DEPARTAMENTO DE BIOLOGÍA
FACULDAD DE CIENCAS DEL MAR Y AMBIENTALES
UNIVERSIDAD DE CÁDIZ

DEMOGRAPHY AND EVOLUTIONARY ECOLOGY OF THE CARNIVOROUS SUBSHRUB *DROSOPHYLLUM LUSITANICUM* (L.) LINK (DROSOPHYLLACEAE)

DEMOGRAFÍA Y ECOLOGÍA EVOLUTIVA DEL SUBARBUSTO CARNÍVORO *DROSOPHYLLUM LUSITANICUM* (L.) LINK. (DROSOPHYLLACEAE)

MARIA SIMPSON PANIW
CÁDIZ, 2016

UNIVERSIDAD DE CÁDIZ
FACULDAD DE CIENCAS DEL MAR Y AMBIENTALES

DEMOGRAPHY AND EVOLUTIONARY ECOLOGY OF THE CARNIVOROUS SUBSHRUB *DROSOPHYLLUM LUSITANICUM* (L.) LINK (DROSOPHYLLACEAE)

DEMOGRAFÍA Y ECOLOGÍA EVOLUTIVA DEL SUBARBUSTO CARNÍVORO *DROSOPHYLLUM LUSITANICUM* (L.) LINK. (DROSOPHYLLACEAE)

Maria Simpson Paniw
Cádiz, 2016

Cover illustration by Vanessa González Ortiz

This doctoral thesis was carried out in the framework of the research project “*Geographical barrier, habitat fragmentation and vulnerability of endemics: Biodiversity patterns of the Mediterranean heathland across the Strait of Gibraltar (BREATHAL)*” (CGL2011-28759) and was financed by a predoctoral FPI (Formación de Personal Investigador) grant from the Spanish Ministry of Economy and Competitiveness (BES-2012-053075).

Esta tesis doctoral ha sido realizada dentro del proyecto nacional “*Barrera geográfica, discontinuidad del hábitat y vulnerabilidad de los endemismos: Patrones de Biodiversidad del Brezal Mediterráneo a través del Estrecho de Gibraltar (BREZAL)*” (CGL2011-28759) y ha sido financiada por una beca predoctoral FPI (Formación de Personal Investigador) del Ministerio de Economía y Competitividad (BES-2012-053075).

Memoria presentada por Maria Simpson Paniw para optar al grado de Doctor por la Universidad de Cádiz

Fdo. Maria Simpson Paniw

DR. FERNANDO OJEDA COPETE, Profesor Titular del Departamento de Biología de la Universidad de Cádiz, y DR. ROBERTO SALGUERO-GÓMEZ, *independent research fellow* en el *Department of Animal and Plant Sciences* de la *University of Sheffield* (UK) y *honorary research fellow* de la *University of Queensland* (AUS), como sus directores,

HACEN CONSTAR:

Que esta memoria titulada "Demography and Evolutionary Ecology of the Carnivorous Subshrub *Drosophyllum lusitanicum* (L.) Link. (Drosophyllaceae)", presentada por Dña. Maria Simpson Paniw, resume su trabajo de Tesis Doctoral y, considerando que reúne todos los requisitos legales, autorizan a su presentación y defensa para optar al grado de Doctor por la Universidad de Cádiz.

Cádiz, Septiembre 2016

Dr. Fernando Ojeda Copete　　　　Dr. Roberto Salguero-Gómez

Aufklärung ist der Ausgang des Menschen aus
seiner selbst verschuldeten Unmündigkeit.

- Immanuel Kant, 1784

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my two thesis advisors for getting me through all the up and downs of ecological research. Fernando, thank you for giving me the opportunity to work with you at UCA – besides a greater curiosity for fire-adapted ecosystems, all our field trips awoke a passion for hiking in me. Roberto, thank you for always setting the bar high and for introducing me to the wonderful international community of plant and animal demographers. Both of you have made me a better scientist.

A special thank you is also reserved for my collaborators, in particularly Pedro F. Quintana-Ascencio and Arpat Ozgul. My academic progress was starkly improved while working in their respective groups during short stays. I very much enjoyed interacting with members of their groups making new friends and future collaborators.

My doctoral work could not have been accomplished without the support of the Spanish Navy, who allowed me to perform annual demographic censuses and various experiments at the Retín Military Base (Barbate, Spain). It has been a great pleasure to interact with military personnel interested in the preservation of local biodiversity. At the same time, my work could not have been done without the hands-on support of a small but dedicated group of undergraduate and graduate helpers: Mike Scott, Miguel Collado, Jaime Rodríguez, Beatriz Gasalla, Kamila Antunes, and Manu Gil-López. Manu deserves a special thanks because, despite being busy with his own thesis, he helped me out in the field numerous times. In addition, I am grateful to José-Ramón Aracama for provining us with a steady supply of fruit flies.

Being a PhD student can be stressful at times – to say the least. So it is only thanks to my wonderful friends that I came out of this experience more or less sane. I feel grateful towards pretty much all of the biology department. A special thanks also goes to my four-legged best friend, Pistaccio. Being forced to leave behind hours of R coding to clear your head during a nice walk in the park can prove strangely productive.

Lastly, it is no doubt Pablo who, with his unconditional support, valuable advice, and ingenuity of handling Spanish bureaucracy, made this 4-year adventure a true bliss. Thank you very much.

SUMMARY

Natural disturbances occur in various ecosystems and have resulted in the evolution of life histories to buffer or even benefit from disturbance regimes. However, human activities increasingly interact with natural disturbances, posing potentially significant threats to the viability of disturbance-adapted species and therefore causing biodiviersity loss. With fires regularly affecting 50 % of the Eath's surface, such compounded effects of disturbance interactions are particularly prominent in fire-prone ecosystems. Using the rare carnivorous subshrub *Drosophyllum lusitanicum* (Drosophyllaceae), endemic to Mediterranean heathlands under increasing human pressure in the southwestern Iberian Peninsula and northern Morocco, this doctoral work illustrates how interactions between fire and small-scale human disturbances affect population dynamics and the potential evolutionary trajectory of populations. Greenhouse and *in-situ* field experiments and stochastic demographic models quantified biological and ecological characteristics of the study species that could be linked to an important, positive role of recurrent fires in population dynamics. At the same time, population censuses across the species range revealed that small-scale human disturbances related to removal of competitively superior shrub neighbors significantly increased the probability of population occurrence and the abundance of several life-cycle stages. Subsequently, stochastic integral projection models confirmed that moderate interactions between human and fire disturbances may significantly improve species viability in the absence of fires. However, a crucial finding of this work was that frequent human disturbances as well as frequent interactions between fires and chronic vegetation removal may be detrimental to population viability because the two fundamentally different disturbance types exert opposing selection pressures on populations. These findings are of potentially great importance for the management of disturbance-adapted species because they highlight the importace of including compounding effects of environmental drivers into demographic models and the need to consider the local disturbance history when designing conservation strategies of species exposed to various disturbance types.

RESUMEN

Las perturbaciones naturales que afectan a los distintos ecosistemas conllevan a la evolución de las características de los ciclos vitales para soportarlas e incluso beneficiarse de las mismas. Sin embargo, las actividades antropogénicas interaccionan con las perturbaciones naturales y pueden amenazar potencialmente a las especies adaptadas a estas perturbaciones y resultar en una pérdida de biodiversidad. Las interacciones entre ambos tipos de perturbaciones (naturales y antropogénicas) son particularmente importantes en ecosistemas adaptados al fuego, el cual afecta al 50 % de la superficie terrestre. Esta Tesis Doctoral se centra en cómo las interacciones entre el fuego y perturbaciones antropogénicas a pequeña escala pueden afectar la potencial trayectoria evolutiva de las poblaciones de *Drosophyllum lusitanicum* (Drosophyllaceae), una planta carnívora endémica de los brezales mediterráneos en el suroeste de la Península Ibérica y norte de Marruecos. Para ello se han combinado experimentos *in situ* y en invernadero, así como modelos demográficos estocásticos, con el fin de cuantificar las características biológicas y ecológicas de la especie bajo estudio que puedan ser relacionadas con la dinámica de sus poblaciones y el papel del fuego sobre las mismas. Así mismo, los resultados procedentes del censo de las poblaciones de *Drosophyllum lusitanicum* muestras que las perturbaciones antropogénicas a pequeña escala incrementan la probabilidad de la presencia de poblaciones de esta planta y la abundancia de varios estadios de su ciclo vital. En este sentido, los modelos estocásticos de proyección integral confirman que interacciones moderadas entre las perturbaciones antropogénicas y el fuego podrían incrementar de forma significativa la viabilidad poblacional de esta especie. Sin embargo, uno de los puntos claves de esta Tesis es que las perturbaciones antropogénicas frecuentes y las interacciones entre fuegos y eliminación recurrente de la vegetación pueden influir negativamente en la viabilidad de las poblaciones debido a que las perturbaciones de diferente naturaleza ejercen presiones selectivas opuestas sobre las poblaciones. Estos hallazgos tienen, potencialmente, una gran importancia para la gestión de especies adaptadas a perturbaciones debido a que señalan la importancia de incluir diversos componentes relaciondos con efectos ecológicos en modelos demográficos, así como la necesidad de tener en cuenta la historia de las perturbaciones locales a la hora de diseñar estrategias de conservación para especies expuestas a distintos tipos de perturbaciones.

TABLE OF CONTENTS

Chapter 1: General Introduction and Objectives

Chapter 2: Attract them anyway - Benefits of large, showy flowers in a highly autogamous, carnivorous plant species

Chapter 3: Effective prey attraction in the rare *Drosophyllum lusitanicum* (L.) Link (Drosophyllaceae), a flypaper-trap carnivorous plant

Chapter 4: Plant carnivory beyond bogs: reliance on prey feeding in *Drosophyllum lusitanicum* (Drosophyllaceae) in dry Mediterranean heathland habitats

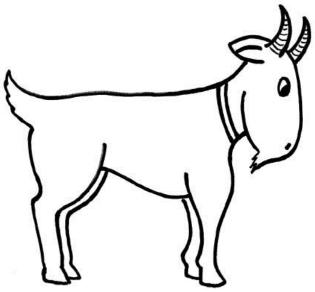
Chapter 5: Accounting for uncertainty in dormant life stages in stochastic demographic models

Chapter 6: Local-scale disturbances can benefit an endangered, fire-adapted plant species in Western Mediterranean heathlands in the absence of fire

Chapter 7: Interacting livestock and fire may both threaten and increase viability of a fire-adapted Mediterranean carnivorous subshrub

Chapter 8: Transient facilitation of resprouting shrubs in fire-prone habitats

Chapter 9: Discussion and Conclusions



CHAPTER 1

General Introduction and Objectives

CHAPTER 1

General Introduction and Objectives

1.1 Introduction

1.1.1 The increasing interest in disturbance ecology

Virtually all natural ecosystems are exposed to periodic or stochastic environmental fluctuations shaping life histories of species and community composition (Benton and Grant 1996; Boyce *et al.* 2006; Turner 2010). Such fluctuations can take on many forms, including seasonal temperature cycles, random interannual climatic variation, or multidecadal oscillations (Stenseth *et al.* 2002; Marshall and Burgess 2015). Among these various forms, natural or seminatural disturbances, most commonly defined as "any relatively discrete event in time that disrupts ecosystem, community, or population structure and that changes resources, substrate availability or condition, or the physical environment" (White and Pickett 1985, p. 7), have increasingly gained importance among ecologists (Turner 2010). This is in part due to the fact that recurrent disturbance events such as floods, fires, or severe windstorms (Fig. 1) have spurred the evolution of some of the most perplexing life-history strategies in plants, including seed dormancy, resprouting, and vegetative dormancy (Murphy 1968; Tuljapurkar 1990; Benton and Grant 1996). These adaptations translate into life-cycle transitions, *i.e.*, survival, growth, reproduction, and recruitment, cued to disturbance regimes (*e.g.*, Horvitz and Schemske 1995; Quintana-Ascencio *et al*. 2003; Mandle *et al*. 2015).

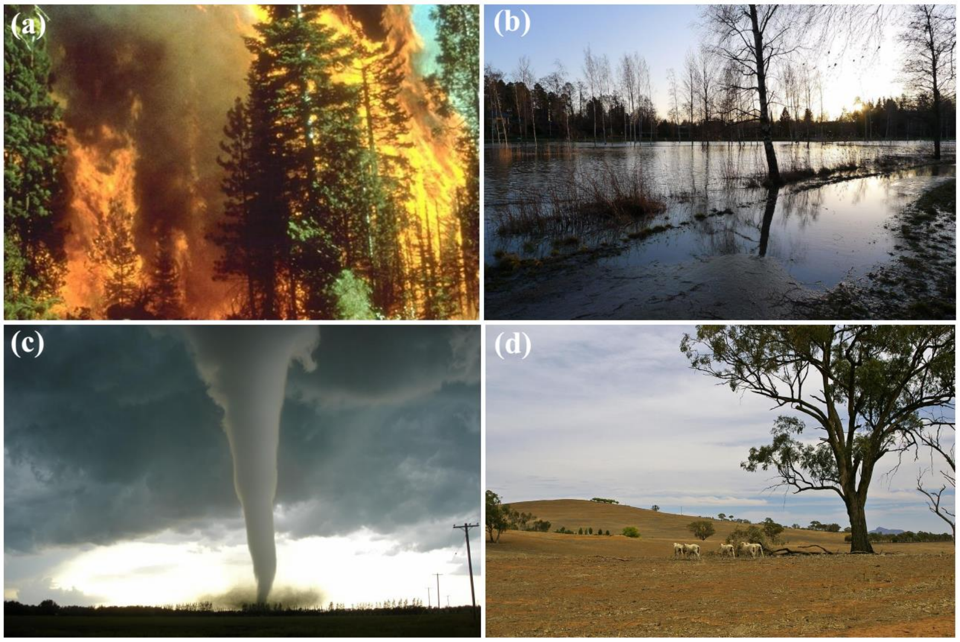


Figure 1 Examples of natural or seminatural disturbances: wildfires (a), flooding (b), severe wind storms (c), or drought (d); Source: Wikipedia.org

The increasing interest in disturbance ecology may also be explained by the fact that natural disturbance regimes are increasingly altered by direct or indirect human action, with potentially severe consequences for disturbance-adapted species and entire ecosystems (Paine *et al.* 1998; Turner 2010; Walker 2012; Frishkoff *et al*. 2016). For example, changes to flooding regimes may severely disrupt riparian communities (Fraaije *et al*. 2015; Soriano-Redondo *et al*. 2016) and cause extinction of plant species that rely on recurrent floods for seedling recruitment (Smith *et al.* 2005). In addition to changes in disturbance regimes, an emerging challenge is the introduction of novel anthropogenic disturbances and their interaction with natural disturbance regimes (Turner 2010; Ehrlén *et al*. 2016). These novel human pressures to ecosystems are of higher frequency than natural disturbance regimes and typically include some form of vegetation removal or harvest of plant parts and/or introduction of diseases or invasive species (Paine *et al.* 1998; Duwyn and MacDougall 2015). For example, in forest ecosystems where early-successional species germinated mostly into patches cleared of dominant trees after major windstorms, forestry practices largely control the patchiness in these systems today (Rogers 1996). The compounded effects of natural and human-driven disturbances affect population dynamics of disturbance-adapted species in all disturbance-prone ecosystems (e.g., Elderd and Doak

2006; Mandle *et al.* 2015; Tye *et al.* 2016), but are most prominent in fire-prone areas, which are not only the most common but also among the the most biodiverse type of disturbance-prone ecosystems across the globe (Chuvieco *et al.* 2008; Keeley *et al.* 2012).

1.1.2 Disturbance interactions in fire-prone Mediterranean ecosystems

Fires regularly affect 50 % of the Earth's surface (Chuvieco *et al.* 2008), and fire regimes in ecosystems characterized by seasonal drought and biomass fuel accumulation, *i.e.*, grasslands, boreal forests, and Mediterranean shrublands, are the main determinants of species composition and interactions (Bond *et al.* 2005; Thuiller *et al.* 2007; Walker 2012). Recurrent fires typically increase spatial habitat heterogeneity and biodiversity, particularly in Mediterranean shrublands, which comprise only 2 % of the global land area (Fig. 2) but provide habitat for 15 % of the total vascular plant flora, much of it adapted to a wide range of fire regimes (Rundel 2004, Keeley *et al.* 2012).

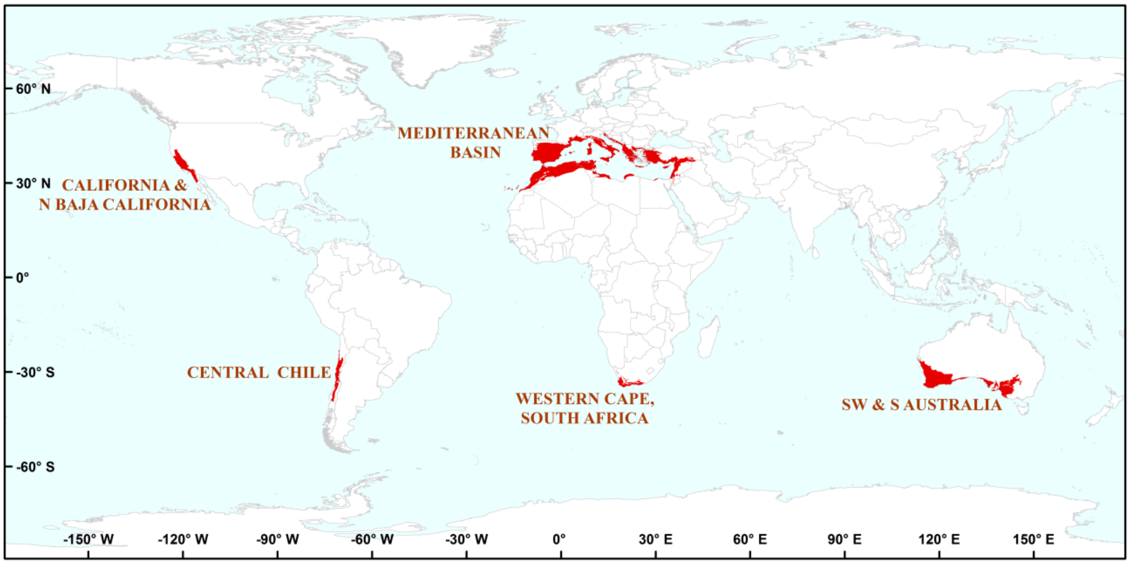


Figure 2 Location of Mediterranean ecosystems (red) around the globe; Source: The Nature Conservancy (2013)

Fire has shaped the evolution of plants for more than 300 million years (Pausas and Keeley 2009), promoting the spread of the angiosperms in the Cretaceous (Bond and Scott

2010). The two main adaptations are mass germination from either persistent soil seed banks or serotinous reproductive structures (e.g., pine cones) and resprouting from dormant meristems in fire-resistant vegetative organs triggered by fire-related cues (Keeley *et al.* 2011). Such cues can be direct (i.e. pyrogenic), such as heat shock and/or smoke-derived compounds (Keeley and Bond, 1997; Moreira *et al.*, 2010), or indirect, such as marked increases in light levels (Keeley 1987; Ooi *et al.* 2014) or drastic reductions of allelochemical inhibitors following the removal of aboveground vegetation and litter by fire (Preston and Baldwin 1999). Post-fire regeneration strategies typically co-occur across fire-prone Mediterranean ecosystems (Moreira *et al.* 2010). In Mediterranean shrublands such as the South African fynbos, for example, the relative abundance of resprouter *vs*. seeder (recruiting from a persistent seed bank) populations may be related to extrinsic factors such as fire frequency and precipitation (Ojeda *et al.* 2005) or intrinsic ones such as life history strategies and resource-allocation tradeoffs (Keeley *et al*. 2011). Because the life cycles of many species are closely linked to fire regimes in Mediterranean ecosystems, human-driven changes to these regimes can have severe effects on population dynamics.

