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Germination patterns throughout an insular altitudinal gradient: The case of the Macaronesian endemic plant *Rubia fruticosa* Ait. (Rubiaceae) in El Hierro (Canary Islands)

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Abstract

Seed germination percentage and rate of *Rubia fruticosa*, an endemic Macaronesian shrub was assessed with seed lots collected at three levels of an altitudinal gradient (200–800 m asl) located in El Hierro Island (Canarian Archipelago). Both seeds collected from control plants and those found in lizard droppings were studied. A significant decrease in the germination percentage was observed with control seeds and those seeds which are previously eaten by lizards if collected at 800 m asl. This indicates that *R. fruticosa* is a plant whose optimal germination environment is located between 200 and 500 m asl, occurring in higher altitudes only under sub-optimal conditions. The effect of lizard gut on *R. fruticosa* seeds was variable in the two study years: while germination percentage of seeds having passed through the lizards was not significantly different to that of control seeds in the first year, an increased germination was found for the animal-treated seeds in all three altitudinal zones in the second year. In general, germination rate of control seeds was rather variable between years. However, improvement of *R. fruticosa* seed germination caused by lizards may be important for its fitness; and a co-evolution may have occurred, since lizards and seeds of this endemic plant have been intensively interacting for millions of years in the lower zones of the Canary Islands.
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Introduction

Germination capability and rate (speed of germination) are two of the most important factors that affect the recruitment of a given plant population and species (Baskin and Baskin, 1998; Kitijama and Fenner, 2000). In addition to the seed germination percentage, the time at which a seed germinates can also determine its future success (Karlsson and Milberg, 2008; Verdú and Traveset, 2004; Weaver and Cavers, 1979) and has important consequences for the evolution of plant life histories (Donohue et al., 2005a). Geber and Griffen (2003) have

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found that early germination of seeds is a heritable trait that is subjected to the strong selection pressure and is mediated by competitive interactions. In this respect, Donohue et al. (2005b) have recently demonstrated that some annual weeds, such as *Arabidopsis thaliana* (Brassicaceae), are capable of rapidly evolving an appropriate germination response in new environments, facilitating their colonisation across a broad geographic range. Therefore, the rate at which germination timing evolves may strongly influence the speed at which a species can expand or shift their geographic range (Donohue et al., 2005c).

The seed germination rate is an important trait in plants adapted to xeric environments, where subsequent fitness directly depends on the arrival of rains and the rapid germination of seeds (Gutterman, 1993; Whitford, 2002). Seed germination in plants from arid zones tends to be faster than in other species which inhabit environments with high water availability (Flores and Jurado, 1998; Jurado et al., 2000; Meyer and Monsen, 1992). Evaluation of the germination timing is especially interesting in steep oceanic islands where plant species can be found at different altitudes in quite various environments. Research in these places can determine if natural selection pressures have modulated differences on the germination percentage and the rate of conspecific plants which inhabit different altitudinal zones and are subjected to a different strong climatic gradient.

Another ecological factor that influences early germination in some plants, such as *R. fruticosa*, the object of the present study (Nogales et al., 2006), is the intervention of frugivorous vertebrates which modify the duration of seed dormancy (Izhaki and Safriel, 1990; Traveset et al., 2001). An asynchrony in germination caused by different disperser types has been interpreted as an adaptation to geographical zones where rain is unpredictable (in timing and quantity), as occurs in some habitats of the Mediterranean Region (Giménez-Benavides et al., 2005; Izhaki and Safriel, 1990; Traveset et al., 2001) and in lowland zones of subtropical oceanic Atlantic islands (Nogales et al., 2006). Harper (1977) noted that this asynchrony guarantees that seedling mortality is spread over a long period, thus providing a higher probability of success for some seedlings.

This study aimed to evaluate the seed germination percentage and the rate of *R. fruticosa*, an endemic Macaronesian shrub plant, under controlled, but semi-natural conditions. Furthermore, it was assessed whether one of the most important short-distance disperser animals there, the Canarian endemic lizard *Gallotia caesaris*, produces asynchronies in germination rate with respect to seeds from control plants. For this purpose a lot of dispersed seeds were extracted from lizard droppings collected at different altitudinal zones and tested for germination.

