

Ecology of fruit-colour polymorphism in *Myrtus communis* and differential effects of birds and mammals on seed germination and seedling growth

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Summary

1 The fruit-colour polymorphism of *Myrtus communis*, a common Mediterranean shrub, is examined. We investigate whether frugivores affect the maintenance of the polymorphism, whether morphs differ in germination or seedling growth, and whether passage through frugivores' gut affects seed behaviour and seedling growth.

2 Blue berries are very similar in morphological traits and nutrient composition to the rarer white morph. Rates of fruit removal by birds, the main dispersers of this species, did not differ between morphs, suggesting that a colour preference is not involved in maintenance of the polymorphism.

3 Seeds of the two morphs showed the same germinability (final percentage germination) as well as similar rates of germination under controlled conditions (in growth chamber and greenhouse). Outdoors, seeds from blue berries tended to germinate slightly faster (which might give them an early advantage) but differences between morphs disappeared after several weeks of growth.

4 The germination of myrtle seeds is differently affected by passage through the digestive tract of birds, which appeared to significantly increase germinability, and of the carnivorous pine marten, *Martes martes*, which did not. The different seed retention time in the gut or the chemical composition of the food ingested along with the seeds may be responsible for such differences.

5 The detection of a positive effect of bird ingestion in outdoor conditions, but not in the growth chamber or in the greenhouse, demonstrates the importance of performing germination tests in the natural habitat.

6 Seed size, irrespective of morph, affects the response to passage through the bird gut. Large seeds, which are more rapidly ejected, and are thus less abraded, germinated at a similar speed to non-ingested controls. Variation in seed size within a single species should thus be considered in future studies.

Key-words: avian fruit removal, Balearic Islands, Mediterranean vegetation, pine marten, seed passage through digestive tracts

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Introduction

Colour polymorphisms are widespread in nature, both in animals and plants, and their prevalence over long periods of time suggests that they are maintained by natural selection. Although the study of such polymorphisms has received particular attention during the last few decades (e.g. Jones *et al.* 1977; Golding 1992;

Gillespie & Oxford 1998, for animals; Stanton *et al.* 1989; Levin & Brack 1995; Fry & Rausher 1997, for flowers; Willson & O'Dowd 1989; Puckey *et al.* 1996; Traveset & Willson 1998, for fruits), the mechanisms that explain their persistence are still relatively unknown.

Identifying such mechanisms often depends on associating changes in colour with shifts in environmental variables and therefore on how strongly and in what direction selection acts in space or time (Brodie *et al.* 1995). It has often been hypothesized that selection for colour-polymorphism is mediated in fruits by vertebrate frugivores (Willson & O'Dowd 1989; Willson &

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Whelan 1990; Traveset & Willson 1998), in the same way that flower-colour polymorphisms are thought to respond to selective pressures exerted by pollinators (e.g. Waser & Price 1981; Stanton *et al.* 1989; Levin & Brack 1995). The preferences of vertebrate frugivores, however, may be only one of several selective pressures that determine the frequency distribution of fruit colour (Willson & Comet 1993; Willson 1994; Traveset & Willson 1998).

We focus on the fruit-colour polymorphism of the shrub *Myrtus communis* L. (Myrtaceae), which is widespread around the Mediterranean basin, but also extends to eastern Iran and Afganistan (Bonafé 1979). We determine whether frugivores exhibit preference for a particular morph and whether morphs differ in their seed germination and subsequent growth. Finally, we examine the effect of passage through the digestive tract of dispersers on seed germination and seedling growth.

Methods

STUDY PLANT

Myrtus communis (myrtle) is an evergreen shrub that can reach up to 5 m in height. It occurs in Mediterranean woodlands, maquis and garrigues, and it is the only species of the genus found in the Northern Hemisphere (Bonafé 1979). Berries are usually dark blue but may be yellowish-white (blue and white, hereafter). This polymorphism is rarely described in botanical floras but has been found on the island of Mallorca (Bonafé 1979) in Italy (Milia *et al.* 1996) and in the southern and eastern

Iberian Peninsula (P. Jordano & P. García-Fayos, personal communication). The two morphs are apparently similar in all traits describing plant size and fecundity and their fruits do not differ in either morphology or nutrient composition of the pulp (Table 1). The ellipsoidal berries turn from green to pale yellow and finally, in the common morph, to dark blue when completely ripe. In the rare morph, fruits either remain pale yellow or get darker but never turn blue.

Ripe fruits appear by mid-November and can remain on the plants until mid-February. The main dispersers are birds, although carnivorous mammals (such as foxes, martens and weasels) have been reported to consume the fruits and disperse the seeds intact (Aronne & Russo 1997). In Mallorca, faeces of pine marten (*Martes martes*) found in areas with myrtle shrubs usually contain large amounts (up to 500) of its seeds, although this varies much among years. In this region, single myrtle fruits bear, on average, about 12 seeds, a significantly higher figure than the 3–3.5 seeds per fruit reported by Aronne & Russo *et al.* (1997) for Italy. Fruit diameter is significantly correlated with number of seeds, although the correlation is not very high ($r = 0.34$, $n = 200$ and $r = 0.19$, $n = 250$, $P < 0.05$, for the white and blue morph, respectively). The snail-shaped seeds possess a thick coat and an elaiosome, which suggests secondary dispersal by ants (Aronne & Wilcock 1994), although this has not been studied.

Seeds of this species are non-dormant (Baskin & Baskin 1998), germinating in the spring following production, with 100% germination under light conditions (Ozturk *et al.* 1983).