Humans have for millennia used fire as a management tool (Hobbs *et al*. 1995; Blondel 2006) and have used both deliberate burning to convert forests and shrublands to pastures (Grove and Rackham 2001) and fire suppression via intensive grazing and land management to protect infrastructure (Keeley 2006; Pausas and Keeley 2009). Although in recent decades fire frequency in Mediterranean ecosystems overall has increased due to land abandonment (Pausas and Fernández-Muñoz 2012), introduction of invasive species (Brooks *et al.* 2004), or changes to land management such as afforestation campaigns (Moreira *et al*. 2001), active fire suppression policies dominate fire management in many regions of the globe (Keeley *et al.* 2012; Fernandes *et al.* 2013) and have contributed to decreases in annuals fires in some areas (Turco *et al*. 2016). Both increases and decreases in fire frequency may pose severe threats to species which cued their life histories to certain fire regimes and face extinction if fire intervals are shorter than required to produce viable propagules or longer than survival of adults or seed banks (Quintana-Ascencio *et al.* 2003; Lawson *et al.* 2010; Buma *et al.* 2013).

However, beyond merely changing fire regimes, it is increasingly recognized that anthropogenic disturbances, typically involving vegetation removal, harvesting of plant parts, or intense livestock browsing (Fig. 3), interact with fire regimes, posing significant challenges to the management of fire-prone species (Walker 2012; Mandle *et al.* 2015; Darabant *et al.* 2016; Tye *et al.* 2016). Moderate interactions of fire and human disturbances may not necessarily be detrimental to fire-adapted species, as Mediterranean ecosystems have been exposed to human activities for millennia (Bartolomé *et al.* 2005; Velle *et al.* 2014). For example, when post-fire regeneration (either from vegetative resprouting or recruitment from seed banks) is triggered by indirect cues such as increased light levels or removal of allelopathic compounds (Ooi *et al.* 2014; Renne *et al.* 2014), human disturbances that mimic the effect of fire of removing vegetation may allow for persistence of some species even as fire return intervals decrease (Bond and Kelley 2005; Quintana-Ascencio *et al.* 2007). However, disturbances such as livestock browsing or mechanical vegetation removal typically occur at different frequencies and intensities than fire regimes and may therefore drastically alter population dynamics of fire-adapted species (Keeley *et al.* 2012).

Figure 3 Examples of human disturbances in Mediterranean ecosystems include habitat conversion and vegetation removal to suppress fires (a) and livestock browsing (b). (Fotos: M. Paniw)

One important way in which human disturbances alter population dynamics of fire-adapted species is by fundamentally changing their population structure. For example, post-fire recruiting, even-aged cohort populations of seeders may become mixed-aged if vegetation removal promotes germination and establishment in long-unburned habitats (Quintana-Ascencio *et al.* 2007; see also Garrido *et al*. 2003). The increase of mixed-aged populations is typically accompanied by a diminishing role of the seed bank and large seedling mortality in repeated germination events (Quintana-Ascencio *et al.* 2007; Tye *et al.* 2016). This loss of propagules may mean the loss of an important buffer from genetic drift (Uchiyama *et al.* 2006; Dolan *et al.* 2008; Honnay *et al.* 2008). By fundamentally changing population dynamics, human disturbances may create opposing selection pressures compared with natural fire regimes and put species unable to adapt or migrate at higher risk of local extinction, thereby decreasing biodiversity in fire-prone ecosystems. However, the population-level consequences of disturbance interactions in fire-prone ecosystems remain little explored (but see Quintana-Ascencio *et al.* 2007; Lawson et al. 2010; Mandle *et al.* 2015; Tye *et al.* 2016).

In addition to changing population structure and selection pressures, human disturbances such as livestock herbivory and harvesting typically occur in early post-fire years and may threaten the post-fire recovery of species (Winter *et al.* 2011). For example, harvesting of resprouting green parts of plants may weaken plant fitness and hence recovery from fire (Paula and Ojeda 2006; Mandle and Ticktin 2012; Mandle *et al.* 2015). Lastly, frequent human disturbances also increase the interannual environmental variation populations are exposed to and may thus exacerbate effects of climate change on population dynamics (Moreno *et al.* 2011). Many post-fire dwelling species depend on predictable regeneration patterns after fires and are extremely sensitive to environmental fluctuation (Ojeda *et al.* 2005; Moreno *et al.* 2011).

1.2 Disturbance interactions in the population dynamics of a model system – *Drosophyllum lusitanicum*[1]

As human disturbances are unlikely to decrease in disturbance-prone ecosystems, assessing the population-level impacts of increasing interactions of historic natural and novel human disturbance regimes are ever more important for conservation and evolutionary biologists alike (Syphard *et al*. 2009). The main aim of this doctoral work was therefore to contribute to the important discipline of disturbance ecology by assessing various aspects of the biology and population ecology of the rare carnivorous subshrub *Drosophyllum lusitanicum* (L.) Link (Drosophyllaceae). This seeder species (*Drosophyllum*, hereafter) is endemic to fire-prone Mediterranean heathlands on highly acidic, nutrient-poor soils of the southwestern Iberian Peninsula and northern Morocco (Fig. 4a, b). However, due to habitat degradation and fire suppression policies, most populations persist in habitats where human disturbances chronically mimic the vegetation removal effect of fire (Müller and Deil 2001; Garrido *et al.* 2003). These populations in human-disturbed habitats do occasionally burn however, making the species a suitable model organism to investigate the role of disturbance interactions on population dynamics.

Plant carnivory is an adaptation to nutrient-poor soils and is often tightly associated with fire-prone habitats (Juniper *et al.* 1989). Carnivorous plants, typically growing slowly due to inefficient prey nutrient allocation (Ellison and Gotelli 2009), benefit from fires, which remove competitively superior interspecific neighbors (Brewer 2001). Despite this widespread association with fires among carnivorous genera, the fate of *Drosophyllum* populations under increasing alterations of fire regimes and introduction of novel disturbances across its range has received little attention. More generally, the evolutionary biology and ecology of this rare species has remained largely unexplored albeit the fact that many populations have gone extinct (Correia and Freitas 2002; Garrido *et al*. 2003) and this alarming trend is continuing (M. Paniw, pers. obs.).

---

[1] The parts of this subchapter that are related to describing *Drosophyllum lusitanicum* will be published in the upcoming book "*Carnivorous Plants: Physiology, Ecology, and Evolution*" (Ellison, A.M. and Adamec, L. eds.)

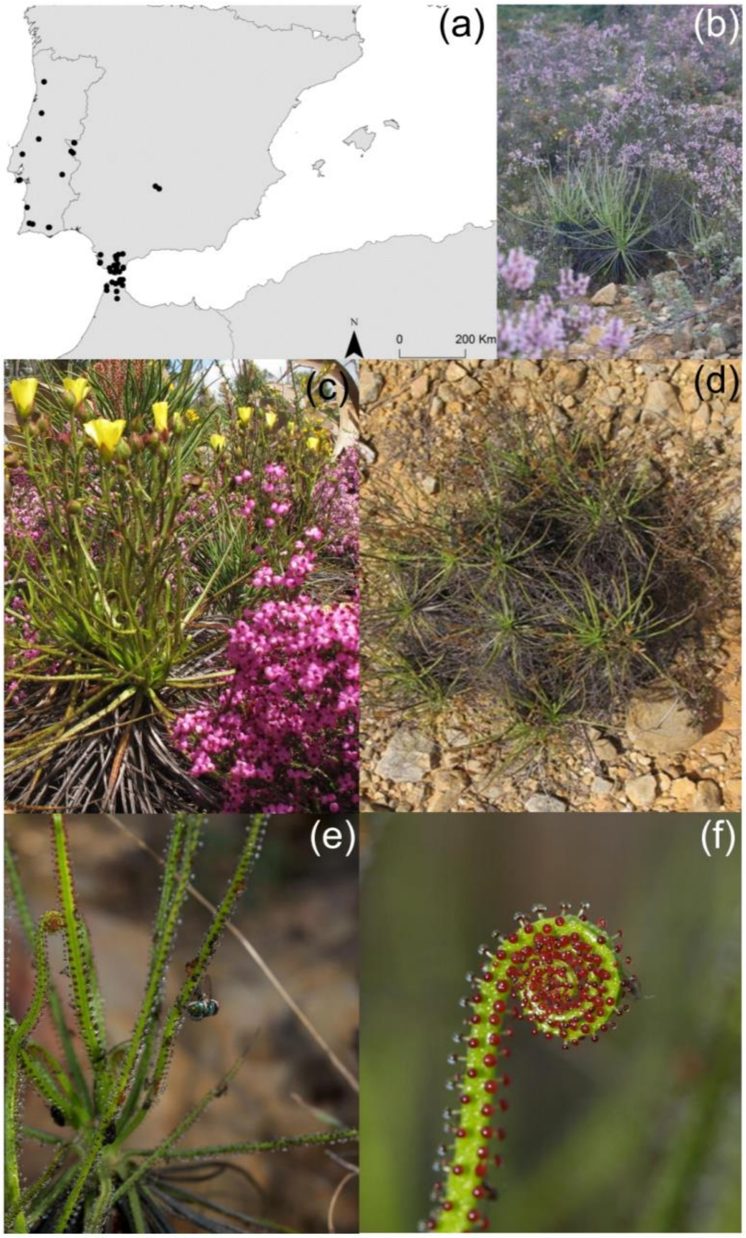


Figure 4 Distribution (a) and physiological/ecological characteristics (b-f) of *Drosophyllum lusitanicum*. Black points show presence of populations and were obtained from historical records and population censuses in 2013 and 2014. Individuals occur in communities dominated by Ericaceae (e.g., *Calluna vulgaris* in b) and Fabaceae shrubs (b); grow in rosettes (c) in nutrient-poor sandstone soils (d). Plants acquire nutrients from prey insects (e) by trapping insect prey with complex galnds on their leaves (f).

The few investigations on the biology and ecology of *Drosophyllum* point to the importance of disturbances in population dynamics of this ecologically and taxonomically unique, endemic species (Heubl *et al*. 2006). The monospecific *Drosophyllum* forming the monogeneric family *Drosophyllaceae* is a short-lived perennial, woody subshrub (Garrido *et al.* 2003) that grows in rosettes attached to secondary stems branched from a woody main stem (Fig. 4c, d; Correia and Freitas 2002). Each rosette contains 10-30 erect, narrowly linear leaves with thread-like tips. The leaves are circinate when young (Fig. 4f) and can reach a length of ca. 25 cm when erect (Correia and Freitas 2002). All leaves are densely covered by stalked sessile and pedunculated glands, secreting carbohydrate-rich mucilage to capture insects and are located at the abaxial surface and along the leaf margins (Fig. 4d, f; Lloyd 1942). The glands on *Drosophyllum* leaves are complex and have been known to be highly efficient in catching a wide variety of prey (Adlassnig *et al.* 2006). *Drosophyllum* begins flowering two years after emergence, with one stalked inflorescence produced on each rosette. Each stalk contains 4-7 flowers arranged in cymose, bracteate inflorescences with a pseudocorymbose appearance (Ortega-Olivencia *et al.* 1995). The flowers are hermaphroditic, actinomorphic, and pentameric. The species is highly autogamous (Ortega-Olivencia *et al.* 1995), and both the strong reliance on prey capture and high levels of autogamy indicate that individuals may have adapted to maximize seed production in the first years after fire, before populations are outcompeted by surrounding, regenerating shrubs.

Contrary to the common association of carnivorous plants with wet habitats (Juniper *et al.* 1989; Brewer et al. 2011), *Drosophyllum* occurs on dry, nutrient-poor sandstone soils in fire-prone, Mediterranean heathlands (Fig. 4d). These heathland communities are dominated by species of Ericaceae (*Calluna vulgaris*, *Erica australis*, *E. umbellata*, and *E. scoparia*) and Fabaceae (e.g. *Stauracanthus boivini* and *Genista tridens*) (Garrido *et al.* 2003). *Drosophyllum* plants are rare in mature, unburned heathlands (M. Paniw, pers. obs.), and the formation of extensive soil seed banks facilitates population persistence (Correia and Freitas 2002), but the role of the seed bank in population dynamics remains unknown.

Despite the association of *Drosophyllum* with Mediterranean heathland habitats, most extant populations occur in secondary habitats, where small-scale human disturbances such as mechanical uprooting or hand-slashing of shrubs to create fire-break lines have replaced the role of fire in vegetation removal (Garrido *et al.* 2003). Some populations in such secondary habitats have persisted above-ground for decades (Adlassnig *et al.* 2006), but it is not known whether populations in human-disturbed habitats are stable and how occasional fires may affect them. It is also unclear whether the changes in community structure (by removal of shrub vegetation) may potentially negatively affect *Drosophyllum* plants.

Despite declining population numbers and its phylogenetic and ecological uniqueness, *Drosophyllum* does not appear in the European Red List of Vascular Plants (Bilz *et al.* 2011). The conservation status of populations varies across the species' range. Populations are largely protected in southern Spain where the species is listed as vulnerable in the Andalusian Red List of Threatened Plants (BOJA 1994). In Morocco, *Drosophyllum* is considered rare and protected locally (Fennane and Ibn Tattou 1998). In Portugal however, where populations are in a strong decline (Correia and Freitas, 2002), the species does not have a formal protection status. This work is therefore not only aimed to quantify how disturbance interactions may lead to biodiversity loss by potentially causing local extinctions of populations of a rare, fire-adapted plant species but also to inform future conservation management of *Drosophyllum* populations.

1.3 Thesis objectives

The general objectives of this thesis were the following:

Assess key biological and ecological characteristics of Drosophyllum *and link these characteristics to the role of fires in population dynamics*. The first part of this thesis focuses on filling some major gaps related to the evolutionary ecology of *Drosophyllum* and link the findings to adaptations to fire regimes. **Chapter 2** addresses the role of pollinators in the reproductive biology of the study species and discusses why the maintenance of large, showy flowers despite high autogamy may be favored in light of life-history adaptations to recurrent fires. **Chapters 3 and 4** discuss the carnivorous syndrome in *Drosophyllum*, highlighting not only the clear separation of prey and pollinator fauna but also the complex carnivorous structures that allow *Drosophyllum* to persist in the extremely nutrient-poor Mediterranean heathland habitats.

Quantify population dynamics in fire-disturbed habitats under the consideration of critical parameters and assessment of their uncertainty. After assessing important aspects of the evolutionary ecology of *Drosophyllum*, **chapter 5** focuses on quantifying critical life stages that inform on population dynamics in fire-disturbed habitats. Assuming that the persistent seed bank is critical for the viability of fire-disturbed populations, a strong focus of this chapter is the quantification of uncertainty around model parameters describing seed-bank transitions. It is hypothesized that the uncertainty in data-limited estimates of important parameters can cause high uncertainty in the estimates and interpretation of population dynamics.

Quantify the structure of populations in human-disturbed habitats as a function of environmental variables at different spatial scales. Given that most extant populations of *Drosophyllum* are found in human-disturbed habitats, population dynamics in fire-disturbed habitats may not be representative. It is not clear however, whether human-disturbed habitats are a step toward extinction of populations or a refuge for the species when natural fire regimes cannot be restored. In this context, **chapter 6** examines to what extent the occurrence and abundance of populations correlate with predictor variables related to human disturbance regimes at two spatial scales (regional and local).

Assess population dynamics and selection pressures under interactive effects of fire and human disturbances. Understanding population dynamics under changing disturbance regimes will prove critical for the future management of disturbance-adapted species. Since many populations of *Drosophyllum* are exposed to both human disturbances and fires, two emerging questions are (i) how increasing human disturbances change demographic rates, selection pressures, and population dynamics of a fire-adapted species, and (ii) whether disturbance interactions increase the risk of extinction. **Chapter 7** adresses these questions by modeling stochastic population dynamics under various scenarios of disturbance interactions. This chapter also quantifies the different selection pressures exerted on individuals by different disturbance types.

Assess how the community structure may mitigate effects of human disturbances: In human disturbed habitats, *Drosophyllum* populations are not only exposed to different disturbance frequencies and intensities than in fire-disturbed heathlands, they are also found in different, less diverse communities, with shrub neighbors largely removed. **Chapter 8** assesses how shrub neighbors may affect several vital rates of *Drosophyllum* throughout post-fire habitat succession and discusses the potential consequences of removing these neighbors on the performance of *Drosophyllum* individuals.

1.4 General organization

The following chapters 2-8 are organized in a typical article format including an abstract, introduction, materials and methods, results, discussion, and references. This organization stems from the fact that chapter 2-8 have been either submitted to or published (chapters 2, 3, and 5) in peer-reviewed journals. Chapters 6 and 7 contain detailed appendices relevant to the population models fit in this study. The appendices include descriptions of all populations used, the study designs, and details on the modelling approaches. In addition, fully commented *R* scripts and data sets to run the population models are available on a CD attached to the back cover, at the Dryad Data Depository (doi:10.5061/dryad.rq7t3; chapter 5), and at https://github.com/MariaPaniw/Drosophyllum-Population-Models (chapter 7).

1.5 References

Adlassnig W, Peroutka M, Eder G, Pois W, Lichtscheidl IK. 2006. Ecophysiological observations on *Drosophyllum lusitanicum*. *Ecological Research* **21**:255-262.

Bartolomé J, Plaixats J, Fanlo R, Boada M. 2005. Conservation of isolated Atlantic heathlands in the Mediterranean region: effects of land-use changes in the Montseny biosphere reserve (Spain). *Biological Conservation* **122**:81–88.