Study areas and methods

The Island of El Hierro and the plant species

The Canarian Archipelago is situated in the Atlantic Ocean some 100 km northwest off the African continent. The volcanic island of El Hierro is located in the south-western extreme of the Canaries, lying between 17°52′–18°09′ W and 27°38′–27°51′ N. This island measures 278 km^2 and has a maximum altitude of 1500 m asl, with abundant steep slopes. Therefore, a strong altitudinal climatic gradient exists, both in rainfall and temperature. El Hierro is the most oceanic island of the archipelago and geologically is the youngest (less than 1 My; Fuster et al., 1993). Vegetation cover of natural and semi-natural areas is characterised by forest habitats (15.6% of the island's land area), while mixed habitats occupy 7.1% of the land, and the remaining 70.8% is comprised by open habitats. The latter habitats are characterised by xerophytic shrubs at the lowest altitude (mainly consisting of *Euphorbia obtusifolia*, *Kleinia neriifolia* and *Rubia fruticosa*), and by agriculture and grazing activity at middle altitudes (400–800 m asl). Forested areas include groves of the endemic *Pinus canariensis*, *Juniperus turbinata* and laurel forest (see Pérez de Paz et al., 1981; Santos, 1980).

R. fruticosa Aiton is an endemic plant distributed in the lowlands of the central archipelagos of Macaronesia (Madeira, Salvages and Canaries). It inhabits all of the main Canary Islands, and can reach about 0.5 m^2 of plant cover and half a meter in height. Fleshy fruits are spherical, translucent berries with an average length of 7.5 mm, a diameter of 5.6 mm an average fresh weight of 37 mg, and a typical water content of approximately 80%. The mean number of spherical seeds per fruit is 1.4 and the average seed weight is 8.2 mg (*n*: 40 fruits; Nogales et al., 1999). Seeds from this plant show a relatively high proportion of germination and dormancy is scarce. Fruits of *R. fruticosa* are consumed by several vertebrates, including native lizards and birds, and introduced non-native mammals (Nogales et al., 2005). According to the data obtained in the latter contribution, while lizards and passerine birds are legitimate seed dispersers, introduced mammals clearly reduce seed viability and germination of *R. fruticosa*. Furthermore, two long-distance dispersers also take part in the seed dispersal system of this species: the Raven *Corvus corax* (Nogales et al., 1999), and the Gull *Larus michahellis* (Nogales et al., 2001). These two species have important populations in El Hierro, which move frequently between altitudes and transport a relatively high number of seeds produced by *R. fruticosa* (M. Nogales, pers. obs.).

Study areas and procedures

Study areas of the present work are located in a continuous patch of *R. fruticosa* located in the northeast

of the island (Timijiraque–Isora) that reaches from about 200 to 800 m asl (in a longitudinal distance of only 3 km). The climate is variable in altitude, ranging between 100 and 200 mm/year and approximately 22 °C at 200 m asl (Timijiraque) to 400–500 mm/year and about 17 °C at 800 m asl (Isora) (Marzol Jaén, 2000).

To assess the germination percentage and the rate, we collected seeds from both control plants and lizard droppings in three different altitudinal subpopulations along linear transects of 300 m at 200, 500 and 800 m asl, respectively. A total of 250 fruits were directly taken from plants ($n \approx 70$) and approximately 200 fresh lizard droppings were collected in each locality during the springs of 2005 and 2006. In each locality, this material was collected from a surface area of 1000 m^2 in order to minimise the plant's mother effect, to evaluate the real consequences of the lizard gut effect on the germination of seeds. Seeds from lizard droppings were manually extracted in dry conditions and were then counted. We separated visually undamaged seeds using a stereomicroscope (10 × magnification). We performed a germination experiment in a greenhouse over a period of 5 months during each year (1 October 2005–28 February 2006 and 1 October 2006–28 February 2007), following the recommendations of Traveset and Verdú (2002). We selected these dates because they coincide with the plant's natural germination process in nature. We used a total of 170 seeds in 2005 and 230 seeds in 2006 for both treatments (control and seeds from lizard droppings, respectively) and for each altitudinal zone. Each seed was planted independently in a 4 cm^2 pot (n: 230 pots per batch) using a standard substrate (25% turf, 25% ravine sand and 50% culture soil) and each seed was buried about 5 mm deep. The experiment was performed at Tagoro (Tenerife Island; 300 m asl) where the night–day period was similar to that found in the study areas. All pots were watered every two days. Germination was defined as the emergence of any seedling part from the soil surface. Data were monitored daily and Kolmogorov–Smirnov tests were employed to evaluate differences in the germination rate between the three study areas different in altitudes. To avoid a possible bias in calculating germination rate, caused by the different percentage of seed germination between altitudinal areas and experimental years, only those data that comprised the period from the first seedling emerged (≈ 15 days) until the germination curve was first established (≈ 35 days) were considered.