Table 1 Characteristics of blue and white colour-morphs of myrtle fruits (mean \pm SE) collected from different individuals at the study site

Trait	Blue morph	<i>n</i>	White morph	<i>n</i>	F (or T)	<i>P</i>
Fruit length (mm)	11.03 \pm 0.09	250	10.87 \pm 0.11	200	0.06	0.80
Fruit width (mm)	10.21 \pm 0.06	250	10.58 \pm 0.09	200	0.74	0.40
Fruit mass (g)	0.54 \pm 0.009	250	0.58 \pm 0.01	200	0.33	0.57
Pulp mass (g)	0.34 \pm 0.004	50	0.31 \pm 0.004	50	1.40	0.17
No. of seeds per fruit	12.06 \pm 0.34	250	11.23 \pm 0.32	200	0.30	0.59
Seed weight (mg)	7.16 \pm 0.23	25	7.02 \pm 0.27	25	0.37	0.71
Seed length (mm)	3.33 \pm 0.06	25	3.27 \pm 0.07	25	0.60	0.55
% Lipid (pulp)*	1.02	2	0.79	2	–	–
% Protein (pulp)*	2.97	3	2.28	3	–	–
% Sugar (pulp)*	48.2	3	44.4	3	–	–
% Water (pulp)	65	3	69	3	–	–
Macroelements (p.p.m.)*						
N	0.310	3	0.313	3	–	–
P	0.043	3	0.035	3	–	–
K	0.750	3	0.643	3	–	–
Ca	0.274	3	0.287	3	–	–
Mg	0.131	3	0.132	3	–	–
Na	0.192	3	0.087	3	–	–
Oligoelements (p.p.m.)*						
Cu	3.5	3	4.0	3	–	–
Fe	32	3	28	3	–	–
Mn	9	3	6	3	–	–
Zn	7	3	6	3	–	–

*Calculated from dry mass.

Table 2 Measurements on seedling growth (mean \pm SE) of the two seed size categories for each treatment

Treatment	Trait	All seeds	Seeds > 3.5 mm long
Control white morph	Seedling length	7.09 \pm 0.49	8.90 \pm 0.63*
	No. of leaves	48 \pm 6.15	78.50 \pm 13.73*
Control blue morph	Seedling length	8.39 \pm 0.52	10.57 \pm 1.96
	No. of leaves	80.57 \pm 12.73	76.29 \pm 13.35
Ingested by birds	Seedling length	7.01 \pm 0.49	9.91 \pm 0.70***
	No. of leaves	53.45 \pm 6.13	85.75 \pm 7.67***
Ingested by pine martens	Seedling length	7.71 \pm 0.49	9.13 \pm 0.95
	No. of leaves	66 \pm 8.62	1102.33 \pm 15.42(*)

* $P < 0.05$; *** $P < 0.01$; marginal significance is indicated by (*) ($P = 0.08$).

STUDY SITE

We examined the myrtle population found at sea level, at Port des Canonge, north-western Mallorca. The dominant species in the area are *Pinus halepensis*, *Pistacia lentiscus*, *Phillyrea* spp., *Olea europea*, *Cistus monspeliensis* and *Myrtus communis*. Myrtle shrubs grow mainly along the edges of the two torrents that flow into the sea. The blue morph is more common, but here is only twice as frequent as the white morph (81 blue individuals vs. 44 white individuals were recorded in fruit in 1998). In other populations within the island, the white morph is rarer (A. Traveset, personal observation).

FRUIT REMOVAL EXPERIMENTS

On 19 November 1997, we haphazardly selected 10 blue and eight white fruiting plants. For each individual, we marked four branches and counted all the fruits on them. Fruit removal was periodically monitored until 16 January 1998, when most berries had been removed or had dried on the branches. The same individuals plus another two of the white morph were monitored again from 10 November 1998. This time plants bore berries for a longer period and fruit removal was monitored until 2 February 1999.

We evaluated the species composition of avian frugivores over 5 days during each fruiting period (a total of 14 h 30 min of observation in 1997 and 9 h 15 min in 1998), recording the presence of any birds known to consume fleshy fruits. We observed a total of eight such species (in order of abundance: *Erithacus rubecula*, *Sylvia atricapilla*, *S. melanocephala*, *Phoenicurus ochruros*, *Parus major*, *Turdus merula*, *Fringilla coelebs* and *Ficedula hypoleuca*). Of these species, only *E. rubecula*, the two *Sylvia* species and *T. merula* regularly include fruits in their diet. The dense foliage made it very difficult to observe birds removing berries directly and our estimates of 'colour preferences' are therefore based on the removal rates by the whole assemblage of birds. We consider that this is unlikely to obscure species-specific preferences. Data from captive birds (*S. melanocephala* and *T. merula*) (A. Traveset & R. E. Mas, unpublished

data) and findings of other studies on fruit colour preferences by birds (e.g. Willson & Comet 1993; Willson 1994; Traveset & Willson 1998) indicate that variation in fruit choice is greater between individuals of the same species than among species, being usually associated with the previous experience a given individual has had with a particular colour. The four principal species are territorial and presumably have similar patterns of seed deposition.

SEED GERMINATION EXPERIMENTS

Germination chamber studies

In the first year we compared germination under controlled conditions. Seeds were extracted from randomly chosen blue and white berries collected from at least 10 different individuals of each morph. Seeds that had been ingested by birds (of unknown morph, as they are then indistinguishable) were gathered from bird scats. On 12 February 1998, we placed 200 seeds of each morph and 200 ingested seeds in Petri dishes (25 seeds per dish) in a germination chamber, at 25 °C and with a photoperiod of 12 h (conditions known to be optimum for germination of this species; Mitrakos 1981). Seeds were soaked for 10 min in sodium hypochloride (a common pre-treatment used to avoid infection by fungi), before transfer to filter paper saturated with distilled water. Dishes were monitored every 1–3 days and germinated seeds (i.e. those from which the radicle had emerged) were removed until 6 April, when no new seeds had germinated for 2 weeks. At the end of the experiment, viability of the remaining seeds was examined by opening them under the dissecting microscope to see whether they still had a white endosperm or if they had been aborted (with a dark and shrunken endosperm).