Benton TG, Grant A. 1996. How to keep fit in the real world : Elasticity analyses and selection pressures on life histories in a variable environment. *The American Naturalist* **147**:115–139.

Bilz M, Kell SP, Maxted N, Lansdown RV. 2011. *European Red List of Vascular Plants*. Luxembourg: Publications Office of the European Union.

Blondel J. 2006. The ‘design’of Mediterranean landscapes: a millennial story of humans and ecological systems during the historic period. *Human Ecology* **34**:713-729.

BOJA no. 107 (1994) Decreto 104/1994, de 10 de Mayo, por el que se establece el Catálogo Andaluz de la Flora Silvestre Amenazada. Junta de Andalucía, Sevilla.

Bond WJ, Keeley JE. 2005. Fire as a global ‘herbivore’: the ecology and evolution of flammable ecosystems. *Trends in Ecology and Evolution* **20**:387-394.

Bond WJ, Scott AC. 2010. Fire and the spread of flowering plants in the Cretaceous. *New Phytologist* **188**:1137-1150.

Bond WJ, Woodward FI, Midgley GF. 2005. The global distribution of ecosystems in a world without fire. *New Phytologist* **165**:525-538.

Boyce MS, Haridas CV, Lee CT, NCEAS Stochastic Demography Working Group. 2006. Demography in an increasingly variable world. *Trends in Ecology and Evolution* **21**:141-148.

Brewer JS. 2001. A demographic analysis of fire-stimulated seedling establishment of *Sarracenia alata* (Sarraceniaceae). *American Journal of Botany* **88**:1250-1257.

Brewer JS, Baker DJ, Nero AS, Patterson AL, Roberts RS, Turner LM. 2011. Carnivory in plants as a beneficial trait in wetlands. *Aquatic Botany* **94**:62–70.

Brooks ML, D'Antonio CM, Richardson DM, Keeley JE, DiTomaso JM, Hobbs RJ, Pellant M, Pyke D. 2004. Effects of invasive alien plants on fire regimes. *BioScience* **54**:677–688.

Buma B, Brown CD, Donato DC, Fontaine JB Johnstone JF. 2013. The impacts of changing disturbance regimes on serotinous plant populations and communities. *BioScience* **63**:866-876.

Correia E, Freitas H. 2002. *Drosophyllum lusitanicum*, an endangered West Mediterranean endemic carnivorous plant: threats and its ability to control available resources. *Botanical Journal of the Linnaean Society* **140**:383–390.

Chuvieco E, Giglio L, Justice C. 2008. Global characterization of fire activity: toward defining fire regimes from Earth observation data. *Global Change Biology* **14**:1488-1502.

Darabant A, Staudhammer CL, Rai PB, Gratzer G. 2016. Burning for enhanced non-timber forest product yield may jeopardize the resource base through interactive effects. *Journal of Applied Ecology*, in press.

Dolan RW, Quintana-Ascencio PF, Menges ES. 2008. Genetic change following fire in populations of a seed-banking perennial plant. *Oecologia* **158**:355–360.

Ehrlén J, Morris WF, Euler T, Dahlgren JP. 2016. Advancing environmentally explicit structured population models of plants. *Journal of Ecology* **104**:292-305.

Elderd BD, Doak DF. 2006. Comparing the direct and community-mediated effects of disturbance on plant population dynamics: flooding, herbivory and *Mimulus guttatus*. *Journal of Ecology* **94**:656-669.

Ellison AM, Gotelli NJ. 2009. Energetics and the evolution of carnivorous plants—Darwin's 'most wonderful plants in the world'. *Journal of Experimental Botany* **60**:19-42.

Fennane M, Ibn Tattou M. 1998. Catalogue des plantes vasculaires rares, menacées ou endémiques du Maroc. *Bocconea* **8**:1–243.

Fernandes PM, Davies GM, Ascoli D, Fernández C, Moreira F, Rigolot E, Stoof CR, Vega JA, Molina D. 2013. Prescribed burning in southern Europe: developing fire management in a dynamic landscape. *Frontiers in Ecology and the Environments* **11**:e4–e14.

Fraaije RG, Braak CJ, Verduyn B, Breeman L, Verhoeven JT, Soons MB. 2015. Early plant recruitment stages set the template for the development of vegetation patterns along a hydrological gradient. *Functional Ecology* **29**:971-980.

Frishkoff LO, Karp DS, Flanders JR, Zook J, Hadly EA, Daily GC, M'Gonigle LK. 2016. Climate change and habitat conversion favour the same species. *Ecology Letters* **19**:1081-1090.

Garrido B, Hampe A, Marañón T, Arroyo J. 2003. Regional differences in land use affect population performance of the threatened insectivorous plant *Drosophyllum lusitanicum* (Droseraceae). *Diversity and Distributions* **9**:335–350.

Grove AT, Rackham O. 2001. *The Nature of Mediterranean Europe: an Ecological History*. Yale University Press.

Heubl G, Bringmann G, Meimberg H. 2006. Molecular phylogeny and character evolution of carnivorous plant families in Caryophyllales—revisited. *Plant Biology* **8**:821-830.

Hobbs RJ, Richardson DM, Davis GW. 1995. Mediterranean-type ecosystems: Opportunities and constraints for studying the function of biodiveristy. In Richardson DM, Davis GW (eds.) *Mediterranean-type Ecosytems: The Function of Biodiveristy*. Springer, 1-42.

Honnay O, Bossuyt B, Jacquemyn H, Shimono A, Uchiyama K. 2008. Can a seed bank maintain the genetic variation in the above ground plant population? *Oikos* **117**:1–5.

Horvitz CC, Schemske DW. 1995. Spatiotemporal variation in demographic transitions for a tropical understory herb: projection matrix analysis. *Ecological Monographs* **65**:155-192.

Juniper BE, Robins RJ, Joel DM. 1989. *The Carnivorous Plants*. Associated Press.

Keeley JE. 1987. Role of fire in seed germination of woody taxa in California chaparral. *Ecology* **68**:434-443.

Keeley JE. 2006. South coast bioregion. In Sugihara NG *et al*. (eds.). *Fire in California's Ecosystems*. University of California Press, 350-390.

Keeley JE, Bond WJ. 1997. Convergent seed germination in South African fynbos and Californian chaparral. *Plant Ecology* **133**:153-167

Keeley JE, Bond WJ, Bradstock RA, Pausas JG, Rundel PW. 2012. *Fire in Mediterranean Ecosystems: Ecology, Evolution and Management*. Cambridge University Press.

Keeley JE, Pausas JG, Rundel PW, Bond WJ, Bradstock RA. 2011. Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Sciences* **16**:406–411.

Lawson DM, Regan HM, Zedler PH, Franklin J. 2010. Cumulative effects of land use, altered fire regime and climate change on persistence of *Ceanothus verrucosus*, a rare, fire-dependent plant species. *Global Change Biology* **16**:2518–2529.

Lloyd FE. 1942. *The Carnivorous Plants*. The Ronald Press Company.

Mandle L, Ticktin T. 2012. Interactions among fire, grazing, harvest and abiotic conditions shape palm demographic responses to disturbance. *Journal of Ecology* **100**:997-1008.

Mandle L, Ticktin T, Zuidema PA. 2015. Resilience of palm populations to disturbance is determined by interactive effects of fire, herbivory and harvest. *Journal of Ecology* **103**:1032-1043

Marshall DJ, Burgess SC. 2015. Deconstructing environmental predictability: seasonality, environmental colour and the biogeography of marine life histories. *Ecology Letters* **18**:174-181.

Moreira F, Rego FC, Ferreira PG. 2001. Temporal (1958–1995) pattern of change in a cultural landscape of northwestern Portugal: implications for fire occurrence. *Landscape Ecology* **16**:557-567.

Moreira B, Tormo J, Estrelles E, Pausas JG. 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany* **105**:627–635.

Moreno JM, Zuazua E, Pérez B, Luna B, Velasco A, Resco de Dios V. 2011. Rainfall patterns after fire differentially affect the recruitment of three Mediterranean shrubs. *Biogeosciences* **8**:3721-3732.

Müller J, Deil U. Ecology and structure of *Drosophyllum lusitanicum* (L.) Link populations in the south-west of the Iberian Peninsula. *Acta Botanica Malacitana* **26**:47-68.

Murphy GI. 1968. Pattern in life history and the environment. *The American Naturalist* **102**:391-403.

The Nature Conservancy. 2013. TNC terrestrial ecoregions – vector digital data. <http://maps.tnc.org/gis_data.html#TerrEcos>.

Ojeda F, Brun FG, Vergara JJ. 2005. Fire, rain and the selection of seeder and resprouter life-histories in fire-recruiting, woody plants. *New Phytologist* **168**:155-165.

Ooi MKJ, Denham AJ, Santana VM, Auld TD. 2014. Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. *Ecology and Evolution* **4**:656-671.

Ortega-Olivencia A, Carrasco Claver JP, Devesa Alcaraz JA. 1995. Floral and reproductive biology of *Drosophyllum lusitanicum* (L.) Link (Droseraceae). *Botanical Journal of the Linnean Society* **118**:331–351.

Paine RT, Tegner MJ, Johnson EA .1998. Compounded perturbations yield ecological surprises. *Ecosystems* **1**:535–545.

Paula S, Ojeda F. 2006. Resistance of three co-occurring resprouter *Erica* species to highly frequent disturbance. *Plant Ecology* **183**:329-336.

Pausas JG. 2004. Changes in fire and climate in the eastern Iberian Peninsula (Mediterranean basin). *Climatic Change* **63**:337-350.

Pausas JG, Fernández-Muñoz S. 2012. Fire regime changes in the Western Mediterranean Basin: from fuel-limited to drought-driven fire regime. *Climatic Change* **110**:215-226.

Pausas JG, Keeley JE. 2009. A burning story: the role of fire in the history of life. *BioScience* **59**:593-601.

Preston CA, Baldwin IT. 1999. Positive and negative signals regulate germination in post-fire annual, *Nicotiana attenuata*. *Ecology*, **80**:481–494.

Quintana-Ascencio PF, Menges ES, Weekley CV, 2003. A fire-explicit population viability analysis of *Hypericum cumulicola* in Florida rosemary scrub. *Conservation Biology* **17**:433–449.

Renne IJ, Sinn BT, Shook GW, Sedlacko DM, Dull JR, Villarreal D, Hierro JL. 2014. Eavesdropping in plants: delayed germination via biochemical recognition. *Journal of Ecology* **102**:86-94.

Rogers P. 1996. *Disturbance Ecology and Forest Management: A Review of the Literature*. US Department of Agriculture, Forest Service.

Rundel PW. 2004. Mediterranean-climate ecosystems: defining their extent and community dominance. In Arianoutsou A, Panastasis, VP (eds.) *Ecology, Conservation, and Management of Mediterranean Climate Ecosystems*. Millpress, 1-12.

Ruokolainen L, Linden A, Kaitala V, Fowler MS. 2009. Ecological and evolutionary dynamics under coloured environmental variation. *Trends in Ecology* and *Evolution* **24**:555-63.

Smith M, Caswell H, Mettler-Cherry P. 2005. Stochastic flood and precipitation regimes and the population dynamics of a threatened floodplain plant. *Ecological Applications* **15**:1036–1052.

Soriano-Redondo A, Bearhop S, Cleasby IR, Lock L, Votier SC, Hilton GM. 2016. Ecological responses to extreme flooding events: A case study with a reintroduced bird. *Scientific Reports* **6**:285-295.

Stenseth NC, Mysterud A, Ottersen G, Hurrell JW, Chan KS, Lima M. 2002. Ecological effects of climate fluctuations. *Science* **297**:1292-1296.

Syphard AD, Radeloff VC, Hawbaker TJ, Stewart SI. 2009. Conservation threats due to human-caused increases in fire frequency in Mediterranean-climate ecosystems. *Conservation Biology* **23**:758-69.

Thuiller W, Slingsby JA, Privett SD, Cowling RM. 2007. Stochastic species turnover and stable coexistence in a species-rich, fire-prone plant community. *PloS One* **26**:e938.

Tuljapurkar S. 1990. Delayed reproduction and fitness in variable environments. *Proceedings of the National Academy of Sciences* **87**:1139-1143.

Turco M, Bedia J, Di Liberto F, Fiorucci P, von Hardenberg J, Koutsias N, Llasat MC, Xystrakis F, Provenzale A. 2016. Decreasing fires in Mediterranean Europe. *PLoS One* **16**:e0150663.

Turner MG. 2010. Disturbance and landscape dynamics in a changing world. *Ecology* **91**:2833-2849.

Tye MR, Menges ES, Weekley C, Quintana-Ascencio PF, Salguero-Gómez R. 2016. A demographic ménage à trois: interactions between disturbances both amplify and dampen population dynamics of an endemic plant. *Journal of Ecology*, in press.

Uchiyama K, Goto S, Tsuda Y, Takahashi Y, Ide Y. 2006. Genetic diversity and genetic structure of adult and buried seed populations of Betula maximowicziana in mixed and post-fire stands. *Forest Ecology and Management* **237**:119-126.

Velle LG, Nilsen LS, Norderhaug A, Vandvik V. 2014. Does prescribed burning result in the homogenization of coastal heathlands? *Global Change Biology* **20**:1429-1440.

Walker LR. 2012. *The Biology of Disturbed Habitats*. Oxford University Press.

White PS, Pickett STA. 1985. Natural disturbance and patch dynamics: an introduction. In: Pickett STA, White PS (eds). *The Ecology of Natural Disturbance and Patch Dynamics*. Academic Press, 3–13.

Winter SL, Fuhlendorf SD, Goad CL, Davis CA, Hickman KR, Leslie, DM Jr. 2011. Restoration of the fire-grazing interaction in *Artemisia filifolia* shrubland. *Journal of Applied Ecology* **49**:242–250.

CHAPTER 2

Attract them anyway - Benefits of large, showy flowers in a highly autogamous, carnivorous plant species

CHAPTER 2

Attract them anyway - Benefits of large, showy flowers in a highly autogamous, carnivorous plant species

Antonia Salces-Castellano*, Maria Paniw*, Ramón Casimiro-Soriguer, and Fernando Ojeda

*These authors have contributed equally

This chapter was published in *AoB Plants* (2016), plw017.

2.1 Abstract

Reproductive biology of carnivorous plants has largely been studied on species that rely on insects as pollinators and prey, creating potential conflicts. Autogamous pollination, or autonomous selfing, although present in some carnivorous species, has received less attention. In angiosperms, autonomous selfing is expected to lead to a reduction in flower size, thereby reducing resource allocation to structures with the no-longer-used task of attracting insect pollinators. A notable exception is the carnivorous pyrophyte *Drosophyllum lusitanicum* (Drosophyllaceae), which has been described as an autonomous selfing species but produces large, yellow flowers. Using a flower removal and a pollination experiment, we assessed, respectively, whether large flowers in this species may serve as an attracting device to prey insects or whether previously reported high selfing rates for this species in peripheral populations may be lower in more central, less isolated populations. We found no differences between flower-removed plants and intact, flowering plants in numbers of prey insects trapped. We also found no indication of higher outcrossing rates or fitness costs of selfing, in terms of either seed set or seed size. However, our results showed significant increases in seed set of bagged, hand-pollinated flowers and unbagged flowers exposed to insect visitation compared with bagged, non-manipulated flowers that could only self-pollinate autonomously. Considering that the key life-history strategy of this pyrophytic species is to maintain a viable seed bank, any increase in seed set through insect pollinator activity would increase plant fitness. This in turn would explain the maintenance of large, conspicuous flowers in a highly autogamous, carnivorous plant.

Keywords**:** autonomous selfing, *Drosophyllum lusitanicum*, floral display, pollination biology, prey capture, pyrophyte, seed set.

2.2 Introduction

Carnivorous plants have long captivated naturalists and scientists worldwide (Chase *et al.* 2009; Król *et al.* 2012). Charles Darwin himself was most fascinated by them and was the first to demonstrate plant carnivory experimentally (Darwin 1875). Carnivory has evolved several times independently in the angiosperms and approximately 600 species of carnivorous plants can be found today across the globe, most prominently in tropical and temperate regions (Heubl *et al.* 2006; Ellison and Gotelli 2009). They are largely restricted to infertile, wet, open habitats (Givnish *et al.* 1984) where they have adapted to extremely low soil nutrient levels by evolving elaborately modified leaves that trap small animals, mainly insects, as prey (Ellison and Gotelli 2001, 2009; Gibson and Waller 2009) and absorb the necessary mineral nutrients from them, particularly nitrogen and phosphorus (Adamec 1997).

Since most carnivorous plants are also entomophilous (i.e. they rely on pollinating insects to facilitate sexual reproduction), a pollinator-prey conflict might occur if they trapped potentially efficient pollinators (Zamora 1999; Ellison and Gotelli 2001). However, there are mechanisms in carnivorous plants to avoid or minimize this conflict, such as separation (spatial or temporal) of flowers from leaf traps to avoid pollinators being trapped as prey, or the occurrence of autonomous self-pollination to become somewhat independent of the role of insect vectors for reproduction (Ellison and Gotelli 2001; Jürgens *et al.* 2012). Autonomous self-pollination is actually common in some species from different carnivorous genera (see references in Jürgens *et al.* 2012).

Drosophyllum lusitanicum (L.) Link (Drosophyllaceae), the only extant species of the family Drosophyllaceae (Heubl *et al.* 2006), is a striking example of autonomous self-pollination in carnivorous plant species (Ortega-Olivencia *et al.* 1995, 1998). This species

(*Drosophyllum*, hereafter) is endemic to the western Iberian Peninsula and northern Morocco (Garrido *et al.* 2003; chapter 6), where it is restricted to highly acidic, nutrient-poor Mediterranean heathlands (Müller and Deil 2001; Adlassnig *et al.* 2006) and tightly associated to post-fire habitats (Correia and Freitas 2002; chapter 5). *Drosophyllum* is a short-lived subshrub up to 45 cm tall with circinate, linear leaves grouped in dense rosettes and covered with stalked mucilage-producing glands (Paiva 1997). It produces large, sulphur-yellow, hermaphrodite flowers, radiate and pentamerous, borne in stalked, cymose inflorescences (Paiva 1997; Correia and Freitas 2002; Figure 1). Flowers are homogamous, i.e., possess a spatial and temporal closeness between dehiscing anthers and receptive stigmas, with high selfing capability even in pre-anthesis (Ortega-Olivencia *et al.* 1995, 1998).