Results and discussion

After 5 months of the germination experiment, a significant decreased germination percentage of control seeds, collected at 800 m asl, was observed in both years (2005: 73.5%, 2006: 29.1%) with respect to the other two altitudinal zones, 200 m asl (2005: 84.1%, 2006: 36.5%) and 500 m asl (2005: 87.6%, 2006: 80.9%) (2005, G_2: 12.0, p: 0.012; 2006, G_2: 152.1, $p < 0.001$). A similar decreased pattern of germination of those seeds extracted from lizard droppings at 800 m asl was observed both in 2005 (76.5% vs. 85.9% at 200 m asl and 80.6% at 500 m asl) (G_2: 4.87, p: 0.027) and 2006 (43.2% vs. 71.8% at 200 m asl and 67.8 at 500 m asl) (G_2: 40.6, $p < 0.001$). These data seem to indicate that *R. fruticosa* is a plant that optimally germinates between 200 and 500 m asl and is found in sub-optimal conditions at higher altitudes. This conforms to the view that species perform better in the centre of their altitudinal range (lowlands in the case of *R. fruticosa*) than at the boundaries (Giménez-Benavides et al., 2007: *Silene ciliata* from Mediterranean high mountains).

It is important to consider that the vegetation of the Island of El Hierro has suffered strong changes during the last 500 years after the conquest of the islands by the Europeans and the spread of their associated fauna (Pérez de Paz et al., 1981). A large part of the former forests, probably comprised of *J. turbinata* (Cupressaceae), which occupied the middle zones of the island (≈ 300–700 m asl) (Santos, 1980), were cleared for agriculture, pasture, firewood and timber harvest. Remains of this forest still persist in the zone, currently represented by isolated trees. Furthermore, the effects of large alien herbivores, such as goats, sheep or cattle, over the centuries, have also helped to provoke a substantial impoverishment of the vegetation. These important changes in the original vegetation of El Hierro have probably caused the disappearance of the strong-structured belt vegetation according to altitude and orientation, which is typical for the Canarian climax vegetation (see Ceballos and Ortuño, 1951). Probably as a response to these strong environmental changes, some plants such as *R. fruticosa* that commonly occupy in the lowland zones, were able to reach higher altitudes. In this respect, it is also interesting to note that the first human settlement (the "Bimbaches"), also brought goats, sheep and pigs to the islands, more than 2000 years ago (Navarro et al., 1990), which gives us an idea that some vegetation changes must have occurred since this time. Therefore, although *R. fruticosa* can colonise relatively high-altitudinal open environments, these populations seem to show some limitations in important biological factors such as germination.

When data were analysed comparing altitudinal provenances and seed collection years germination of control seeds did not present a clear pattern between the 2 years. In 2005 no significant differences were found in the three altitudinal populations, while in 2006, we found such differences between all of them (Table 1). Therefore, the germination rate does not seem to be a consistent genetic variable trait in the different

Table 1. Results of germination rates (Kolmogorov–Smirnov tests) obtained on both seed treatments (control and seeds from lizard droppings) and years in the different altitudinal zones.

| Compared treatments	Z	p
2005		
Control 200 m asl vs. Control 500 m asl	0.77	0.59
Control 200 m asl vs. Control 800 m asl	0.92	0.35
Control 500 m asl vs. Control 800 m asl	0.77	0.59
Gallotia 200 m asl vs. Gallotia 500 m asl	0.61	0.84
Gallotia 200 m asl vs. Gallotia 800 m asl	0.46	0.98
Gallotia 500 m asl vs. Gallotia 800 m asl	0.30	1.00
Control 200 m asl vs. Gallotia 200 m asl	1.69	0.006[a]
Control 500 m asl vs. Gallotia 500 m asl	0.61	0.84
Control 800 m asl vs. Gallotia 800 m asl	1.08	0.19
2006		
Control 200 m asl vs. Control 500 m asl	2.00	0.001[a]
Control 200 m asl vs. Control 800 m asl	2.16	<0.001[a]
Control 500 m asl vs. Control 800 m asl	2.93	<0.001[a]
Gallotia 200 m asl vs. Gallotia 500 m asl	0.92	0.35
Gallotia 200 m asl vs. Gallotia 800 m asl	1.85	0.002[a]
Gallotia 500 m asl vs. Gallotia 800 m asl	1.85	0.002[a]
Control 200 m asl vs. Gallotia 200 m asl	1.54	0.017[a]
Control 500 m asl vs. Gallotia 500 m asl	1.38	0.042[a]
Control 800 m asl vs. Gallotia 800 m asl	1.85	0.002[a]