We repeated this test in 1999 with seeds ingested by pine martens as a fourth treatment. Faeces, which were composed mainly of fruit pulp and myrtle seeds, were rinsed and passed through a sieve to extract the seeds. Seeds from the other treatments were also rinsed and allowed to dry at air temperature for 2–3 days before placing 100 seeds of each treatment in Petri dishes (20

seeds per dish). Germination was monitored from 1 February until 16 June, when no more seeds had emerged for *c.* a month. This time we did not sterilize the seeds; instead, we periodically sprayed fungicide (COMPO, BASF) into the dishes to enable long-term control of infection.

In order to test whether the presence of pulp inhibits germination, on 14 January 2000 we placed 10 fruits of each morph in each of 10 Petri dishes under the same conditions as before. We cut berries in half, placed the flat surface upwards, and monitored germination periodically until 5 May. We sprayed fungicide and removed fungal hyphae as soon as they appeared in the dish. Seeds were removed as they germinated, and we counted the total number of seeds tested at the end of the experiment to obtain germination percentage.

Greenhouse and experimental garden conditions

Differences in seed germination rate among the four treatments (seeds ingested by birds, by marten, and the two controls) were examined by planting seeds (200 seeds per treatment; five seeds per pot; diameter of each pot was 5.5 cm) in potting soil (horticultural mixture) and placing half of the pots in a greenhouse (with an average temperature of 20 °C) and the other half outdoors, in an experimental garden, where the average temperature ranged from 14 °C (April) to 30 °C (August). We placed 20 pots per treatment, randomly positioned within trays, in each of the two conditions, on 29 January 1999. All pots were watered every 2 or 3 days during the experiment.

After observing considerable differences in seed size (see Fig. 1), we further wanted to investigate how this trait affects germination performance and/or seedling growth. We thus selected, from the remaining pool of seeds collected for each treatment, a total of 50 from among the largest (all longer than 3.5 mm). These seeds were significantly heavier (mean = 13 mg; $n = 25$) than seeds selected at random (7 mg; $n = 50$) ($t = 15.6$, $P < 0.001$). On 4 February, five seeds per pot (i.e. 10 pots per treatment) were planted, placed in a tray in the experimental garden and watered uniformly as above.

SEEDLING GROWTH MEASURES

Seedling growth rates were compared among treatments, both in the greenhouse and outdoors. In the greenhouse, we began measuring seedling size (stem length and number of leaves) on 12 April 1999, and then every 15–20 days until 4 June. In the garden, seeds germinated much later and we measured growth from 15 June to 24 September. At the end of each experiment we randomly chose 10 seedlings per treatment and measured dry weight of the aerial part (by drying them in an oven at 60 °C for 76 h).

All those seedlings that died within the period of observation, including those seeds that died a few days

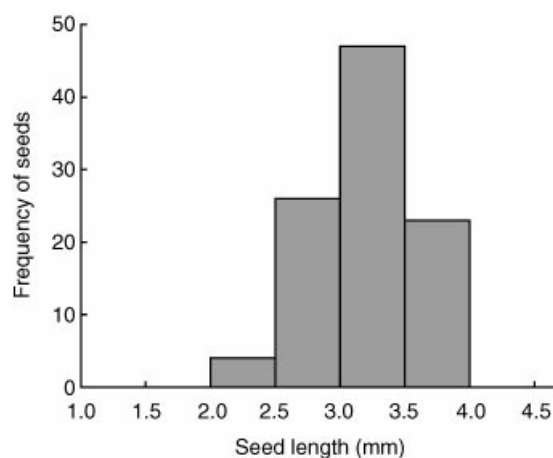


Fig. 1 Frequency distribution of seed size (of the two morphs together) of *Myrtus communis* in the study site. $n = 75$.

after germinating, were discarded from the analyses. A few seedlings appeared to be of the rare 'microphylla' form (Bonafé 1979), which grows shorter but bears more abundant, smaller leaves than the normal myrtle; these seedlings were also excluded from the analyses.

DATA ANALYSES

The proportions of fruits removed in the field as well as the seed germination and seedling growth curves were analysed by performing repeated-measures analyses of variance. Each dish or pot was considered as a replicate, as all tested seeds were selected at random from the whole pool of seeds collected from many different individuals. The angular (arc-sin square root) transformation was used to normalize the proportions. Since the number of seedlings growing per pot varied among pots, and some pots had none, the analyses of seedling measurements were done on an individual basis. These data were transformed either logarithmically (for seedling length measurements) or with the square root (for leaf counts). An analysis of covariance tested the effect of seedling density in pots on growth, using day of seed germination as the covariate; this test was done after checking for homogeneity of slopes. Seedling mortality was compared among treatments by means of the Kruskal–Wallis test, as data could not be normalized with any transformation. All means are accompanied by their standard errors unless otherwise indicated.

Results

FRUIT REMOVAL

The removal rate of fruits in the field was similar between morphs, in both 1998 ($F = 1.37$, d.f. = 1, 16, $P = 0.26$) and 1999 ($F = 2.21$, d.f. = 1, 16, $P = 0.16$). Both years showed a significant time effect but the time–morph interaction was not significant (Fig. 2). Plants were depleted of fruits much earlier in 1998 than