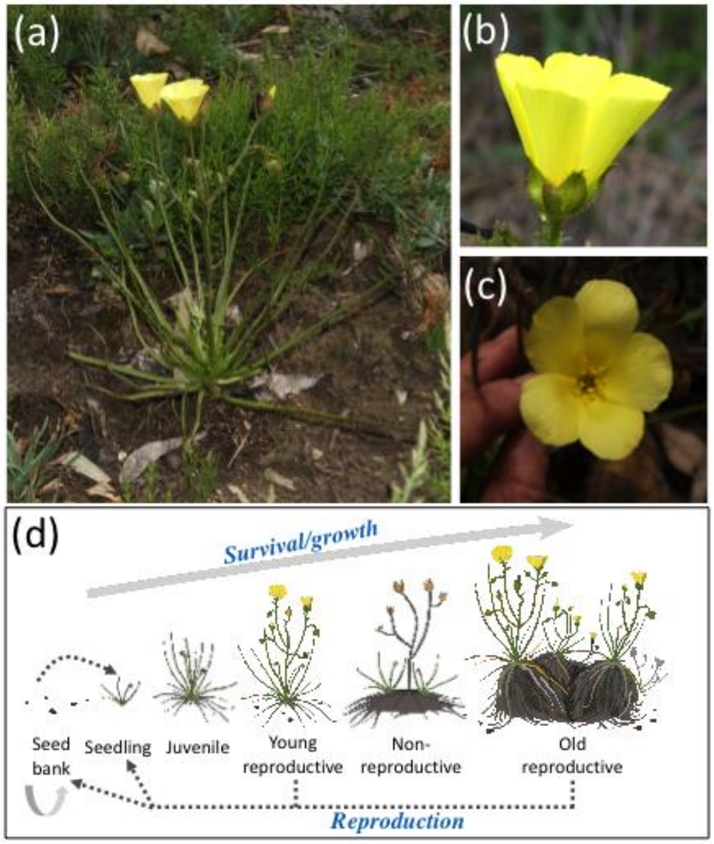


Figure 1 Visual description of *Drosophyllum*. (a) Young reproductive individual with a single rosette of leaves and a stalked inflorescences with two open flowers. (b) Lateral view of the flower showing the five large, bright yellow petals. (c) Frontal view of the flower, showing the homogamous lack of separation between anthers and stigmas. (d) Schematic description of the plant's life-cycle.

It is well established that autonomous selfing in angiosperms is favoured under pollinator limitation (Schemske and Lande 1985; Morgan and Wilson 2005), and it is usually accompanied by morphological changes in floral traits such as the occurrence of homogamy and a dramatic reduction in corolla size (Goodwillie *et al.* 2010; Sicard and Lenhard 2011). This reduction in flower size and other floral traits (e.g. showiness) is explained as a way to minimize resource allocation to floral display when pollinator attraction is no longer necessary (e.g. Andersson 2005; Celedón-Neghme *et al.* 2007). However, one of the noticeable features of the highly autogamous *Drosophyllum* is the production of still large, showy flowers on peduncled inflorescences (Figure 1). Therefore, considering the high allocation costs of flower production (Galen 1999; Andersson 2005), what are the benefits of large, conspicuous flowers in a carnivorous plant species presumably independent of the role of pollinating insects for reproduction (Ortega-Olivencia *et al.* 1995)?

Here, we present a field experimental study on the floral and reproductive biology of *Drosophyllum* aimed to determine fitness benefits from the production of large, conspicuous flowers. First, assuming independence of pollinating insects for reproduction (Ortega-Olivencia *et al.* 1995), we explored whether large, bright-yellow corollas in this carnivorous species act as attracting devices for enhancing prey capture onto the sticky leaf traps, thereby supporting plant growth. Although there is virtually no overlap between prey and flower-visiting insect faunas (chapter 3), it is well established that the bright yellow colour is attractive to many insect species, particularly flies (e.g. Neuenschwander 1982; Yee 2015), which are the most common prey in *Drosophyllum* (chapter 3). Specifically, we hypothesized that flowering *Drosophyllum* plants whose flowers are removed would trap less prey insects than co-occurring, intact flowering plants, which would indicate an increase in plant fitness through insect capture resulting from maintenance of large, yellow flowers.

Second, we conducted a controlled pollination experiment to investigate the actual contribution of pollinators to the fecundity (*i.e.* seed production) of this species. Previous pollination experiments on this species (Ortega-Olivencia *et al.* 1995, 1998) have been performed in geographically isolated, small populations (Garrido *et al.* 2003; Chapter 7).

Since marginal populations of normally outcrossing plant species frequently show a considerable increase in the selfing rate (Lloyd 1980; Pujol *et al.* 2009), the highly autonomous self-fertilization of *Drosophyllum* reported previously might be contingent on geographical isolation. Therefore, we predicted that attraction of pollinating insects by *Drosophyllum* flowers would increase fitness through an increase in fecundity in this carnivorous species, thus accounting for its large, conspicuous flowers.

2.3 Materials and methods

2.3.1 Ecological aspects of Drosophyllum

Drosophyllum is a disturbance-adapted, carnivorous species, colonizing (from a persistent seed bank) recently burned heathlands or heathland patches where small-scale disturbances create open space (Garrido *et al.* 2003; chapter 5). Within 4-6 years after fire, regenerating heathland shrubs outcompete above-ground *Drosophyllum* individuals, making the formation of a seed bank − in which populations may persist for several decades until another fire − a critical life-history strategy (chapter 5). Individuals grow in rosettes, and number of rosettes in this species is a good proxy for age. Plants 1-2 rosettes in size initially reproduce in the second year after emergence and gain 1-2 rosettes per previous rosette each growing season (Ortega-Olivencia *et al.* 1995; Garrido *et al.* 2003; Figure 1D). The maximum observed lifespan of individuals is approximately 10 years (Juniper *et al.* 1989), although most plants die before reaching four years of age (chapter 5). Demographic censuses of populations across southern Spain determined that each rosette produces one floral scape with an average (± SD) of 3.5 ± 2.1 flowers (M. Paniw *et al.* in prep.). Bright sulfur-yellow flowers on each scape open gradually and last one day in full anthesis, so that no more than two flowers per rosette are in anthesis at the same time (Figure 1). Flowers are large (Correia and Freitas 2002), with an average petal length of 2.84 ± 0.21 cm and petal width of 1.89 ± 0.17 cm (chapter 2). Each flower produces a dehiscent capsule with an average of 9.8 ± 2.4 hard seeds (chapter 7).

2.3.2 Study region and sites

Two field experiments were conducted in five natural *Drosophyllum* populations, located at five sites within the southern Aljibe Mountains, at the European side of the Strait of Gibraltar (Table 1; Figure 2): Monte Murta, Montera del Torero, Sierra Carbonera, Monte Retin North and Monte Retin South. This region is characterized by a mild Mediterranean climate (ca. 18 ºC mean annual temperature and ca. 1200 mm annual rainfall) and a rough topography dominated by Oligo-Miocene sandstone mountains and hills, which produce acidic, nutrient-poor soils in ridges and upper slopes (Ojeda *et al.* 2000). These infertile soils are covered by Mediterranean heathlands, dominated by dwarf shrubs like *Erica australis*, *Pterospartum tridentatum, Quercus lusitanica, Calluna vulgaris* and *Halimium lasianthum*, and are the primary habitat of *Drosophyllum* (Müller and Deil 2001; chapter 6). Although this species is highly pyrophytic and therefore threatened by large-scale anthropogenic disturbances such as afforestation (Andrés and Ojeda 2002) and fire suppression (Correia and Freitas 2002), it profits from small-scale vegetation clearances, where populations can still thrive (Garrido *et al.* 2003; chapter 6).

Table 1 Description of sites used in the flower removal and pollination experiments quantifying the role of *Drosophyllum* flowers in prey capture and pollinator attraction, respectively. N – total number of *Drosophyllum* individuals found in 2014.

Site	**Location**	**Experiment**	**Site characteristics**	**Population characteristics**
Monte Murta	36° 19' 16" N 5° 33' 03" W	Flower removal	open, rocky sandstone ridge	N = 5000; mixed-aged population
Monte Retin North	36° 11' 53" N 5° 49' 25" W	Flower removal	open heathland patch	N = 1500; mixed-aged population
Monte Retin South	36° 10' 23" N 5° 50' 53" W	Flower removal	Post-fire regenerating heathland (fire 2010); browsed and trampled by cattle	N = 500; mixed-aged population
Sierra Carbonera	36° 12' 35" N 5° 21' 37" W	Pollination	Post-fire regenerating heathland (fire 2011)	N = 3000; mainly young reproductive individuals
Montera del Torero	36° 13' 35" N 5° 35' 08" W	Pollination	Mechanically built firebreak	N = 3700; mainly old reproductive individuals

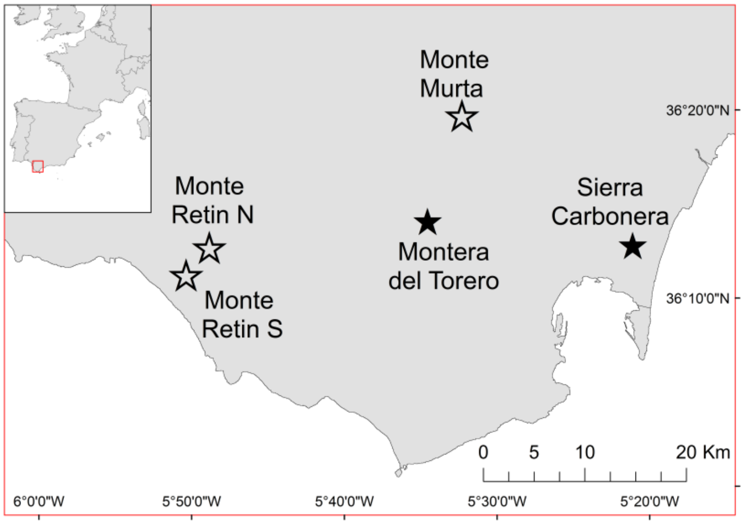


Figure 2 Study area and location of the sites where the flower contribution to prey attraction (open star symbols) and pollination experiments (closed star symbols) were performed. See Table 1 for detailed description of the *Drosophyllum* populations at each site**.**

We chose the study sites to represent the most common habitats of *Drosophyllum* populations (chapter 6). Monte Murta (36° 19' 16" N; 5° 33' 03" W; 380 m) is an open, rocky sandstone ridge with sparse heathland vegetation, which had been mechanically removed about 30 years ago for pine afforestation. In 2014, its *Drosophyllum* population consisted of ca. 5000 individuals, where young flowering plants, consisting of 1-2 rosettes, and old flowering plants (> 2 rosettes) co-occurred. Sierra Carbonera (36° 12' 35" N; 5° 21' 37" W; 273 m) is a regenerating heathland patch from a fire suffered in early autumn 2011. The *Drosophyllum* population here was also large (ca. 3000 individuals) and consisted mainly of young flowering plants, plus juveniles and a few seedlings. Montera del Torero (36° 13' 35" N; 5° 35' 08" W; 136 m) is an old firebreak line across a heathland created by mechanical clearance of the vegetation. The *Drosophyllum* population at this site consisted of ca. 3700 individuals and has persisted for > 30 years, being dominated by very old flowering plants. Lastly, two populations with different relative abundance of old reproductive individuals were encountered in Monte Retin. The population in Monte Retin North (36° 11' 53" N; 5° 49' 25" W; 246 m) has persisted for > 20 years in an open heathland on a rocky sandstone ridge. It consisted of ca. 1500 individuals where old and young flowering individuals co-occurred. The population in Monte Retin South (36° 10' 23" N; 5° 50' 53" W; 284 m) is found on a regenerating heathland patch from a fire suffered in early autumn 2010. This population, which has been heavily disturbed by cattle grazing and trampling, consisted of ca. 500 individuals, with an even distribution of young and old reproductive individuals.

2.3.3 Flower contribution to prey attraction

In order to test whether flowers in *Drosophyllum* contributed to attract and trap insects as prey by the sticky leaves, we carried out a field experiment at three of the five study sites, Monte Murta, Monte Retin North and Monte Retin South (Figure 2) in April 2014, during peak flowering. At each site, we located 'isolated' flowering plants growing in open microhabitats (> 1 m from the nearest conspecific and > 30 cm from the nearest interspecific neighbour). We randomly marked 14 plants that fulfilled this isolation requirement and recorded the number of rosettes and leaves per rosette of each plant. All prey insects were then carefully hand-removed with tweezers from each plant. Next, we randomly selected seven plants out of those 14 and removed all their flowers by cutting off the inflorescence stalks with scissors. After one week, we returned to each of the three populations and recorded the number of prey insects attached to the leaves of the 14 plants.

We analyzed the differences in insect capture between flower-removed plants (treatment) and intact ones (control) for each site separately by fitting a generalized linear model (GLM) with a Poisson error distribution on the total number of insects, using treatment as fixed effect and total number of leaves per plant as the offset.

2.3.4 Pollination experiment

We carried out an experiment at two of the five study sites, Sierra Carbonera and Montera del Torero (Figure 2), to investigate the contribution of pollinators to *Drosophyllum* fecundity (i.e. seed production). In mid-April 2014, at the beginning of the flowering season for this species at the two sites, we labeled 56 and 43 plants in Sierra Carbonera and Montera del Torero, respectively. On each plant, flowers were randomly assigned to one of four treatments: hand cross-pollination (HCP), hand self-pollination (HSP), spontaneous self-pollination (SSP) and control or open pollination (OP). In the first three treatments, flowers were covered with nylon-mesh bags (0.15-mm mesh) before anthesis to exclude potential insect visitors. For the two hand-pollination treatments, HCP and HSP, we collected ripe anthers from plants separated > 300 meters (HCP) or from the same flower (HSP) and brushed the stigmas with them, taking care of bagging them back after this artificial pollination. Flowers in the SSP treatment were not hand-pollinated and remained bagged in order to account for spontaneous autogamy. Finally, flowers in the OP treatment (control) were left exposed to natural pollinator activity. In most plants, there were more than one flower for each treatment (Table 2). We also collected a single petal from an extra flower per plant to measure petal length as a surrogate for flower size.

In July 2014, soon after fruit (capsule) ripening and before seed dispersal (dehiscence), we collected the fruits of the four treatments on each individual plant from the two sites. They were stored individually in labelled paper bags and taken to the lab, where we calculated fruit set (percentage of flowers within each treatment developing into fruits) and seed set (percentage of ovules per flower maturing into seeds) per treatment. Additionally, three randomly chosen seeds per fruit were weighed on an electronic balance to the nearest 0.1 mg and their length (as a surrogate for size) measured using an image analyzer (Leica Application Suite v4.4.0, LAS v4.4, Leica Microsystems).

We searched for differences in fruit set, seed set, seed weight and seed size among pollination treatments by means of a generalized mixed effect model (GLMM) with a binomial error distribution where we considered treatment (OP, HCP, SCP and SSP) as fixed effect and plant individual (ID) as a random effect. We fitted a GLMM for each of the two sites separately.

All analyses were performed with R software (R Core Team 2015). We used the R package *lme4* (Bates 2013) to fit the mixed effect models. When significant differences between treatment levels were found, a *post-hoc* Tukey's honestly significant difference (HSD) test was applied to the linear predictors using the R package *multcomp* (Hothorn *et al.* 2008) to detect significant pairwise differences between treatments.

2.4 Results

2.4.1 Flower contribution to prey attraction

Overall, insect capture levels differed between the three sites used for this study, being considerably higher in Monte Retin South than in the other two sites (Figure 3). However, we detected no significant differences in insect capture rates between *flower-removed* plants and control plants across the three sites (Likelihood ratio tests, D = 2.2 for Murta, D = 1.9 for Retin North, and D = 1.8 for Retin South; *d.f.* =1, $P > 0.1$ for the three sites; Figure 3).

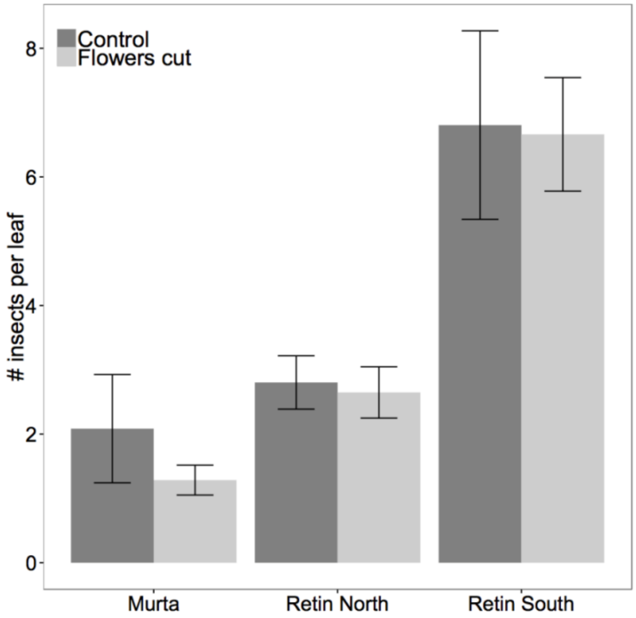


Figure 3 Average number of insects per leaf (± S.E.) at three different sites (Monte Murta, Monte Retin North, Monte Retin South) caught by seven intact flowering plants (control; dark grey bar) and seven plants whose flowers were removed (flower-cut; light grey).

2.4.2 Pollination experiment

Flowers had an overall smaller size (i.e. petal length) in *Drosophyllum* plants from Montera del Torero (average petal length ± SD: 2.64 ± 0.89 cm) than in those from Sierra Carbonera (2.98 ± 0.59 cm; Welch's *t*-test: $t_{64.57} = 6.46$, $P < 0.0001$).

Fruit set was very high in *Drosophyllum*, with almost 100% flowers across the four treatments developing into fruits in the two sites (Table 2). By contrast, we detected significant differences in seed set among treatments in the two study sites (Likelihood ratio test, D = 140.38 for Sierra Carbonera and D = 198.28 for Montera; *d.f.* =3, $p < 0.05$ for the two sites; Figure 4). These significant differences were due to the OP (open pollination) treatment, which produced significantly higher seed set values than the other three treatments in Montera del Torero (but not in Sierra Carbonera), and particularly to the SSP (spontaneous self-pollination) treatment, which produced significantly lower seed set values than the other three treatments at both sites (Table 2; Figure 4). Regarding seed dimensions, seeds were overall larger and heavier in Sierra Carbonera than in Montera del Torero (Table 2). However, while seeds from the OP (open pollination) treatment in Montera del Torero produced slightly but significantly smaller and lighter seeds, no differences in seed size nor weight were detected among treatments in Sierra Carbonera (Table 2).

Table 2. Fecundity variables (fruit set, seed set, seed weight and seed length; mean ± sd) of *Drosophyllum lusitanicum* per treatment in the two sites. Pairwise significant differences ($P < 0.05$; Tukey's HSD tests) between treatments are indicated by different superscript letters. HCP, hand cross-pollination (HCP); HSP, hand self-pollination; SSP, spontaneous self-pollination; OP, control, open pollination.