[a]Indicates significant differences, considering the Bonferroni correction for avoiding differences by chance.

altitudinal populations. Perhaps as previously mentioned, the presence of *R. fruticosa* in high-altitude environments could be relatively recent and there has not been adequate time for evolutionary events to occur. This contrasts with data obtained in some annual weeds which are capable of evolving rapidly to an appropriate germination response in novel environments (Donohue et al., 2005b). However, it is important to consider that the evolutionary rate is slower in shrub plants than in weeds (Rice and Emery, 2003). Another factor that does not suggest that seed germination is a variable trait in the different populations of *R. fruticosa* in El Hierro, is the existence of important populations of long-distance dispersers, such as ravens and gulls, which move a substantial number of seeds between different altitudinal zones (Nogales et al., 1999; Nogales pers. obs.). This moving of seeds does not favour the isolation of plant populations and the adaptive divergence of life-history traits.

When the germination rate of control seeds and those found in lizard droppings were compared, an important differential pattern was appreciated between years. While in the experiments performed in 2005–2006, seed passage through lizards increased the germination rate significantly only at the lowest altitude (200 m asl), in the experiment of 2006–2007 such a fate of seeds led to an increased germination rate at the three altitudes. Furthermore, considering all data of the three altitudinal
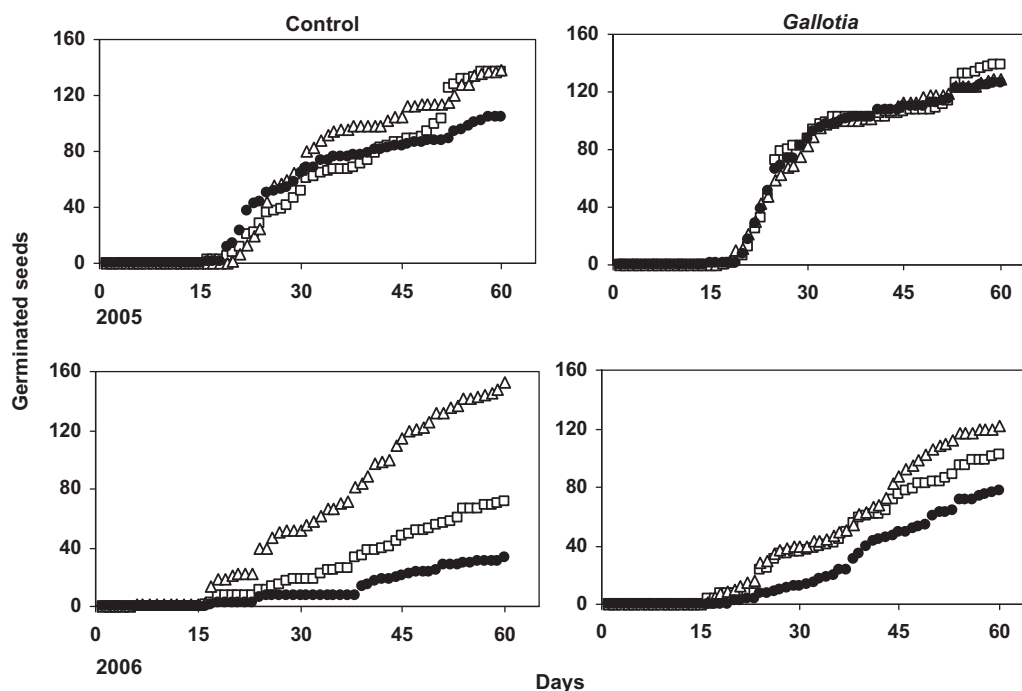
Fig. 1. Germination rate obtained in each treatment (control and lizard droppings) in the different altitudinal zones of El Hierro in 2005 and 2006. White squares: 200 m asl, white triangles: 500 m asl and black circles: 800 m asl
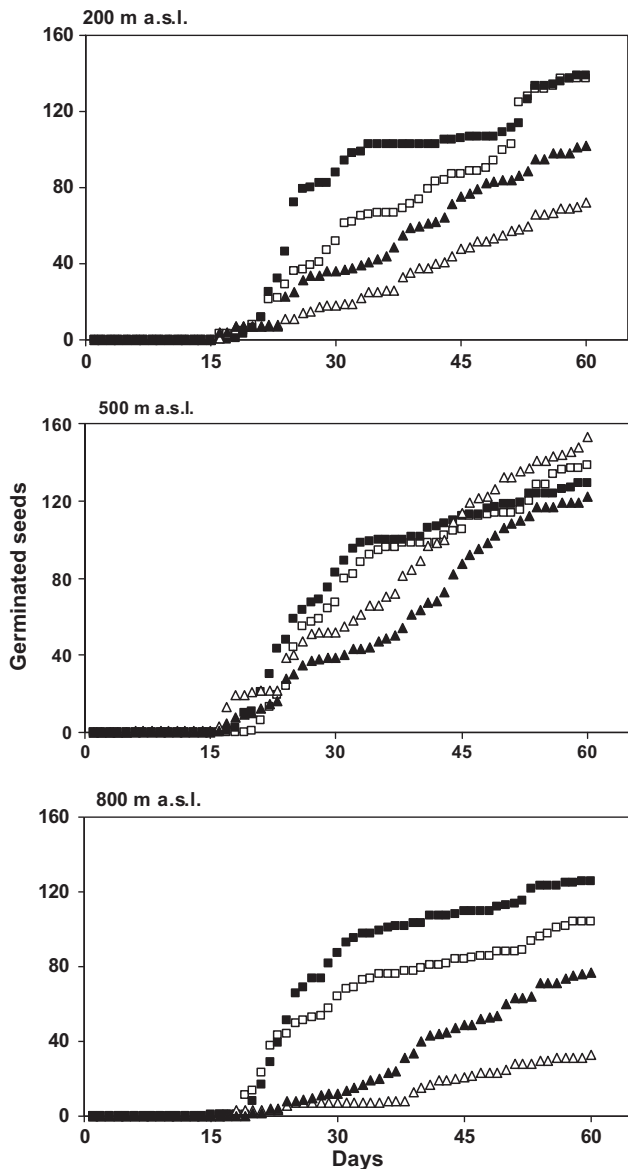
Fig. 2. Comparison of the seed germination rate in both treatments (control and lizard droppings) in the different altitudinal zones of El Hierro in 2005 and 2006. Data 2005: squares, white (control) and black (seeds from *Gallotia* droppings); data 2006: triangles, white (control) and black (seeds from *Gallotia* droppings).