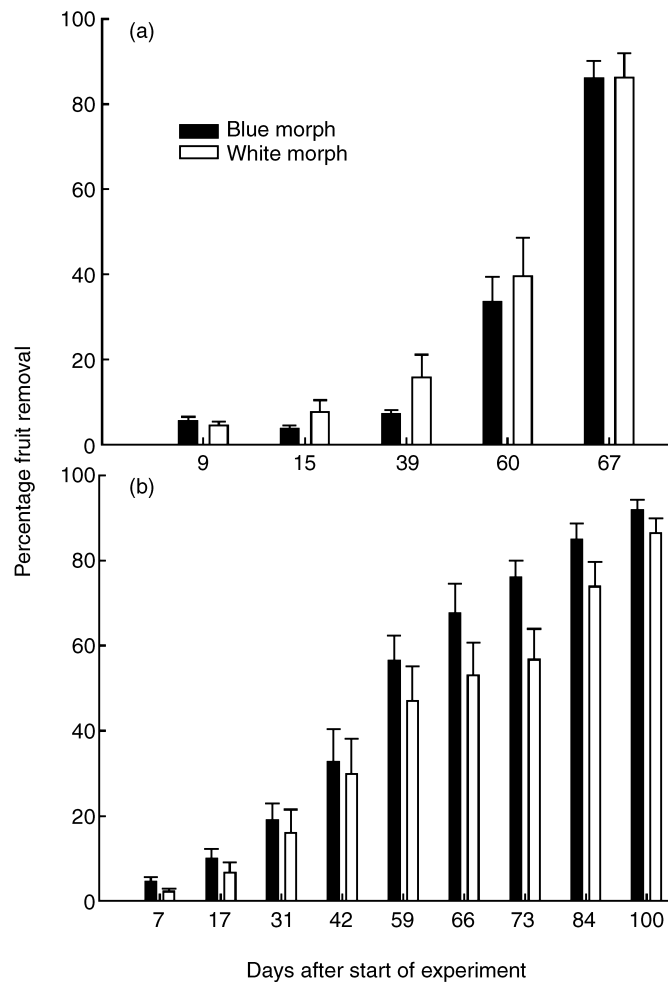


Fig. 2 Avian fruit removal observed in the field for the two morphs during (a) 1997–98 season and (b) 1998–99. Bars represent standard errors.

the following year. Bird abundance appeared similar and we attribute the difference to the apparently greater fruit production of *Myrtus communis* in 1999, although it is also possible that less fruit of other species was available in 1998. The fact that in our study area bird faeces with myrtle seeds were much more abundant than marten faeces suggests that birds were responsible for most of the fruit removal in our labelled branches. Furthermore, martens presumably gather fruits primarily from the lowest branches, which formed only a proportion of our sample.

SEED GERMINATION

In the germination chamber, seeds of the two morphs emerged at similar rates (Fig. 3). Moreover, seed ingestion by either birds or pine martens did not affect germination success (1998: $F = 1.35$, d.f. = 2, 21, $P = 0.28$; 1999: $F = 1.41$, d.f. = 3, 16, $P = 0.28$; time–morph interaction not significant). Most seeds of all treatments germinated during the first 10 days, although they continued to emerge for several weeks. Final percentage germination did not differ among treatments in either year, although in 1998 seeds from bird faeces tended to germinate more than non-ingested seeds of the white morph

($F = 2.76$, d.f. = 2, 21, $P = 0.09$; Fig. 3a). Ninety per cent of the seeds that failed to germinate were found not to be viable.

Germination of seeds embedded in fruit pulp was found to begin considerably later than ‘clean’ seeds (18 ± 6 (SD) vs. 7 ± 1 days). Seeds of the two morphs did not differ in germination rate ($F = 0.36$, d.f. = 1, 16, $P = 0.56$; time–morph interaction not significant) and final percentage germination was similar between blue (18.78 ± 2.17 ; $n = 9$) and white berries (17.33 ± 1.78 ; $n = 9$), much lower than the figures observed for seeds clean of pulp (Fig. 3), which suggests that pulp contains a compound that inhibits germination, as found in a number of species (e.g. Izhaki & Safriel 1990; Barnea *et al.* 1991; Ladley & Kelly 1996).

Our results in the greenhouse were consistent with those found in the chamber. Neither fruit morph nor seed ingestion by animals appeared to affect germination patterns ($F = 0.07$, d.f. = 3, 76, $P = 0.97$; Fig. 4). Under these conditions, seeds germinated over a period of about 2 months, although mostly during the first 10 days after planting. Final percentage germination was not significantly different among treatments ($F = 0.35$, d.f. = 3, 76, $P = 0.79$), ranging from 67% to 73%.

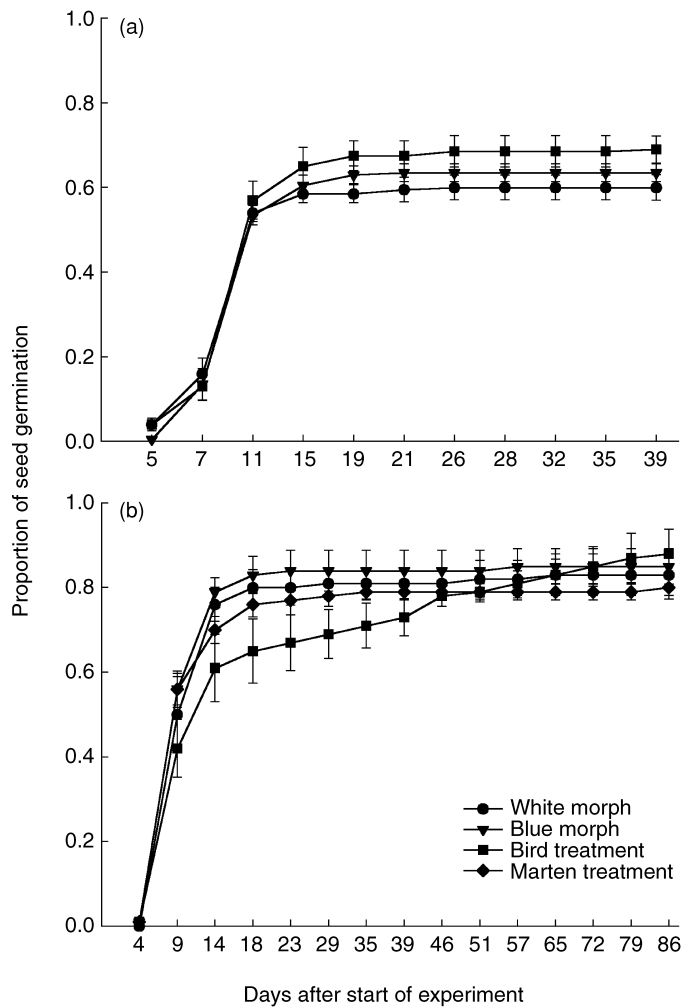


Fig. 3 Proportion of seed germination in the growth chamber (at 25 °C and 12 h photoperiod) for the two morphs and for unclassified seeds ingested by birds or martens. Data from (a) 1998 and (b) 1999.