Treatment	N flowers	Fruit set (%)	Seed set (%)	Seed weight (mg)	Seed length (mm)
Sierra Carbonera					
HCP	67	98.5 (± 12.2)	77.7 (± 18.9) ^{A}	4.36 (± 0.35)	2.48 (± 0.13)
HSP	36	100 (± 0.0)	77.4 (± 22.6) ^{A}	4.40 (± 0.31)	2.48 (± 0.15)
SSP	167	99.4 (± 7.7)	61.0 (± 30.7) ^{C}	4.35 (± 0.45)	2.50 (± 0.16)
OP	76	100 (± 0.0)	70.6 (± 29.7) ^{B}	4.39 (± 0.41)	2.49 (± 0.19)
Montera del Torero					
HCP	43	100 (± 0.0)	60.0 (± 29.1) ^{a}	3.29 (± 0.32) ^{a}	2.15 (± 0.13) ^{a}
HSP	24	100 (± 0.0)	54.6 (± 28.2) ^{a}	3.28 (± 0.23) ^{a}	2.15 (± 0.12) ^{a}
SSP	135	99.3 (± 8.6)	47.0 (± 31.5) ^{c}	3.38 (± 0.37) ^{b}	2.15 (± 0.17) ^{a}
OP	65	100 (± 0.0)	73.0 (± 25.8) ^{b}	3.16 (± 0.31) ^{a}	2.10 (± 0.13) ^{b}

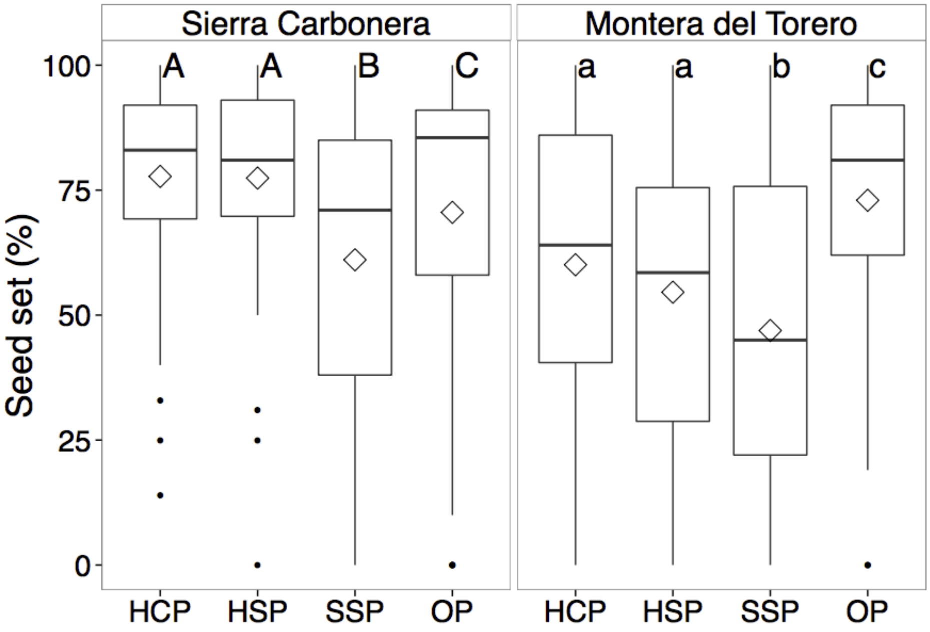


Figure 4 Boxplots of seed set of *Drosophyllum lusitanicum* after hand cross-pollination (HCP), hand self-pollination (HSP), spontaneous self-pollination (SSP) and control, open pollination (OP) across two experimental sites (Sierra Carbonera and Montera del Torero). Different letters represent significant pairwise differences (Tukey´s HSD, $p < 0.05$) of group means between the four pollination treatments at each site.

2.5 Discussion

Although there are no closely related extant species to *Drosophyllum* for comparison (Heubl *et al.* 2006), its large, bright-yellow flowers seem to contradict the paradigm of dramatic flower size reduction in highly autogamous angiosperms (Goodwillie *et al.* 2010; Sicard and Lenhard 2011). Considering the presumably high allocation costs of flower production (e.g. Galen 1999; Andersson 2005), we have explored the advantages or benefits that large, conspicuous flowers confer on this highly autogamous, carnivorous plant species.

Since small Diptera (flies) are the main prey insects in *Drosophyllum* (chapter 3), and the yellow colour is particularly attractive to flies (Neuenschwander 1982; Yee 2015), we tested the hypothesis that large, showy flowers might not be directly related to reproduction, but would instead support plant growth by enhancing prey capture. An increase in prey capture might cause an increase in seed production, as it has been reported in *Drosera* species (Thum 1988), and would therefore have indirect benefits on the reproductive output. However, insect capture rates between intact blooming plants and those plants whose flowers were removed did not differ in any of the three populations (Figure 3), so we rejected the role of large yellow flowers as significant contributors to prey attraction in *Drosophyllum*.

Considering that the *Drosophyllum* population at Montera del Torero was dominated by old plants while most reproductive individuals in Sierra Carbonera were young (Table 1), the differences in flower size between both populations can be explained as an allometric effect of plant age. Branching (i.e. number of rosettes) in this species increases with age (Ortega-Olivencia *et al.* 1995; Garrido *et al.* 2003), and flower (or inflorescence) size is known to decrease with branching (Midgley and Bond 1989).

Regarding the controlled pollination experiments, fruit set was very high, with nearly 100% of the flowers developing into fruit in the four treatments at the two sites (Table 2). Therefore, our results concur with those of Ortega-Olivencia *et al.* (1995, 1998), suggesting that *Drosophyllum* is a highly autogamous species regardless of geographic isolation and population size (Garrido *et al.* 2003; chapter 6). However, when looking at seed production, some interesting patterns emerged. First, seeds were overall smaller in size and weight in plants from Montera del Torero than in those from Sierra Carbonera (Table 2). Again, this can be attributed to an allometric effect derived from plant age (see above), as there is a strong direct relationship between petal size and seed size in angiosperms (Primack 1987). The slightly but significantly smaller and lighter seeds from the OP (open pollination) treatment in Montera del Torero (Table 2) might be due to the existence of a trade-off between seed number per fruit and seed size/weight (e.g. Baker *et al.* 1994).

Second, while seed set values after the two hand-pollination treatments (HCP and HSP) were remarkably high in Sierra Carbonera, significantly higher than after control open pollination (OP), they were significantly lower than after OP in Montera del Torero (Figure 4). These differences could also be explained by the overall large differences in plant age between reproductive plants of the two populations (Table 1). Since most reproductive plants from Montera del Torero were old, their siring ability might be low, as pollen viability in plants decreases with aging (Aizen and Rovere 1995; Marshall *et al.*

2010). As only a single anther brush was applied to stigmas of flowers in both HCP and HSP hand-pollination treatments, this could have been sufficient in Sierra Carbonera, where all reproductive plants were young, but not in Montera del Torero. At the same time, the lack of differences in seed set between both HCP and HSP treatments in the two populations confirms that no mechanism of self-incompatibility is operating in this species (Ortega-Olivencia *et al.* 1998).

But the most remarkable result found in this study has been the significantly lower seed set values in the SSP (spontaneous self-pollination) treatment at both sites (Table 2; Figure 4). This means that, even though *Drosophyllum* flowers are readily able to self-pollinate spontaneously, as Ortega-Olivencia *et al.* (1995) had already reported, insect visitation significantly increases seed production by 15−25% in this species, either by cross- or insect-assisted self-pollination.

However, considering the relatively high rates of seed set after spontaneous self-pollination in *Drosophyllum* (Ortega-Olivencia *et al.* 1995; this study), may a 15-25% increase in seed set through insect-assisted pollination offset the costs associated with maintaining large, showy flowers in this highly autogamous species? Its life history and population dynamics suggest an affirmative answer. Adult individuals of this early-successional pyrophyte species cannot persist in mature, dense vegetation stands, whose germination and growth are largely confined to a short post-fire window (M. Paniw *et al.*, in prep). In this short temporal window, producing seeds to replenish the seed bank is critical for *Drosophyllum*, as it happens in other pyrophytes (Quintana-Ascencio *et al.* 2003; Menges and Quintana-Ascencio 2004). Therefore, any increase in seed set over autonomous selfing caused by insect visitation would increase plant fitness. This, in turn, would account for the large, conspicuous flowers to attract pollinating insects.

2.6 Acknowledgements

We thank the Spanish military *Campo de Adiestramiento de la Armada* in *Sierra del Retín* (Barbate, Cádiz) for allowing access to the Monte Retin sites and facilitating the study. This study was supported by the *Ministerio de Economía y Competitividad*, (project *BREATHAL*; CGL2011-28759).

2.7 References

Adamec L. 1997. Mineral nutrition of carnivorous plants: a review. *Botanical Review* **63:** 273-299.

Adlassnig W, Peroutka M, Eder G, Pois W, Lichtscheidl IK. 2006. Ecophysiological observations on *Drosophyllum lusitanicum*. *Ecological Research* **21:** 255-262.

Aizen MA, Rovere AE. 1995. Does pollen viability decrease with aging? A cross-population examination in Austrocedrus chilensis (Cupressaceae). *International Journal of Plant Sciences* **156**: 227–231.

Andersson S. 2005. Floral costs in *Nigella sativa* (Ranunculaceae) compensatory responses to perianth removal. *American Journal of Botany* **92**: 279–283

Andrés C, Ojeda F. 2002. Effects of afforestation with pines on woody plant diversity of Mediterranean heathlands in southern Spain. *Biodiversity and Conservation* **11**: 1511–1520.

Baker K, Richards AJ, Tremayne M. 1994. Fitness constraints on flower number, seed number and seed size in the dimorphic species *Primula farinosa* L. and *Armeria maritima* (Miller) Willd. *New Phytologist* **128**: 563-570.

Bates D, Maechler M, Bolker B, Walker S. 2013. lme4: Linear mixed-effects models using Eigen and S4. *R package version*, 1(4).

Celedón-Neghme C, Gonzáles WL, Gianoli E. 2007. Cost and benefits of attractive floral traits in the annual species *Madia sativa* (Asteraceae). *Evolutionary Ecology* **21**: 247-257.

Chase MW, Christenhusz MJ, Sanders D, Fay MF. 2009. Murderous plants: Victorian Gothic, Darwin and modern insights into vegetable carnivory. *Botanical Journal of the Linnean Society* **161**: 329 – 356.

Correia E, Freitas H. 2002. *Drosophyllum lusitanicum*, an endangered West Mediterranean endemic carnivorous plant: threats and its ability to control available resources. *Botanical Journal of the Linnean Society* **140**: 383-390.

Darwin C. 1875. Insectivorous plants. John Murray, London, UK.

Ellison AM, Gotelli NJ. 2001 . Evolutionary ecology of carnivorous plants. *Trends in Ecology* and *Evolution* **16**: 623-629.

Ellison AM, Gotelli NJ. 2009. Energetics and the evolution of carnivorous plants–Darwin's 'most wonderful plants in the world'. *Journal of Experimental Botany* **60**: 19 – 42.

Galen C. 1999. Why do flowers vary? The functional ecology of variation in flower size and form within natural plant populations. *Bioscience* **49**: 631-640.

Garrido B, Hampe A, Marañón T, Arroyo J. 2003. Regional differences in land use affect population performance of the threatened insectivorous plant *Drosophyllum lusitanicum* (Droseraceae). *Diversity and Distributions* **9**: 335 – 350.

Gibson TC, Waller DM. 2009. Evolving Darwin's 'most wonderful' plant: ecological steps to a snap-trap. *New Phytologist* **183**: 575-587.

Givnish TJ, Burkhardt EL, Happel RE, Weintraub JD. 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. The *American Society of Naturalists* **124**: 479-497.

Goodwillie C, Sargent RD, Eckert CG, Elle E, Geber MA, Johnston MO, Kalisz S, Moeller DA, Ree RH, Vallejo-Marin M, Winn AA. 2010. Correlated evolution of mating system and floral display traits in flowering plants and its implications for the distribution of mating system variation. *New Phytologist* **185**: 311–321. doi:10.1111/j.1469-8137.2009.03043.x

Heubl G, Bringmann G, Meimberg H. 2006. Molecular phylogeny and character evolution of carnivorous plant families in Caryophyllales—revisited. *Plant Biology* **8**: 821-830.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* **50**: 346-363.

Juniper BE, Robins RJ, Joel DM. 1989. *The carnivorous plants*. Academic Press, San Diego, California, USA.

Jürgens A, Sciligo A, Witt T, El-Sayed AM, Suckling DM. 2012. Pollinator-prey conflict in carnivorous plants. *Biological Reviews* **87**: 602-615.

Król E, Płachno BJ, Adamec L, Stolarz M, Dziubińska H, Trębacz K. 2012. Quite a few reasons for calling carnivores 'the most wonderful plants in the world'. *Annals of Botany* **109**: 47-64.

Lloyd DG. 1980. Demographic factors and mating patterns in angiosperms. In: Solbrig O.T. (ed.) *Demography and Evolution in Plant populations*. Blackwell, Oxford, UK, pp. 67-88

Marshall DL, Avritt JJ, Maliakal-Witt S, Medeiros JS, Shaner MGM. 2010. The impact of plant and flower age on mating patterns. *Annals of Botany* **105**: 7-22.

Menges ES, Quintana-Ascencio PF. 2004. Population viability with fire in *Eryngium cuneifolium*: deciphering a decade of demographic data. *Ecological Monograph*s **74**: 79-99.

Midgley J, Bond W. 1989. Leaf size and inflorescence size may be allometrically related traits. *Oecologia* **78**: 427-429.

Morgan MT, Wilson WG. 2005. Self-fertilization and the escape from pollen limitation in variable pollination environments. *Evolution* **59**: 1143-1148.

Müller J, Deil U. 2001. Ecology and structure of *Drosophyllum lusitanicum* (L.) Link populations in the south-western of the Iberian Peninsula. *Acta Botánica Malacitana* **26**: 47-68.

Neuenschwander P. 1982. Beneficial insects caught by yellow traps used in mass-trapping of the olive fly, *Dacus oleae*. *Entomologia Experimentalis et Applicata* **32**: 286-296.

Ojeda F, Marañón T, Arroyo J. 2000. Plant diversity patterns in the Aljibe Mountains (S. Spain): a comprehensive account. *Biodiversity and Conservation* **9**: 1323-1343

Ortega-Olivencia A, Carrasco JP, Devesa JA. 1995. Floral and reproductive biology of *Drosophyllum lusitanicum* (L.) Link (Droseraceae). *Botanical Journal of the Linnean Society* **118**: 331-351.

Ortega-Olivencia A, López Paredes JA, Rodríguez-Riaño T, Devesa JA. 1998. Modes of self-pollination and absence of cryptic self-incompatibility in *Drosophyllum lusitanicum* (Droseraceae). *Botanica Acta* **111**: 474-480.

Paiva J. 1997. Drosophyllum Link. In S. Castroviejo, C. Aedo, M. Laínz, F. Muñoz Garmendia, G. Nieto Feliner, J. Paiva, and C. Benedí [eds.], Flora Iberica, 5th ed., 78–80. CSIC, Real Jardín Botánico, Madrid, Spain.

Primack RB. 1987. Relationships among flowers, fruits, and seeds. *Annual Review of Ecology and Systematics* **18**: 409-430

Pujol B, Zhou SR, Vilas JS, Pannell JR. 2009. Reduced inbreeding depression after species range expansion. *Proceedings of the National Academy of Sciences of the USA* **106**: 15379-15383.

Quintana-Ascencio PF, Menges ES, Weekley CW. 2003. A fire-explicit population viability analysis of *Hypericum cumulicola* in Florida rosemary scrub. *Conservation Biology* **17**: 433–449.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.

Schemske DW, Lande R. 1985. The Evolution of Self-Fertilization and Inbreeding Depression in Plants. II. Empirical Observations. *Evolution* **39**: 41-52.

SicardA, Lenhard M. 2011. The selfing syndrome: a model for studying the genetic and evolutionary basis of morphological adaptation in plants. *Annals of Botany* **107**: 1433–1443.

Thum M. 1988. The significance of carnivory for the fitness of *Drosera* in its natural habitat. *Oecologia* **75**: 472-480.

Yee WL. 2015. Commercial Yellow Sticky Strips more attractive than yellow boards to western cherry fruit fly (Dipt., Tephritidae). *Journal of Applied Entomology* **139**: 289-301.

Zamora R. 1999. Conditional outcomes of interactions: The pollinator–prey conflict of an insectivorous plant. *Ecology* **80**: 786–795.

CHAPTER 3

Effective prey attraction in the rare *Drosophyllum lusitanicum* (L.) Link (Drosophyllaceae), a flypaper-trap carnivorous plant

CHAPTER 3

Effective prey attraction in the rare *Drosophyllum lusitanicum* (L.) Link (Drosophyllaceae), a flypaper-trap carnivorous plant

Nils Bertol*, Maria Paniw*, and Fernando Ojeda

*These authors have contributed equally

This chapter was published in *American Journal of Botany*, 182 (2016), 689-694.

3.1 Abstract

Carnivorous plants have unusually modified leaves to trap insects as an adaptation to low nutrient environments. Disparate mechanisms have been suggested as luring traits to attract prey insects into their deadly leaves, ranging from very elaborate to none at all. *Drosophyllum lusitanicum* is a rare carnivorous plant with a common flypaper-trap mechanism. Here we tested whether *Drosophyllum* plants lure prey insects into their leaves or they act just as passive traps. We compared prey capture between live potted plants and *Drosophyllum*-shaped artificial mimics coated with odourless glue. Since this species is insect-pollinated, we also explored the possible existence of a pollinator-prey conflict by quantifying the similarity between the pollination and prey guilds in a natural population. We conducted all experiments in southern Spain. *Drosophyllum*'s sticky leaves captured significantly more prey than mimics, particularly small dipterans. Prey attraction, likely exerted by scent or visual cues, seems to be unrelated to pollinator attraction by flowers, as inferred from the low similarity between pollinator and prey insect faunas found in this species. Our results illustrate the effectiveness of this carnivorous species at attracting insects to their flypaper-trap leaves.

