer. J. Bot. 82: 410–420.

Venable D. L., Levin D. A. (1985) Ecology of achene dimorphism in *Heterotheca latifolia* I. Achene structure, germination and dispersal. J. Ecol. 73: 133–145.

Venable D. L., Lawlor L. (1980) Delayed germination and dispersal in desert annuals: escape in space and time. Oecologia 46: 272–282.

Zammit C., Zedler P. H. (1990) Seed yield, seed size and germination behaviour in the annual *Pogogyne abramsii*. Oecologia 84: 24–28.

Zohary M. (1962) Plant life of Palestine, Israel and Jordan. Ronald, New York.

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