In the experimental garden, the rate at which seeds germinated was much lower than under controlled conditions, either in the chamber or in the greenhouse (Fig. 5). Two and a half months after setting up the experiment, only 17.5% of seeds had emerged. Seeds from all treatments began germinating simultaneously *c.* 68–70 days after planting but stopped by mid-June, after which no new emergences were recorded, even though we continued monitoring during the autumn. In contrast to the results obtained in controlled conditions, we found significant differences among treatments ($F = 3.08$, d.f. = 3, 76, $P = 0.03$, as well as significant time effect and a significant time \times treatment effect). The differences, however, were not among morphs (although the blue morph began germinating at a slightly faster rate than the white one and T_{50} , the time by which half of the final germination had taken place, was earlier, day 68 vs. day 89), but between seeds ingested by birds and those ingested by martens or not ingested, and were detected from about a month after initial germination (Tukey's test) (Fig. 5a). Final percentage germination was significantly greater for the bird treatment than for the other treatments ($F = 6.62$, d.f. = 3, 76, $P < 0.0001$).

Surprisingly, the effect of ingestion by birds disappeared when only large seeds were considered ($F = 0.56$, d.f. = 3, 36, $P = 0.65$; Fig. 5b). Final percentage germination in this case was 23% on average, and, although it tended to be greatest for the bird treatment, the difference was not significant ($F = 0.55$, d.f. = 3, 36, $P = 0.65$; Fig. 5b). Although they began emerging at about the same time as randomly selected seeds, these larger seeds germinated more slowly during the first 2 weeks.

SEEDLING GROWTH

In the greenhouse we found significant differences among treatments in seedling growth rates with regard to both length and number of leaves ($F = 2.86$, d.f. = 3, 210, $P = 0.04$ and $F = 3.54$, d.f. = 3, 210, $P = 0.02$, respectively, but no time–treatment interaction), controlling for date of seed germination. There were no differences between morphs, but seedlings from seeds ingested by birds grew at a significantly slower rate than those of the marten treatment or the blue morph (Tukey's test; Fig. 6). At the end of the experiment, dry weight of seedlings was not significantly different among treatments ($F = 0.29$, d.f. = 3, 36, $P = 0.83$), being

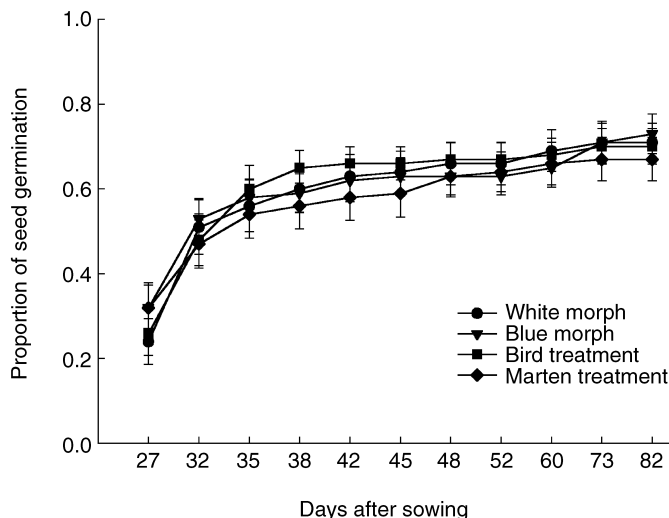


Fig. 4 Proportion of seeds of each morph and of the two frugivores treatments that germinated in the greenhouse in 1999.

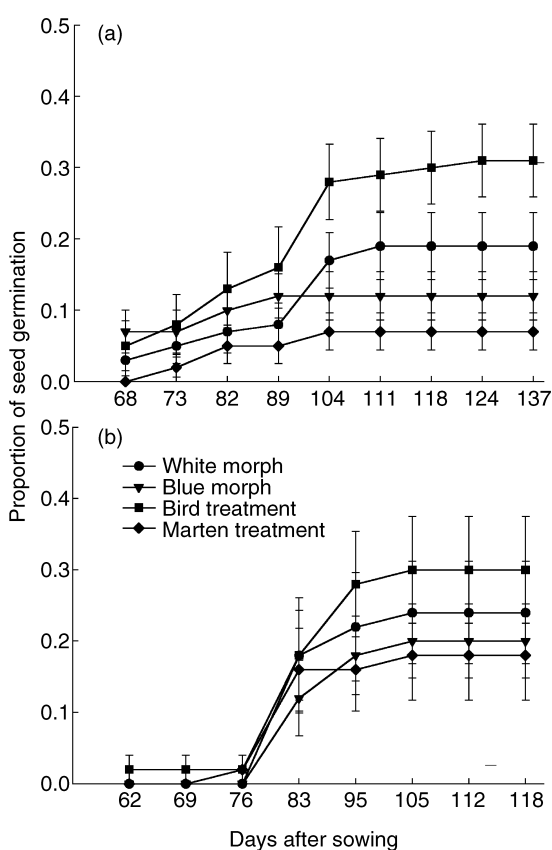


Fig. 5 Proportion of seeds of each morph and of the two frugivores treatments that germinated in the experimental garden for (a) seeds of all sizes and (b) only large seeds (> 3.5 mm).

on average 0.24 ± 0.20 g (SD); seedlings from bird-treated seeds, however, were slightly lighter (0.19 ± 0.16 g) than the rest.