***Keywords*:** adhesive trap; *Drosophyllum lusitanicum*; insect attraction; Mediterranean heathland; plant-artificial mimic comparison; prey-pollinator conflict

3.2 Introduction

Carnivory in plants is an unusual adaptation to low nutrient availability, mostly in wet, acid soils (Benzing 1987; Adamec 1997; Thorén *et al.* 2003). Although there are only about 600 carnivorous species from a total of ca. 300,000 angiosperm species, carnivory has evolved multiple times independently, and carnivorous species are found in most major angiosperm lineages (Chase *et al.* 2009; Ellison and Gotelli 2009). Carnivorous plants have modified leaves to trap small animals as prey, mostly insects and other arthropods (Benzing 1987; Ellison and Gotelli 2001; Chase *et al.* 2009), from which they obtain key mineral nutrients (mainly nitrogen and phosphorus; Adamec 1997; Ellison 2006).

Charles Darwin was the first to actually demonstrate that carnivorous plants assimilate nutrients from captured prey (Darwin 1875). Since then, much has been investigated and learnt about prey capture mechanisms (Chase *et al.* 2009; Król *et al.* 2012) and nutrient uptake (Hanslin and Karlsson 1996; Adamec 1997; Farnsworth and Ellison 2008) in carnivorous plants. But what keeps fascinating scientists is whether and how they lure prey into their deadly leaves. Some authors have suggested that trapping structures produce visual and/or olfactory signals (e.g. Lloyd 1942; Joel *et al.* 1985; Moran 1996; Bennett and Ellison 2009) to attract insects. However, Ellison and Gotelli (2009) highlighted that such attracting devices, although unquestionably existing, do not contribute appreciably to prey capture in carnivorous plants with passive trapping mechanisms, such as pitcher or flypaper traps. Kurup *et al.* (2013), nonetheless, provided experimental evidence for the contrary (i.e. active prey capture attached to colour attraction) in pitcher plants. Chin *et al.* (2014) also showed that pitchers of *Nepenthes* species are not passive sampling traps, and that sympatric *Nepenthes* avoid competition by targeting different Formicid prey taxa. But, what about flypaper plants with adhesive traps? Would they merely function as passive insect traps whose efficiency is determined by shape, size and orientation (e.g. Karlsson *et al.* 1987; Foot *et al.* 2014)?

Drosophyllum lusitanicum (Drosophyllaceae) is a rare carnivorous plant in several aspects. First, it is phylogenetically rare, being the only species in the family Drosophyllaceae (Heubl *et al.* 2006). This species (hereafter referred to as *Drosophyllum*) is also geographically rare, as it is endemic to the western end of the Mediterranean Basin

(Garrido *et al.* 2003). Finally, *Drosophyllum* is also ecologically rare among carnivorous plants because it is found on dry soils (Garrido *et al.* 2003; Adlassnig *et al.* 2006), while the vast majority of carnivorous species are restricted to wet soils (fens or bogs; Givnish 1984; Juniper *et al.* 1989). Morphologically, it is a carnivorous subshrub of up to 45 cm high with circinate, linear leaves ca. 20 cm long grouped in dense rosettes (Paiva 1997; Correia and Freitas 2002) and bright yellow flowers borne in peduncled corymbose inflorescences (Garrido *et al.* 2003). According to Ortega-Olivencia *et al.* (1995, 1998), it is highly self-fertilizing but, contrary to their observations, it benefits markedly from the activity of pollinating insects to produce seeds (seed-set dropped from ca. 80% in open flowers to ca. 55% in bagged, non-pollinated flowers; Chapter 2).

Regarding prey capture, *Drosophyllum* uses a passive, adhesive or flypaper-trap mechanism, common among carnivorous plants (Chase *et al.* 2009). Its leaves trap insects with a carbohydrate-rich, adhesive mucilage produced in large, stalked glands (Darwin 1875; Adlassnig *et al.* 2010) and digest them with enzymes secreted by small, sessile glands (Darwin 1875; Adlassnig *et al.* 2006). In his original description of this species, Charles Darwin remarked that *'*[Drosophyllum] *caught so many insects [...] although the weather was cold and insects scarce, that it must have been in some manner strongly attractive to them'* (Darwin 1875, p. 332). This presumably efficient attraction might be attributable to a noticeable honey-like, sweet scent emitted by this species (Lloyd 1942; Juniper *et al.* 1989; and personal observations by the authors). Unfortunately, this species has never been considered in studies of volatile organic compounds in carnivorous plants (Jürgens *et al.* 2009, 2012).

In this study, we tested whether *Drosophyllum* plants lure prey insects into their flypaper-trap leaves as Darwin (1875) hypothesized or whether they act just as passive traps, as Ellison and Gotelli (2009) generalized for carnivorous plants with flypaper traps. To do so, we measured insect capture by *Drosophyllum* live plants and compared it with that of *Drosophyllum*-shaped artificial traps, formed by green-wire mimics coated with colourless and odourless adhesive, thus serving as a "null expectation" of insect capture determined solely by shape, size and orientation. Since this species, like most carnivorous species, is insect-pollinated, an efficient insect-luring mechanism might bring about a pollinator-prey conflict (Zamora 1999; Anderson and Midgley 2001; Jürgens *et al.* 2012). To explore the existence of such a conflict in *Drosophyllum*, we quantified the similarity between the pollination and prey guilds in a natural population.

3.3 Materials and methods

3.3.1 Prey capture: study site, material and experimental design

To test whether *Drosophyllum* plants attract prey insects into their flypaper-trap leaves or they just act as passive traps, we compared prey capture between live plants and artificial traps, acting as a "null expectation". We performed an outdoor experiment around the campus of the University of Cadiz in Puerto Real, Spain (36.53º N, 5.79º W), whose landscape consists of open lawn with scattered shrubs and trees. The climate is typically Mediterranean, with average annual temperature of ca. 18 ºC and rainfall of ca. 600 mm. We conducted the experiment in mid spring, when insect activity is high.

We used 28 young *Drosophyllum* live plants (6 month old) grown in the glasshouse from seeds in individual 0.5-liter clay pots with a substrate of siliceous sand and peat-moss (50/50 v/v). Seeds had been collected the previous year in several natural populations from the northern side of the Strait of Gibraltar (south Spain). At the onset of the experiment, plants had 7.3 ± 0.3 (mean ± SE) leaves per individual plant and leaf mean length was 12.7 ± 0.4 cm. We then made 28 *Drosophyllum*-shaped artificial adhesive traps (mimics) of similar size, shape, colour and number of leaves by using green wire coated with colourless and odourless glue (mice glue; Temobí®, Impex Europa S.L., Pontevedra, Spain; available at http://impexeuropa.es) and potted them like the live plants. The possible presence of volatile compounds that might attract (or repell) insects in this odourless adhesive was discarded after headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC-MS). So, plants and mimics looked morphologically and chromatically similar to the human naked eye (Figure 1), but mimics were fully odourless and, although we did not measure reflectance spectra, likely differences in UV reflection patterns may be expected between mimics and plants, since *Drosophyllum* leaves produce considerable UV reflection (Joel *et al.* 1985). Therefore, mimics would function as adequate null models against which insect attraction by *Drosophyllum* plants may be tested, although without distinguishing between visual and olfactory cues as potential attraction mechanisms.

Figure 1(a) Detail of *Drosophyllum* leaf with a lacewing (Chrysopidae, Neuroptera) trapped by its mucilage-rich, sticky glands; (b) Potted young plant and (c) potted green-wired, artificial mimic coated with odourles mice glue used in the experiment.

We then chose seven sites around the campus, and four *Drosophyllum* plants and four mimics were randomly placed at each site, separated about 2 m from each other. We counted prey (insect) trapped on *Drosophyllum* plants and mimics after daylight (from 09:00 to 19:00) and night (from 20:00 to 08:00) periods across three days, from May 1st (09:00) to May 4th 2014 (08:00). At the beginning of each daylight and night period across the three days, we hand-removed trapped insects with tweezers from two plants and two mimics per site, making sure that neither leaf droplets of plants nor glue of mimics were spoiled. Removed insects were placed in vials and frozen for later identification, mostly to the order or class level. The two other plants and mimics per site from which insects were not hand-removed were used to explore the existence of differences in the cumulative number of prey captured between *Drosophyllum* plants and mimics during the three days. It shall be stressed that the sticking power of the odourless adhesive used to coat the mimics was much stronger than that of the leaf mucilage of *Drosophyllum* plants. Considering that we used trapped insects as a proxy of ‘attraction’, the attraction values of the mimics (null expectation) will be inflated compared to those of *Drosophyllum* plants.

Strictly speaking, this is an *ex situ* experimental setup and, as such, it might have interpretation caveats, since prey capture by carnivorous plants is contingent on the insect community of their natural habitats (Bennett and Ellison 2009). For instance, pitcher plants in the Asian genus *Nepenthes* are specialized in trapping ants and termites in their native range, but *Nepenthes ventricosa* captured mostly flies in an *ex-situ* field experiment in Germany (Schaefer and Ruxton 2008). However, in our case, the campus falls within the geographic range of *Drosophyllum* (Garrido *et al.* 2003), next to a nature reserve (*Parque Natural Bahía de Cádiz*, Cadiz province, Spain) where natural populations of this species are found, the closest being less than 7 km from campus (M. Paniw, unpublished data). On the other hand, since the aim of the experiment was to compare insect capture between *Drosophyllum* plants and "neutral mimics", we would not expect major limits to interpretation of the results from this *ex situ* experimental setup.

3.3.2 Prey capture: statistical analyses

We analyzed the average number of prey captured using generalized linear mixed models (GLMMs). For these analyses, we used only data from the plants and mimics whose insects were removed after each period (see above). We examined the main effects of the trap (*Drosophyllum* plant or mimic) and period (daylight or night) treatments. To account for differences among the seven sites and repeated insect counts on individual *Drosophyllum* and mimics, we used site and individual (nested within sites) as random effects in the models. We fitted models with a negative binomial error distribution as simple Poisson models showed overdispersion, i.e. the ratio of squared Pearson residuals and residual degrees of freedom was > 1 (χ^2, $p < 0.01$; Ver Hoef *et al.* 2007). We used likelihood ratio tests to determine significant differences between treatments (Vuong 1989). These tests compare increasingly complex, or nested, models to simpler ones (starting with intercept-only models). Number of leaves per plant was used as an offset in all models. We performed all analyses using the R package *lme4* (Bates *et al.* 2013).

We used a permutational MANOVA test to detect differences in relative abundance of prey taxa between *Drosophyllum* plants and mimics within daylight and night periods. The test was implemented in R using the *adonis* function provided in the package *vegan* (Oksanen *et al.* 2013). This function partitioned the sums of squares between trap and period treatments (nested within site) based on the dissimilarity of insects caught in each treatment combination. We chose the Bray-Curtis approach for the dissimilarity matrix (Beals 1984) and determined significance of the treatments based on 100,000 permutations.

Finally, to compare natural prey capture to prey capture under manipulation by the daily removal of insects, we counted the number of trapped insects per leaf after each period (i.e., twice a day) on the plants and mimics whose insects were not hand-removed. Since the *Drosophyllum* plants did not digest completely most prey insects and the mimics remained sticky regardless of the number of insect trapped on them, we could obtain an estimate of the cumulative number of insects caught without manipulation. To test for significant differences in prey capture between non-manipulated *Drosophyllum* plants and mimics, we fitted and compared GLMMs analogously to the insect-removal experiment but excluding the period (daylight vs night) treatment.

3.3.3 Prey-pollinator conflict: study site, data collection and analysis

To explore the existence of a presumed prey-pollinator conflict in *Drosophyllum*, we estimated the degree of overlap between the prey and pollinator guilds in a natural population. To do so, we carried out pollinator and prey censuses on reproductive plants in a natural population from the northern side of the Strait of Gibraltar (*Montera del Torero*, Spain; 36.23° N, 05.59° W), ca. 65 km from the Puerto Real University campus. The population consists of more than 500 individuals in a sparsely vegetated, firebreak area surrounded by dense Mediterranean heathland.

Pollinator censuses were conducted during six days in mid May 2014 on about 50 flowering individuals in an area of ca. 100 m^2. Observations each day started at 08:00h, when flowers opened and finished at about 15:00h, when flowers closed and pollination activity decreased drastically. Censuses were conducted as follows: nine groups of one to eight *Drosophyllum* individuals were chosen and each group was observed 15 min per day, recording insects visiting a flower of any of the plants of the group. Observation order of the nine groups was changed every day. By so doing, we covered a total census time of 13.5 hours. We considered an insect as a pollinator when it visited a flower and was in contact with stigma and anthers. We captured samples of those pollinating insects and stored them in vials for later identification. Prey censuses were conducted by collecting all identifiable, undigested trapped insects from a total of 21 *Drosophyllum* individuals from the same area for the pollinator censuses and during the same six days. Both insect and prey sampled individuals were identified to closest taxonomical level possible (from species to order).

Since pollinator and prey census methods were different, we discarded frequency and used only presence/absence data of insect taxa in each group. Then, we used the Jaccard similarity index, *J* (Jaccard 1901) to estimate the degree of overlap between pollinator and prey groups in the natural population,

$$J = \frac{a}{a + b + c}$$

where *a* is the number of shared insect taxa (i.e. found both as pollinator and prey), and *b* and *c* are the number of taxa observed only as pollinator or as prey, respectively.

3.4 Results

3.4.1 Prey capture

Overall insect capture was significantly higher during daylight than during night in both plants and mimics (Table 1; Figure 2). *Drosophyllum* leaves trapped significantly more insects than mimic leaves, consistently in both daylight and night periods (Figure 2) as indicated by the lack of significance of the *trap* × *period* interaction effect (Table 1). When looking at the cumulative number of prey captured along the three days' experiment, the same pattern was observed, with *Drosophyllum* plants trapping significantly more insects than mimics (Figure 3).

Table 1 Results of likelihood ratio tests for all considered models describing the number of insects caught. All models included number of leaves (on the *Drosophyllum* plants and mimics) as offset and site and individual (nested within site) as random effects.

Model	# of parameters	Chi Square	Deviance	*p*-value
Intercept	4	1363.4		
Trap	5	1344.6	17.7	< 0.01
Trap + period	6	1274.7	69.9	< 0.01
Trap + period + trap × period	7	1274.2	0.5	0.49

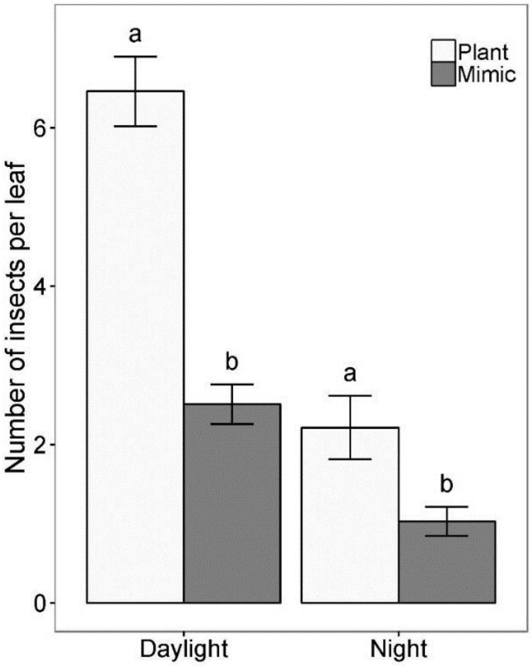


Figure 2 Average number of insects (± S.E.) per leaf trapped by *Drosophyllum* plants and mimics during daylight and night periods. Different letters a and b represent differences in group means within the pairwise trap (plant vs. mimic) treatment. Letters differ if group means are significantly different from each other (likelihood ratio tests; see Table 1).

The majority of insects (> 80%) trapped by *Drosophyllum* plants and mimics across the three days belonged to the Order Diptera (Table 2). Main differences in prey capture between plants and mimics were due to relative abundances of Dipterans, with plants catching a noticeably higher amount of small Dipterans (body length < 5 mm; mostly Drosophilid flies) and a lower amount of large Dipterans (mostly Tachinid flies; Table 2).

3.4.2 Prey-pollinator conflict

A total of 12 different insect species were censused as potential pollinators of *Drosophyllum* flowers. Coleopterans and Hymenopterans were the most abundant flower visitors, while only few Dipterans were censused (Table 3). A total of 18 different taxa were identified as prey, out of which dipterans predominated, followed by Lepidopterans and, to a lesser extent, Coleopterans (Table 3).

The Jaccard similarity index (*J*) between pollinator and prey guilds was very low (*J = 0.04*), indicating a high dissimilarity between both pollinator and prey faunas. Therefore, a presumed prey-pollinator conflict would not exist in *Drosophyllum*.

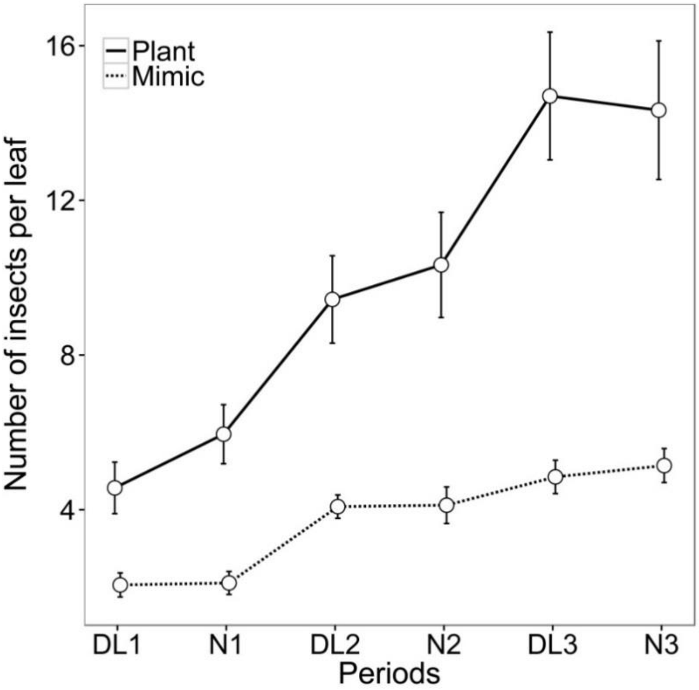


Figure 3 Cumulative number (± S.E.) of insects per leaf on *Drosophyllum* plants and mimics across the three experiment days. DL and N stand for daylight and night periods, respectively. Numbers indicate the experiment day. The differences in insect numbers between plants and mimics are significant (χ^2 deviance = 5.6, df = 1; *p*-value = 0.02).

Table 2 Number (and percentage of total in brackets) of insect taxa removed from *Drosophyllum* plants and mimics over the three days. Overall, *Drosophyllum* plants trapped significantly more insects than mimics during both daylight ($F_{1, 12}$ = 8.1, *p*-value = 0.03) and night ($F_{1, 12}$ = 4.6, *p*-value = 0.02).