zones together, the germination rate of control seeds was variable between years. While germination did not differ among the different altitudinal zones in 2005, there were significant differences among them in 2006 (Table 1; Fig. 1). This between-year variation was also observed for germination of seeds consumed by lizards. Seeds at 200 and 500 m asl germinated significantly earlier than those from 800 m asl in year 2006 (Table 1), which indicates that seeds from the drier and lower altitudinal zones germinated earlier. So, seedlings from the most stressed environment emerged earlier becoming

promptly vigorous and robust enduring by this way better the dry seasons (spring and summer) (see Nogales et al., 2006; Verdú and Traveset, 2004). A rapid germination of those plants that inhabit arid environments may lead to variation in seedling success (growth rate and death likelihood), with later-emerging seedlings being at a disadvantage (Symonides, 1977). The xeric areas of the Canaries are clearly seasonal and there is a predictable period of rainfall (October–March; García et al., 2001), but the amount of yearly rainfall is not really predictable. This asynchrony in the seed germination rate of *R. fruticosa*, where seeds previously consumed by lizards germinated earlier than control seeds, has been also observed in different populations of the eastern Canary Islands (Lanzarote and Fuerteventura; Nogales et al., 2006). It is interesting to note that climatic differences between these two islands are not so straightforward as in the different altitudinal zones studied on El Hierro. Furthermore, this high variability on germination patterns among altitudes and years could be interpreted as an efficient survival strategy for species growing under unpredictable environments, such as occured in the Mediterranean climates (Giménez-Benavides et al., 2005).

In summary, when for the different altitudinal populations the effect of lizards was compared with germination of the control seeds, lizards-accelerated seed germination rate in four out of the six comparisons (three altitudes and 2 years of experiments: 2005 and 2006) (Table 1; Fig. 2). This asynchrony caused by lizards with respect to control seeds seems to be important for plant fitness, if we consider that *R. fruticosa* seeds have been interacting with lizards for millions of years and this interaction is intense in the lower areas of the Canary Islands. This latter fact may affectively contribute that *R. fruticosa* is so successful in the drier environments of the Canaries, while it is less-adapted to live in the higher parts of the islands.

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