However, such lower growth of 'bird seedlings' was due to a higher seedling density in the pots. Due to the greater germination, the bird treatment had a slightly higher frequency of pots with more than two seedlings (84% of the 20 pots) than the other treatments (53% for the marten treatment, and 65% and 63% for the white

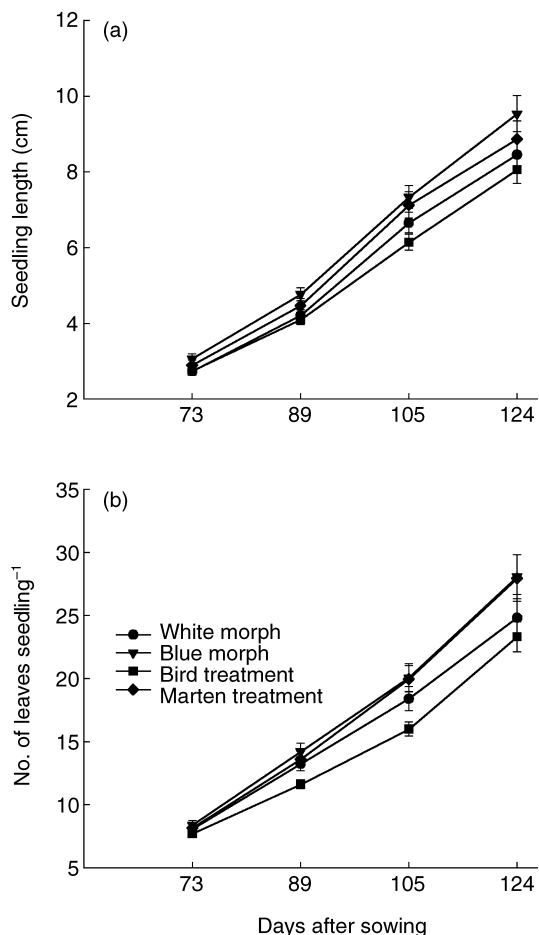


Fig. 6 Growth of germinated seedlings from the two morphs and from seeds ingested by either birds or pine martens in the greenhouse. Only seedlings that survived throughout the period are considered.

and blue morphs, respectively). An effect of seedling density on growth was found when we performed an ANCOVA in which growing period (i.e. time since seed germination) was the covariate. Both seedling length and number of leaves per seedling were greater in those

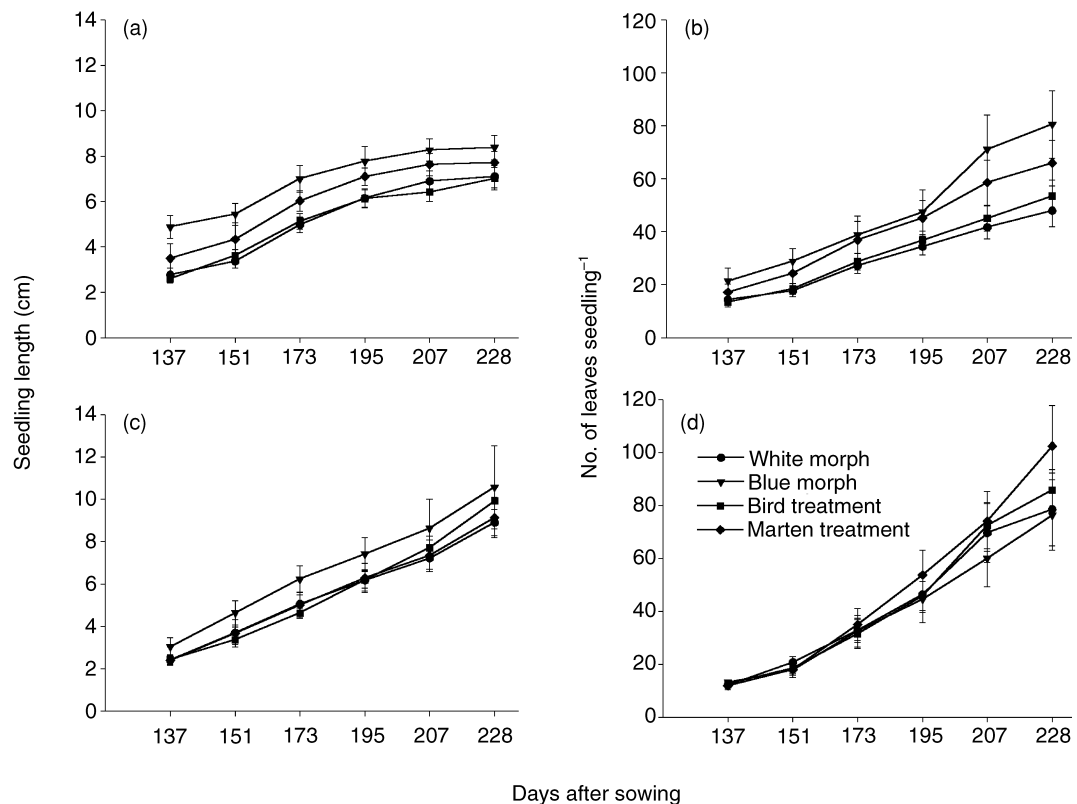


Fig. 7 Seedling growth for the two morphs and seeds ingested by either birds or pine martens in outdoor conditions. Only seedlings that survived throughout the period are considered. a, b: all seed sizes; c, d: only large seeds (> 3.5 mm).

pots with only one or two seedlings (9.83 ± 0.44 cm and 32.73 ± 1.68 leaves; $n = 49$) than in high seedling density pots (8.38 ± 0.24 cm and 23.91 ± 0.91 leaves; $n = 165$) ($F = 8.23$, d.f. = 1, 211, $P = 0.005$ and $F = 21.06$, d.f. = 1, 211, $P < 0.001$). We thus concluded that all seedlings, regardless of treatment, grew at the same rate in the greenhouse.

Seedling mortality, at any stage of growth, in the greenhouse, was relatively low (21.35%) and did not differ significantly among treatments (Kruskal–Wallis test: $\chi^2 = 3.83$, $P = 0.28$).