Period	Trap	Small Diptera (< 5 mm)	Large Diptera	Coleoptera	Hemiptera	Heteroptera	Hymenoptera[1]	Lepidoptera	Araneae	Total
Daylight	*Drosophyllum*	477 (*83.5*)	72 (*12.6*)	3 (*0.5*)	0	9 (*1.6*)	4 (*0.7*)	5 (*0.9*)	1 (*0.2*)	571
	Mimic	138 (*45.7*)	151 (*50*)	4 (*1.3*)	1 (*0.3*)	3 (*1.0*)	3 (*1.0*)	2 (*0.7*)	0	302
Night	*Drosophyllum*	245 (*80.9*)	31 (*10.2*)	5 (*1.7*)	0	12 (*4.0*)	6 (*2.0*)	4 (*1.3*)	0	303
	Mimic	83 (*52.5*)	57 (*36.1*)	6 (*3.8*)	0	9 (*5.7*)	3 (*1.9*)	0	0	158

[1] predominantly bee

Table 3 Pollinator and prey fauna in a natural *Drosophyllum* population (*Montera del Torero*, Spain; 36.23° N, 05.59° W).

	# POLLINATORS (% of total)	PREY (1/0, pres/abs)
Hymenoptera		
Panurgus sp. (Andrenidae)	39 (25.6)	0
Halyctus scabiosae (Halyctidae)	9 (5.9)	0
Panurgus cephalotes (Andrenidae)	4 (2.6)	0
Panurgus banksianus (Andrenidae)	2 (1.3)	0
Andrena sp. (Andrenidae)	2 (1.3)	0
Dasypoda hirtipes (Melitidae)	1 (0.7)	0
Formicidae	0	1
Coleoptera		
Enicopus sp. (Melyridae)	86 (56.6)	1
Oxythyrea funesta (Cetoniidae)	3 (2.0)	0
Omaloplia sp. (Melolonthidae)	2 (1.3)	0
Elateridae	0	1
Lagria sp. (Tenebrionidae)	0	1
Curculionidae	0	1
Coccinellidae	0	1
Diptera		
Drosophila sp.	0	1
Usia sp. (Bombyliidae)	2 (1.3)	0
Episyrphus sp.(Syrphidae)	1 (0.7)	1
Eristalis sp. (Syrphidae)	1 (0.7)	1
Eupeodes sp. (Syrphidae)	0	1
Acalyptratae	0	1
Anthomyiidae	0	1
Siphona sp. (Tachinidae)	0	1
Bombylella atra (Bombyliidae)	0	1
Nephrotoma sp. (Tipulidae)	0	1
Lepidoptera		
Microlepidoptera	0	1
Heteroptera		
Aphididae	0	1
Neuroptera		
Chrysopidae	0	1

3.5 Discussion

Effective prey attraction can be beneficial to carnivorous plants, since an increase in insect capture rates leads to an increase in fitness components (Thum 1988; Zamora *et al.* 1998; Pavlovic *et al.* 2014). However, although it has long been suggested that carnivorous plants might use visual and/or olfactory cues to lure insects into their trap leaves (e.g. Joel *et al.* 1985; Jürgens *et al.* 2009; Kurup *et al.* 2013), Ellison and Gotelli (2009) concluded that prey attraction by pitcher and flypaper carnivorous plants is not more effective than that exerted by a passive trap of similar size and shape. Although this was recently disproven for pitcher plants (Kurup *et al.* 2013; Chin *et al.* 2014), it still appeared to be a reasonable conclusion for flypaper plants with adhesive traps (Karlsson *et al.* 1987; Foot *et al.* 2014).

Diptera (flies) was by far the most common order of insects by number of individuals trapped by both *Drosophyllum* plants and mimics in our *ex situ* outdoor experiment as well as by *Drosophyllum* plants in the natural population at *Montera del Torero*. Flies are certainly predominant as prey for this species across its entire geographic range (authors' personal observations). Thus, despite the caveats of studying prey capture through *ex situ* field designs (Benett and Elison 2009), we are confident of the ecological validity of our results. They illustrate an effective prey luring potential of *Drosophyllum* plants, as they captured significantly more insects than the null expectation (mimics) despite the stronger sticking power of the mimics (see Methods).

Differences were largely attributable to the high quantity of individuals, particularly of small-sized flies, captured by *Drosophyllum* plants compared to mimics, being the taxonomic identity of prey between plants and mimics unimportant (Table 2). Only the amount of large flies (mostly Tachinidae, Muscidae and Calliphoridae) trapped as prey was higher in mimics than in plants (Table 2). However, it shall be stressed that we observed many large flies attracted to but then escaping from *Drosophyllum* leaves, whereas no insect, whatever size, escaped from the strong stickiness of the mimics. We can therefore conclude that *Drosophyllum* − a rare carnivorous species from taxonomical, geographical and ecological points of view but a standard flypaper carnivorous plants in terms of prey capture − effectively attracts insects, mostly dipterans. Interestingly, although such effective prey luring potential in *Drosophyllum* was already proposed by Darwin (1875), and other authors have also mentioned it (e.g. Meyer and Dewèvre 1894; Lloyd 1942), this study provides the first experimental evidence that *Drosophyllum* lures its prey.

Although this study did not assess the role of visual and olfactory cues as potential mechanisms for insect attraction, the noticeable prey attraction by *Drosophyllum* plants compared to the mimics was found not only in daylight periods but also and consistently at night. Even though overall insect activity decreased at night, which was reflected in the overall lower capture values by both plants and mimics, we still detected a consistently higher prey capture in *Drosophyllum* plants during the three nights (Figure 2). At night there is less chance for visual attraction, although at low light intensities (e.g. dawn and dusk) many insect species are strongly attracted by UV light (Weiss *et al.* 1942) and conspicuous UV patterns have been reported in *Drosophyllum* leaves (Joel *et al.* 1985). Insect attraction in *Drosophyllum* is also likely to be exerted by the characteristic sweet, honey-like scent of its leaves (Lloyd 1942; Juniper *et al.* 1989; and personal observations by the authors). Certainly, attraction mechanisms in this species deserve further investigation, which is currently underway in our institution.

An efficient insect-luring mechanism based on an olfactory attraction might cause a pollinator-prey conflict (Jürgens *et al.* 2012). However, although results must be taken as tentative since they come from a single population, the low similarity of the pollinator and prey guilds indicate that such a conflict would not exist. The most frequent pollinators in *Drosophyllum* were small sized Hymenopterans and Coleopterans, whereas the most common prey were Dipterans and, to a lesser extent, Lepidopterans (mostly moths, not observed as flower visitors) and Coleopterans. The sticky mucilage of *Drosophyllum* leaf glands is based on polysaccharides with an acid reaction, producing a noticeable honey-like odour (Adlassnig *et al.* 2010), which is reflected in *Drosophyllum* prey composition: flies, especially fruit flies, moths and green lacewings, all known to be lured by sweet scents (Saad and Bishop 1976; El-Sayed *et al.* 2005; Becher *et al.* 2010). The chemical composition of volatile compounds in *Drosophyllum* is currently being studied (J.M. Igartuburu, unpublished), but it is known that the wonderfully modified leaf traps of the Venus flytrap *Dionaea muscipola* strongly attract fruit flies by producing volatile organic compounds different to those produced by flowers (Kreuzwieser *et al.* 2014).

We conclude that seemingly simple, adhesive traps such as those of *Drosophyllum* are effective at luring prey insects, as Darwin (1875) first noticed. Much more effective than what would be expected from adhesive artificial traps of similar shape and size, unlike what Jürgens *et al.* (2009) suggested and what has been recently shown in another flypaper carnivorous species using a similar experimental approach (Foot *et al.* 2014). Prey attraction seems to be related to visual cues and/or scent, but unrelated to flower scent, as inferred from the virtual lack of prey-pollinator overlap. To explore this further, olfactory bioassays will be performed and volatile organic compounds emitted by leaves and flowers will be analysed by means of mass spectrometry. Such dissimilarity in attracting signals between leaf traps and flowers is surely more widespread among carnivorous plants (e.g. Kreuzwieser *et al.* 2014) than previously thought (Jürgens *et al.* 2012).

3.6 Acknowledgements

We thank Toñi Salces and Ramón Casimiro-Soriguer for help with pollinator censuses. We also thank Ceferino Carrera, from the *Servicio de Invernadero (UCA)* for taking care of the rearing of the plants in the University glasshouse prior to the field experiment. Jose Manuel Igartuburu kindly carried out the HS-SPME-GC-MS assay in his lab to confirm the lack of volatile compounds in the mice glue. Four anonymous referees provided helpful comments on an earlier draft. The Andalusian *Consejería de Medio Ambiente* provided the necessary permits to work with *Drosophyllum lusitanicum*, an endemic, red-listed species. Funding was provided by project BREATHAL (CGL2011-28759/BOS; *Spanish Ministerio de Ciencia e Innovación-MICINN*). MP was supported by a Spanish FPI grant from the *MICINN*.

3.7 References

Adamec L. 1997. Mineral nutrition of carnivorous plants: a review. *Botanical Review* **63**:273-299.

Adlassnig W, Lendl T, Peroutka M, Lang I. (eds.) 2010. Deadly glue—adhesive traps of carnivorous plants. In: *Biological Adhesive Systems*. New York: Springer, 15-28.

Adlassnig W, Peroutka M, Eder G, Pois W, Lichtscheidl IK. 2006. Ecophysiological observations on *Drosophyllum lusitanicum*. *Ecological Research* **21**:255-262.

Anderson B, Midgley JJ. 2001. Food or sex; pollinator-prey conflict in carnivorous plants. *Ecology Letters* **4**:511-513.

Bates D, Maechler M, Bolker B, Walker, S. 2014. *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.0-6. <http://CRAN.R-project.org/package=lme4>.

Beals EW. 1984. Bray-Curtis ordination: an effective strategy for analysis of multivariate ecological data. *Advances in Ecological Research* **14**:1-55.

Becher PG, Bengtsson M, Bill S, Hansson BS, Witzgall P. 2010. Flying the fly: long-range flight behavior of *Drosophila melanogaster* to attractive odors. *Journal of Chemical Ecology* **36**:599–607.

Bennett KF, Ellison AM. 2009. Nectar, not colour, may lure insects to their death. *Biology Letters* **5**:469-472.

Benzing DH. 1987. The origin and rarity of botanical carnivory. *Trends in Ecology* and *Evolution* **2**:364-369.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JS. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology* and *Evolution* **24**:127-135.

Chase MW, Christenhusz MJ, Sanders D, Fay MF. 2009. Murderous plants: Victorian Gothic, Darwin and modern insights into vegetable carnivory. *Botanical Journal of the Linnean Society* **161**:329-356.

Chin L, Chung AYC, Clarke C. 2014. Interspecific variation in prey capture behavior by co-occurring *Nepenthes* pitcher plants. Evidence for resource partitioning or sampling-scheme artifacts? *Plant Signaling* and *Behavior* **9**:e27930,

Correia E, Freitas H. 2002. *Drosophyllum lusitanicum*, an endangered West Mediterranean endemic carnivorous plant: Threats and its ability to control available resources. *Botanical Journal of the Linnean Society* **140**:383-390.

Darwin C. 1875. *Insectivorous Plants*. London: John Murray.

Ellison AM. 2006. Nutrient limitation and stoichiometry of carnivorous plants. *Plant Biology* **8**:740-747.

Ellison AM, Gotelli NJ. 2001. Evolutionary ecology of carnivorous plants. *Trends in Ecology* and *Evolution* **16**:623-629.

Ellison AM, Gotelli NJ. 2009. Energetics and the evolution of carnivorous plants--Darwin's 'most wonderful plants in the world'. *Journal of Experimental Botany* **60**:19-42.

El-Sayed A, Heppelthwaite V, Manning L, Gibb A, Suckling D. 2005. Volatile constituents of fermented sugar baits and their attraction to lepidopteran species. *Journal of Agricultural* and *Food Chemistry* **53**:953-958.

Farnsworth EJ, and Ellison AM. 2008. Prey availability directly affects physiology, growth, nutrient allocation and scaling relationships among leaf traits in 10 carnivorous plant species. *Journal of Ecology* **96**:213-221.

Foot G, Rice SP, Millett J. 2014. Red trap colour of the carnivorous plant *Drosera rotundifolia* does not serve a prey attraction or camouflage function. *Biology Letters* **10**:20131024.

Garrido B, Hampe A, Marañón T, Arroyo J. 2003. Regional differences in land use affect population performance of the threatened insectivorous plant *Drosophyllum lusitanicum* (Droseraceae). *Diversity and Distributions* **9**:335-350.

Givnish TJ, Burkhardt EL, Happel RE, Weintraub JD. 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. *American Naturalist* **124**:479-497.

Hanslin HM, Karlsson PS. 1996. Nitrogen uptake from prey and substrate as affected by prey capture level and plant reproductive status in four carnivorous plant species. *Oecologia* **106**:370-375.

Heubl G, Bringmann G, Meimberg H. 2006. Molecular phylogeny and character evolution of carnivorous plant families in Caryophyllales—revisited. *Plant Biology* **8**:821-830.

Jaccard P. 1901. Etude comparative de la distribution florale dans une portion des Alpes et du Jura. *Bulletin de la Société Vaudoise des Sciences Naturalles* **37**:547-579.

Joel DM, Juniper BE, Dafni A. 1985. Ultraviolet patterns in the traps of carnivorous plants. *New Phytologist* **101**:585-593.

Juniper BE, Robins RJ, Joel DM. 1989. *The Carnivorous Plants*. San Diego: Academic Press Limited.

Jürgens A, El-Sayed AM, Suckling DM. 2009. Do carnivorous plants use volatiles for attracting prey insects? *Functional Ecology* **23**:875-887.

Jürgens A, Sciligo A, Witt T, El-Sayed AM, Suckling DM. 2012. Pollinator-prey conflict in carnivorous plants. *Biological Reviews* **87**:602-615.

Karlsson PS, Nordell KO, Eirefelt S, Svensson A. 1987. Trapping efficiency of three carnivorous *Pinguicula* species. *Oecologia* **73**:518-521.

Kreuzwieser J, Scheerer U, Kruse J, Burzlaff T, Honsel A, Alfarraj S, Georgiev P, Schnitzler JP, Ghirardo A, Kreuzer I, Hedrich R, Rennenberg H. 2014. The Venus flytrap attracts insects by the release of volatile organic compounds. *Journal of Experimental Botany* **65**:755-766.

Król E, Płachno BJ, Adamec L, Stolarz M, Dziubińska H, Trębacz K. 2012. Quite a few reasons for calling carnivores ‘the most wonderful plants in the world’. *Annals of Botany* **109**:47-64.

Kurup R, Johnson AJ, Sankar S, Hussain AA, Kumar CS, Sabulal B. 2013. Fluorescent prey traps in carnivorous plants. *Plant Biology* **15**:611-615.

Lloyd FE. 1942. *The Carnivorous Plants*. New York: The Ronald Press Company.

Meyer DA, Dewèvre DA. 1894. Ueber *Drosophyllum lusitanicum*. *Botanisches Centralblatt* **60**:33-41.

Moran JA. 1996. Pitcher dimorphism, prey composition and mechanisms of prey attraction in the pitcher plant Nepenthes rafflesiana in Borneo. *Journal of Ecology* **84**:515-525.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, Wagner H. 2013. *vegan: Community Ecology Package*. R Foundation for Statistical Computing, Vienna, Austria.

Ortega-Olivencia A, Carrasco Claver JP, Devesa Alcaraz JA. 1995. Floral and reproductive biology of *Drosophyllum lusitanicum* (L.) Link (Droseraceae). *Botanical Journal of the Linnean Society* **118**:331-351.

Ortega-Olivencia A, López Paredes JA, Rodníguez-Riaño T, Devesa JA. 1998. Modes of self-pollination and absence of cryptic self-incompatibility in *Drosophyllum lusitanicum* (Droseraceae). *Botanica Acta* **111**:474-480.

Paiva J. (ed.) 1997. Drosophyllum Link. In: *Flora Iberica*, 5 ed., Castroviejo, S., Aedo, C., Laínz, M., Muñoz Garmendia, F., Nieto Feliner, G., Paiva, J. and Benedí, C., Real Jardín Botánico, Madrid: CSIC, 78-80.

Pavlovic A, Krausko M, Libiaková M, Adamec L. 2014. Feeding on prey increases Photosynthetic efficiency in the carnivorous sundew *Drosera capensis*. *Annals of Botany* **113**:69-78.

Saad AB, Bishop G. 1976. Attraction of insects to potato plants through use of artificial honeydews and aphid juice. *Entomophaga* **21**:49-57.

Schaefer HM, Ruxton GD. 2008. Fatal attraction: Carnivorous plants roll out the red carpet to lure insects. *Biology Letters* **4**:153-155.

Thorén LM, Tuomi J, Kämäräinen T, Laine K. 2003. Resource availability affects investment in carnivory in *Drosera rotundifolia*. *New Phytologist* **159**:507–511.

Thum M. 1988 The significance of carnivory for the fitness of Drosera in its natural habitat. *Oecologia* 75: 472-480.

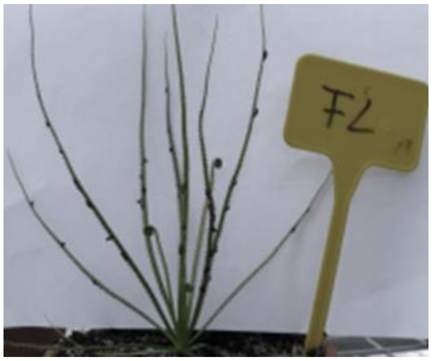
Ver Hoef JM, Boveng PL. 2007. Quasi-Poisson vs. negative binomial regression: how should we model overdispersed count data? *Ecology* **88**:2766-2772.

Vuong HQ. 1989. Likelihood ratio tests for model selection and non-nested hypotheses. *Econometrica* 57: 307–333.

Weiss HB, Soraci FA, McCoy jr EE. 1942. The behaviour of certain insects to various wavelengths of light. *Journal of the New York Entomological Society* **50**:1-35.

Zamora R, Gómez JM, Hódar JA. 1998. Fitness responses of a carnivorous plant in contrasting ecological scenarios. *Ecology* **79**:1630-1644.

Zamora R. 1999. Conditional outcomes of interactions: the pollinator-prey conflict of an insectivorous plant. *Ecology* **80**:786-795.



CHAPTER 4

Plant carnivory beyond bogs: reliance on prey feeding in *Drosophyllum lusitanicum* (Drosophyllaceae) in dry Mediterranean heathland habitats

CHAPTER 4

Plant carnivory beyond bogs: reliance on prey feeding in *Drosophyllum lusitanicum* (Drosophyllaceae) in dry Mediterranean heathland habitats

Maria Paniw, Ester Gil-Cabeza, and Fernando Ojeda

This chapter is under review in *Annals of Botany*.