Outdoors, both seedling length and number of leaves per seedling were found to increase at a similar rate in all treatments ($F = 0.49$, d.f. = 3, 44, $P = 0.69$ and $F = 0.59$, d.f. = 3, 44, $P = 0.63$, respectively), also after controlling for seed germination date. Seedling growth could be measured throughout the period in a total of 48 seedlings, 20 of which belonged to the bird treatment (as this treatment showed the highest germination, see previous section). Here, 38.6% of the germinated seeds died at an early stage, though this mortality occurred irrespective of treatment (Kruskal–Wallis test: $\chi^2 = 3.07$, $P = 0.38$). Seven of the larger seedlings (i.e. longer than 5 cm), of which five were derived from bird-treated seeds, died during the hot months of July and August, when temperatures often reached 37–40 °C in the sun. This loss made ‘bird seedlings’ appear to be smaller than the other treatments during the first half of the growing period (Tukey’s test; Fig. 7a,b). Also during this period, seedlings from the white morph were

shorter and had fewer leaves than those from the blue morph, presumably due to their slightly slower germination of the former morph (Fig. 5a). After mid-August (day 195 after sowing) the differences among treatments became less marked and no longer significant. Dry weight of seedlings at the end of the experiment did not differ among treatments ($F = 1.98$, d.f. = 3, 29, $P = 0.14$), being on average 0.40 ± 0.25 g (SD).

Due to the low germination outdoors, more than half of the pots contained only one seedling and only two pots contained more than two seedlings, and the effect of seedling density need not therefore be considered. For seeds of the blue morph and for seeds ingested by martens, only seven seedlings of each could be measured because of the low germination. Seedlings in the greenhouse conditions appear to invest more in length than in number of leaves than those outdoors, probably due to the lower light availability (compare Figs 6 and 7).

When considering only large seeds, seedlings of the different treatments were also found to grow at the same rate ($F = 0.83$, d.f. = 3, 33, $P = 0.49$ and $F = 0.22$, d.f. = 3, 33, $P = 0.88$, length and number of leaves, respectively; Fig. 7c,d), throughout the measurement period. Seedling density effects on growth were again minimal (never more than two seedlings per pot). Seed and seedling mortality was similar among treatments (Kruskal–Wallis test: $\chi^2 = 4.28$, $P = 0.23$) and averaged 24%. Seed size was positively correlated with seedling growth. We observed significant differences between seedlings derived from all sizes vs. from large seeds (Table 2). For the white

and bird-ingested groups, larger seeds resulted in longer seedlings with more leaves.

Discussion

MAINTENANCE OF THE COLOUR POLYMORPHISM

The only apparent difference between the two morphs of myrtle fruits is colour, and based on the rates of fruit removal in the marked branches, birds did not seem to affect the polymorphism via differences in rates of fruit removal in the marked branches. In general, avian preference for a given colour (of flowers or fruits) has been shown to be labile and variable (e.g. McPherson 1988; Willson *et al.* 1990; Willson & Comet 1993; Puckey *et al.* 1996; Traveset & Willson 1998; Gervais *et al.* 1999) and even the same individual bird can make inconsistent choices when experimentally tested (Stiles 1976; Goldsmith & Goldsmith 1979; Willson & Comet 1993; Traveset & Willson 1998). As well as bearing similar nutrient contents and seed loads, the two fruit morphs require the same handling time from birds, as both are equally likely to be detached from the receptacles once ripe and because of their similar size and shape. The white berries are more conspicuous against the green foliage of the shrubs than the blue, at least to human eyes; however, the greater abundance of the blue morph and the resulting greater bird experience with this colour (found also in mature fleshy fruits of other species such as *Olea* or *Phillyrea*) may well counterbalance such conspicuousness.

Seeds of the two morphs have similar germination rates and final percentage germination, although in outdoor conditions those from blue berries appeared to germinate slightly faster. These results are somewhat inconsistent with those reported for myrtle fruits from Italy by Milia *et al.* (1996), who found a greater germination for the blue (61%) than for the white (46%) morph in a growth chamber but no significant difference outdoors (52 vs. 55%). Germination tests should be performed under a range of conditions and differences between treatments interpreted with caution. The effect of the surrounding pulp is also similar between morphs, lowering germination probably by containing an inhibitor, as found in many other species (e.g. Izhaki & Safriel 1990; Barnea *et al.* 1991; Yagihashi *et al.* 1998, 1999). Frugivores, thus, promote the germination of myrtle seeds simply by separating them from the pulp, regardless of any further effect.

The only two other fruit-colour polymorphic species in which differences in germination behaviour have been examined are *Rhagodia parabolica* (Chenopodiaceae) and *Rubus spectabilis* (Rosaceae). In *R. parabolica*, seeds from the more common (red) morph germinated faster than seeds from a less common (yellow) morph (Willson & O'Dowd 1989). In contrast, no differences between morphs were detected in *R. spectabilis* (Gervais *et al.* 1998; Traveset & Willson 1998), although the type of

soil on which seeds were planted was found to affect the two morphs differently (Traveset & Willson 1998). For both species, seed response to passage through the dispersers' digestive tracts can vary among morphs, which may contribute to the maintenance of the colour polymorphism. In the case of *Myrtus communis*, further experiments with captive animals would be required to determine whether seed ingestion by frugivores, either birds or mammals, has a differential effect on the two morphs.

Myrtle seedlings of the two morphs grew at a similar rate, both in the greenhouse and the experimental garden. However, due to the slightly faster germination of seeds from blue fruit, their seedlings were on average 1–2 cm longer and bore more leaves than those from the white morph, especially outdoors and until mid-August. Such differences in size may well translate into differences in the probability of survival under stressful conditions (for instance, drought or extremely high temperatures). Interestingly, no differences were detected when we compared only large seeds: larger seeds did result in larger seedlings, as found in a great number of empirical studies (Eriksson 1999), irrespective of morph (see Fig. 7). It seems, thus, that the possible differences in seedling growth between genotypes may only be expressed under certain environmental conditions and only in relatively small seeds (admittedly the majority; Fig. 1). Extrapolating to the natural habitat, we could predict that seedlings of the blue morph might eventually outcompete those of the white morph simply because they gain a few days advantage in growth. However, the general similarity of growth rates, germinability and timing of germination, may prevent development of a directional selective force.

EFFECT OF FRUGIVORES ON GERMINATION AND SEEDLING GROWTH

As the faeces of the dispersers were collected in the field, we cannot distinguish between ingested seeds of the two morphs. Even though most of the seeds extracted probably come from blue berries, given their higher abundance, the interpretation of our results is always based on the comparison between ingested seeds, regardless of the morph, and control (non-ingested) seeds of the two morphs separately.

Ingestion by birds accelerated the germination of myrtle seeds, although this was detected only in outdoor conditions. Presumably, the husk of seeds ingested by birds incurs a mild abrasion that enhances water imbibition and the perception of germination cues under field conditions, as reported by Barnea *et al.* (1991). In the more favourable conditions of the greenhouse, the lack of any effect of ingestion by birds confirms that interpretation of germination tests can vary greatly depending on the conditions under which they are carried out (see also Bustamaute *et al.* 1992; Figueiredo *et al.* 1995; Figueiredo & Perin 1995, for different results between laboratory and field). Factors such as

temperature and degree of humidity (as here), as well as chemical properties of the soil may be relevant (Traveset 1998; Traveset & Verdú 2001).

In contrast to the bird treatment, the passage of myrtle seeds by pine marten did not enhance germination. Longer retention in the pine marten gut might actually be detrimental to seeds so that an unknown fraction of seeds in marten faeces might look intact but not be viable. Viability tests should be performed prior to germination tests in future studies. Our results support those reported by Aronne & Russo *et al.* (1997), who examined the effect of ingestion by several *Martes* spp. and by the red fox (*Vulpes vulpes*) on the germination of myrtle seeds in Italy and found no effect of the 'marten' treatment, but a significantly faster germination of seeds that had passed through foxes compared with those extracted from fruits. Differences in seed responses of the same species to ingestion by different frugivores have been found in a variety of plants (Traveset 1998; Traveset *et al.* in press). Seeds often respond differently even when frugivores belong to the same family, which implies that other factors, such as the quality of other food (e.g. its degree of acidity) may affect the structure of the seed coat either physically or chemically, or both. Future research on the chemical composition of ingested food, such as that initiated by Murray *et al.* (1994) and Witmer (1996), and its effect on both seed retention time in the gut and seed coat properties, will certainly shed light on this topic.

Seed size affected the response to passage through avian frugivores. The germination of large seeds ingested by birds was not accelerated compared with controls, in contrast to the effect when all seeds were considered. The size and weight of a seed usually determines the velocity at which it passes through the digestive tract of a bird, with large heavy seeds being defaecated sooner than small light ones (Levey & Grajal 1991; Gardener *et al.* 1993). Large seeds ingested by birds may therefore have remained in their guts for a shorter period so that their coat was less abraded, but the greater amount of resources in large seeds might simply override any effect of ingestion. A positive correlation between seed mass and seedling size has been demonstrated in a number of studies (e.g. Stanton 1984; Wulff 1986; Zhang & Maun 1990; Hendrix *et al.* 1991; Eriksson 1999; Paz *et al.* 1999). We too found that large seeds not only had a slightly higher probability of germination than smaller seeds (23% vs. 17.5%, respectively), but also that their seedlings suffered a lower mortality (24% vs. 39%, respectively) and grew bigger (see Fig. 7c,d). The frequency of large seeds in the population is, however, rather low (Fig. 1).

The few studies that have examined the effect of seed passage through animals on seedling growth (e.g. Dinerstein & Wemmer 1988; Quinn *et al.* 1994; Ocumpaugh *et al.* 1996; Paulsen 1998; Malo & Suárez 1999; Traveset *et al.* 2001) show that seedlings emerging from ingested seeds tend to be more vigorous than controls (but see, for instance, Miller 1995 and Izhaki &

Ne'eman 1997). In most cases, however, the reason is a positive effect of the 'nutritious' faecal material remaining at the time of seed germination (especially in the case of mammal faeces, as bird faeces usually decompose and are washed off in a few days or weeks) and not the actual passage of seeds through the animal's gut. The only study, as far as we know, that examines the effect of seed passage through birds on seedling vigour is that of Paulsen (1998 and unpublished data) who argues that less energy is needed at germination to crack open the coat of ingested seeds than of controls and more resources therefore remain for late growth and leaf development. Although this hypothesis is quite plausible (and testable), we need also to consider the extrinsic factors, such as the physical conditions under which seedlings grow, since these may mask any possible effect of bird ingestion. In our case, for instance, the greater final germination of seeds ingested by birds translated into a larger number of seedlings but not into bigger seedlings, probably due to the stressful conditions in summer.

Myrtus communis seeds have no apparent dormancy, and we could thus predict that fast germination and high germinability are advantageous, in order to maximize reproductive success and seedling recruitment (Harper 1977; Jones *et al.* 1997). However, our experimental garden results show that a high rate of germination, as observed for bird-ingested seeds, does not guarantee a greater probability of survival under unfavourable conditions. We found that mortality was not restricted to the youngest and smallest individuals, with relatively large (> 5 cm) seedlings dying during the hot months of the summer. In the field, seedling mortality can even be greater if rains are scarce and thus water is limiting. Other ecological factors, such as post-dispersal seed predation or seedling herbivory, may also counter-balance any positive effect of birds on germination and seedling growth, and it would thus be necessary to study the seed to seedling stage in natural conditions in order to elucidate the consequences of frugivory on plant reproduction.

In conclusion, on an ecological time-scale, the absence of a colour preference on the part of birds (and similar germinability and growth of the two morphs) makes it less likely that the white morphs will go extinct due to drift than if there were a colour preference. Fruit consumption by birds may well increase germinability as well as promoting dispersal and removing pulp-borne inhibitors. The effects of microhabitat on subsequent growth and survival, and possible associations with different morphs, remain to be studied.

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