4.1 Abstract

From a cost-benefit framework, plant carnivory is hypothesized to be an adaptation to nutrient-poor soils in sunny, wetland habitats. However, apparent exceptions to this cost-benefit model exist, although they have been rarely studied. One of these exceptions is the carnivorous subshrub *Drosophyllum lusitanicum*, which thrives in Mediterranean heathlands on dry sandstone soils and has relatively well developed, xeromorphic roots. Our aim was to assess the role of leaf (carnivory) and root (soil) nutrient uptake in growth promotion of this particular species. In a greenhouse experiment, we fed plants with lab-reared fruit flies (*Drosophila virilis*) and added two concentrations of soil nutrients in a factorial design. We recorded aboveground plant growth and final above- and belowground dry biomass after 13 weeks. We also tested for nutrient uptake via roots using stable nitrogen isotope analysis. Insect feeding resulted in significantly higher growth and above- and belowground biomass compared to soil fertilization. No additional benefits of fertilization were discernable when plants were insect-fed, indicating that roots were not efficient in nutrient absorption. We provide the first evidence of strong reliance on insect prey feeding in a dry-soil carnivorous plant with well-developed roots, suggesting that carnivory *per se* does not preclude persistence in dry habitats. Instead, the combination of carnivory and xeromorphic root features allow *Drosophyllum* to thrive on non-waterlogged soils. This study adds to recent research emphasizing the role of root systems of

carnivorous plants in explaining their distribution, partly challenging the cost-benefit hypothesis.

Keywords: carnivorous plant root, dry-soil carnivorous plant, insect prey, pyrophyte, soil nutrient uptake, stable isotope analysis

4.2 Introduction

Intensively studied by Darwin (1875) in his treatise *Insectivorous Plants*, plant carnivory is arguably the most captivating adaptation to nutrient-poor soils (Adamec 1997; Ellison and Gotelli 2001; Król *et al*. 2012). The uptake and assimilation of nutrients via modified leaf structures has evolved at least nine times independently across the angiosperms (Givnish 2015), with ca. 600 extant species of carnivorous plants in the world's flora (Król *et al*. 2012; Givnish 2015). The nutrition of carnivorous plants has been studied on various species, with a strong focus on sundews (*Drosera* spp.: Darwin 1878; Karlsson and Pate 1992; Adamec 2002; Thorén *et al*. 2003; Millett *et al*. 2012), butterworts (*Pinguicula* spp.: Karlsson and Carlsson 1984; Karlsson *et al*. 1991; Hanslin and Karlsson 1996), and pitcher plants (*Nepenthes*/*Sarracenia* spp.: Schulze *et al*. 1997; Moran *et al*. 2001; Gotelli and Ellison 2002; Butler and Ellison 2007; Farnsworth and Ellison 2008). These studies have supported the hypothesis that carnivorous plants benefit from captured prey insects by acquiring mineral nutrients, mainly nitrogen and phosphorus (Ellison 2006; Farnsworth and Ellison 2008).

Most carnivorous plants are restricted to nutrient-poor, wet soils in sunny habitats (Ellison and Gotelli 2001; Brewer *et al*. 2011; Pavlovič and Saganová 2015). These environmental associations led Givnish *et al*. (1984) to propose a cost-benefit model for the evolution of plant carnivory and its general restriction to sunny, infertile wetlands. According to this model, the net benefit of carnivory, *i.e.*, the photosynthetic gain in terms of leaf production minus the cost of producing and maintaining specialized prey-trapping structures, is predicted to be largest when soil nutrient availability is the major limiting factor to plant growth but light and soil water are readily available. Several studies have since investigated nutrition in carnivorous plants, demonstrating that species vary widely in their capacity to assimilate mineral nutrients from soil (Adamec 1997; Schulze *et al*. 1997; Ellison 2006; Adamec 2010; Król *et al*. 2012). Support for the cost-benefit model comes in particular from studies showing that reliance on prey nutrients decreases with the increase in soil nutrient availability (e.g., Benzing 1987; Karlsson and Pate 1992; Millett *et al*. 2012) or shade (Givnish *et al*. 1984; Schulze *et al*. 2001).

More recently, extensions or alternatives to the cost-benefit model have been proposed (Benzing, 2000; Brewer *et al*. 2011; Abbott and Brewer 2016). Brewer *et al*. (2011), for instance, hypothesized that the characteristic, weakly developed and low-porosity roots, rather than low soil fertility *per se*, might explain the general restriction of carnivorous plants to boggy, waterlogged soils and their disadvantage in drier, non-waterlogged soils. However, carnivorous plant species that thrive in dry habitats and appear to contradict the predictions of the cost-benefit model have received far less attention in the literature, despite potentially providing significant novel insights into the evolution of plant carnivory (Givnish *et al*. 1984; Givnish 2015). One prominent example is the subshrub *Drosophyllum lusitanicum* (L.) Link (Drosophyllaceae). This species (*Drosophyllum*, hereafter) is the only extant species of the family Drosophyllaceae (Heubl *et al*. 2006) and is endemic to the western Iberian Peninsula and northern Morocco (Garrido *et al*. 2003). Across its range, *Drosophyllum* is restricted to fire-prone Mediterranean heathlands on acid, nutrient-poor, dry soils, subject to a moderate summer drought (Adlassnig *et al*. 2006; chapter 6).

Unlike most other carnivorous plant species, many *Drosophyllum* individuals maintain their complex, sticky mucilage on leaves to capture prey even under unfavorable conditions in the dry summer months (Adlassnig *et al*. 2006; Adamec 2009). Another difference between *Drosophyllum* and most other carnivorous plant species is that the root system of the former is relatively well developed, consisting of a branched tap-root with xeromorphic features (Carlquist and Wilson 1995; Adlassnig *et al.* 2005, 2006; Adamec 2009). Despite being one of the few carnivorous plant species with deep, large root systems, no research has been done on the putative role of roots for soil nutrient uptake in this species (Adlassnig *et al*. 2005, 2006). The taxonomic uniqueness and habitat particularity of *Drosophyllum* make the species a valuable system for investigating the importance of leaves *vs*. roots in nutrient acquisition and growth promotion of carnivorous plants in dry habitats.

Here, we studied plant nutrition in *Drosophyllum* plants through leaves (prey insects) and roots (soil nutrients) and the effect of nutrient uptake from the two sources on aboveground growth and above- (leaf) and belowground (root) biomass allocation. Given the scarcity of fine lateral roots in this species (Adamec 2009), we hypothesized that leaf nutrient uptake from trapped insects will determine plant growth, with a low contribution, if any, of soil nutrient uptake from roots, despite their considerable size and depth (Adlassnig *et al*. 2005). To test this hypothesis, we performed a full-factorial greenhouse experiment in which we fed juvenile plants growing on a substrate mixture of siliceous sand and peat moss via leaves (fruit flies) and/or soil (Hoagland's nutrient solution). We recorded aboveground growth as well as final dry biomass of above-ground (leaves) and belowground (roots) plant parts and compared them between treatments. Since the Hoagland's nutrient solution used had an anomalously high δ^{15}N value (see Materials and Methods), we measured δ^{15}N values in the above- (leaves) and belowground (roots) tissue of plants from the different treatments to ascertain the capability of the plants to absorb mineral nutrients from the roots.

4.3 Materials and methods

4.3.1 Growth of plants and experimental design

We grew *Drosophyllum* plants in the University of Cádiz greenhouse from seeds collected in July 2014 from 80 individuals randomly chosen at five sites (16 individuals per site). We mixed all seeds to provide a homogeneous pool and, on February 2nd 2015, we randomly took 200 seeds from the pool and exposed them to dry heat (100º C) during 5 min to break seed dormancy (Correia and Freitas 2002). We then sowed these seeds in seedling trays with a 1:1 mixture of siliceous sand and peat moss and selected the first 120 emerged seedlings for the experiment. The seedlings emerged 20-26 days after sowing and were then individually transplanted into 0.5-L clay pots containing the same mixture of siliceous sand and peat moss. This low fertility soil mixture is commonly used in nutrient addition experiments for carnivorous plants (e.g., Butler and Ellison 2007) and approximates the low fertility conditions of Mediterranean heathland soils (Ojeda *et al.* 2010). The pH of this substrate, measured in a saturated soil paste, was ca. 4.5, also similar to the pH of Mediterranean heathland soils (Ojeda *et al*. 2010).

We grew the 120 potted seedlings in the greenhouse at ambient temperature, but never exceeding 25ºC, and keeping relative humidity around 70-90 % throughout the whole experiment, resembling ambient conditions of natural *Drosophyllum* populations during the spring growing season (chapter 8). During the night, the lowest temperature recorded in the greenhouse was 15ºC. Pots were kept moist via a sprinkling system mounted above the pots that sprayed decalcified water during daytime for 30 s in 2-h intervals. We used decalcified water because soil Ca is toxic to most carnivorous plants (Adlassnig *et al*. 2005), including *Drosophyllum* (Adlassnig *et al*. 2006). We maintained the temperature regime and periodic sprinkling throughout the study. In addition, before initiating the nutrient addition experiment, we watered the pots three-times a week with 50 ml of decalcified water. On March 12th 2015, 14 days after being transplanted, the seedlings were large enough (5-7 leaves of 5.0 (± 0.3 SD) cm length) to start the feeding experiment, which extended for 11 weeks until May 27th 2015, lasting a total of 91 days since seedling emergence.

The experiment was performed in a full-factorial design with “insect feeding” (two treatment levels: *F* and *NF*) and “soil fertilization” (three treatment levels: *H*, *L* and *O*) as fixed factors. The 120 potted seedlings were randomly divided into two equal-sized groups, one of which, the *F* treatment, was supplied with fruit flies (*Drosophila virilis;* ca. 0.3 mg dry weight per fly) and the other, the *NF* treatment, was not. Each plant of the *F* treatment received three flies per leaf in the first two weeks of the experiment, increasing the number of flies by two more per leaf each additional week until the sixth week, when the number of flies per leaf increased to four more each week. The *D. virilis* fruit flies used throughout the experiment were reared in a carbohydrate-rich medium under standard culture conditions and were kept frozen in vials at -20ºC prior to usage.

Plants of the *F* and *NF* groups were further split into three subgroups (20 plants each) for the “soil fertilization” treatments: three times per week for the duration of the experiment, plants in each subgroup received 50 ml of 1/10 strength nutrient solution (*H* treatment), 50 ml of 1/20 strength nutrient solution (*L* treatment) or 50 ml of distilled water

(*O* treatment). We used a balanced nutrient mixture (Hoagland's No. 2 Basal Salt Mixture; Sigma-Aldrich, Saint Louis, USA) to avoid potential deficiencies of some nutrients caused by abundance of another. Similar dilutions have been used in feeding experiments for other carnivorous plant species (e.g., Butler and Ellison, 2007). Plants in the *NF-O* treatment combination, receiving neither flies nor soil nutrients, were considered as control. Each time before treatment application, pots were haphazardly shuffled on the greenhouse bench to avoid a location effect.

In order to assure that the amount of nutrients provided to plants via flies or soil solution did not differ substantially, we determined the amount of nitrogen available to plants from either source. The amount of nitrogen in flies was measured as described for plant samples in "*$\delta^{15}N$ analysis*" below. Throughout the nutrient-addition experiment, plants in the corresponding treatment groups were weekly supplied with approximately 3.1 (H treatment) and 1.05 (L treatment) mg N through the soil. The fine texture of the moss peat in the soil medium aided in retaining the nutrient solution and water. Plants in the FO treatment received a total of approximately 2.1 mg of N from insects, which corresponded to 60 % (range: 52 % - 69 %) of their total N pool. We assumed that the relative concentrations of other nutrients to N were similar between flies and fertilizer.

To track the aboveground growth of plants under different treatment combinations, we counted the number of fully developed leaves and measured the length (cm) of the longest leaf on each plant at the beginning of the experiment (day 14 after emergence) and every week or second week until the end of the experiment (11 weeks later; day 91 after emergence). We then defined *size* as the number of leaves × length of longest leaf (cm). This size measure is biologically significant as it approximates the available leaf area for prey capture and has been used in other studies of this species (M. Paniw, unpublished). Once the experiment was terminated, we removed plants from the pots, washed them in distilled water to remove fruit flies from leaves and soil from roots, separated above-ground (shoot) and below-ground (root) material of each plant, and oven-dried them for 72 h at 65ºC to constant weight . We then weighed the shoot and root dry biomass of each plant to the nearest 0.01 mg.

4.3.2 δ^{15}N analysis

Previous analyses found an average δ^{15}N signature of 18.6 ‰ (range: 18.0-19.0 ‰) in the Hoagland's nutrient solution used in this study (Hoagland's No. 2 Basal Salt Mixture; Sigma-Aldrich, Saint Louis, USA), an anomalously high value for standard synthetic fertilizers ($\delta^{15}N = -0.2 \pm 2.1$ ‰, mean ± SD; Bateman and Kelly, 2007), and much higher than the δ^{15}N signature detected in *Drosophila virilis* flies (range: 2.8-3.0 ‰). This highly δ^{15}N enriched nutrient solution provided an excellent means to explore whether *Drosophyllum* plants were able to take up and assimilate soil nutrients through the roots. After being weighed, shoot and root dry biomass samples of all *Drosophyllum* plants from the nutrient addition experiment were separately placed into plastic vials (up to three samples per plant part if enough biomass was produced), ground to powder using stainless steel beads on a Mixer Mill MM400 cell disrupter (Retsch, Llanera, Spain), and analyzed for % N and δ^{15}N using combustion in the elemental analyzer Flash EA1112 interfaced with Finnigan Tracer Mass Isotope Ratio Mass Spectrometer. Analyses were performed at the Analytical Service Laboratory from the University of A Coruña (Spain). Results of δ^{15}N are expressed in per mil (‰) where $\delta = [(^{15}N/^{14}N) - 1] \times 1000$. All δ^{15}N values had a precision of 0.3 ‰.

4.3.3 Statistical analysis

The overall effects of insect feeding (*F*, *NF*), soil fertilization (*H*, *L*, *O*) and their interaction on above-ground size changes over time were determined by means of a two-way repeated-measures ANOVA. The plant size variable was log-transformed prior to analysis to meet the homoscedasticity assumption. We also explored the effects of insect feeding (*F*, *NF*) and soil fertilization (*H*, *L*, *O*) on the final dry biomass (g) of the aboveground (shoot) and below-ground (root) portions of the plants by performing a two-way MANOVA. Shoot and root dry biomass variables were previously log-transformed to ensure the homoscedasticity assumption of MANOVA. Post-hoc comparisons through Tukey's HSD tests to search for pairwise differences between the six treatment combinations were implemented separately for shoot and root dry biomass variables. An equivalent analysis for whole-plant biomass can be found in Appendix S1.

In order to explore whether plants provided with soil nutrients changed their root-to-shoot allocation patterns, we calculated the percentage contribution of roots to the total plant dry biomass, and tested significant differences between the six treatments by using a non-parametric Kruskal-Wallis rank test. Finally, we also used the Kruskal-Wallis rank test to search for differences in the $\delta^{15}N$ signature of the above- (shoot) and belowground (root) tissue of plants between the six treatment combinations. As the Kruskal-Wallis rank test corresponds to a nonparametric one-way ANOVA, subsequent post-hoc pairwise comparisons between treatment combinations were done using Bonferroni-corrected Mann-Whitney U-tests. All statistical analyses were performed in R (R Core Team 2015).

4.4 Results

Insect-fed plants grew more than four times as much as not insect-fed plants during the experiment (Fig. 1) and produced a more than five times higher dry biomass, both above- and belowground (Fig. 2), regardless of soil fertility conditions.

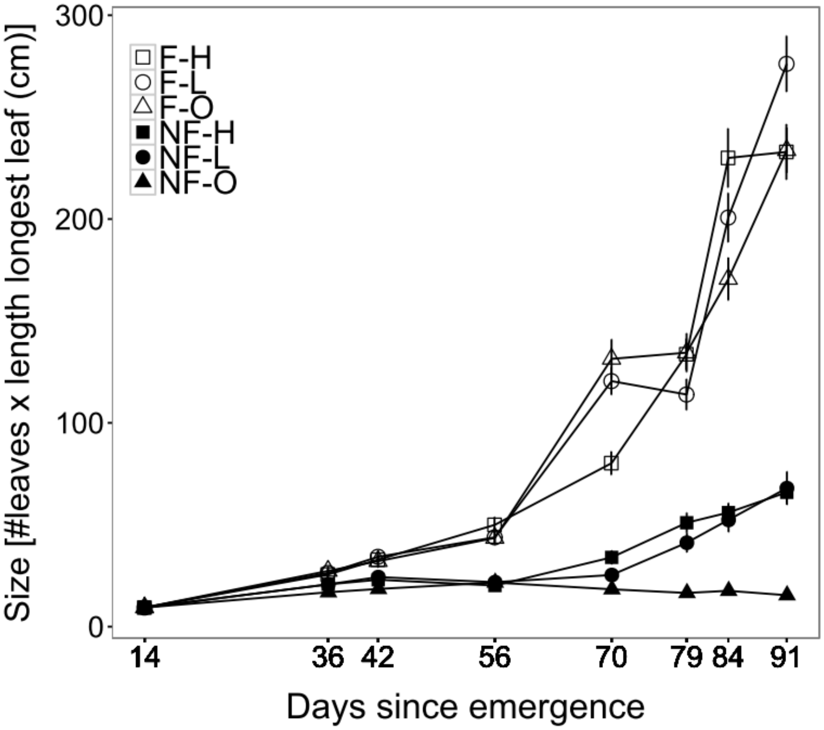


Figure 1 Changes in size, defined as # of leaves × length of longest leaf (cm), of *Drosophyllum* plants through time (days) as a function of two treatments, insect feeding (F / NF) and soil fertilization (H / L / O), and their interaction, resulting in six treatment groups.

The two-way repeated-measures ANOVA detected significant effects of the two factors, insect feeding and soil fertilization, on relative plant growth (Table 1; Fig. 1). In addition, plant size changed significantly with time (days after sowing), with plants growing significantly faster when fed with flies compared with unfed plants (Table 1; Fig. 1). Correspondingly, the two-way MANOVA showed significant effects of both factors on the final dry biomass of above- (shoot) and belowground (root) portions of plants, and a significant interaction between the two factors (Table 2; Fig. 2). The significant interaction effect stemmed from soil fertilization having a slight but significant effect on final dry biomass only when plants were not supplied with fruit flies (Table 2). No significant differences in final dry biomass were detected between the *H* and *L* soil fertilization levels (Fig. 2). Insect-fed plants grew much larger, both above- and below-ground, than soil-fed plants, and no additive effects of soil fertilization on them were detected (Fig. 2).

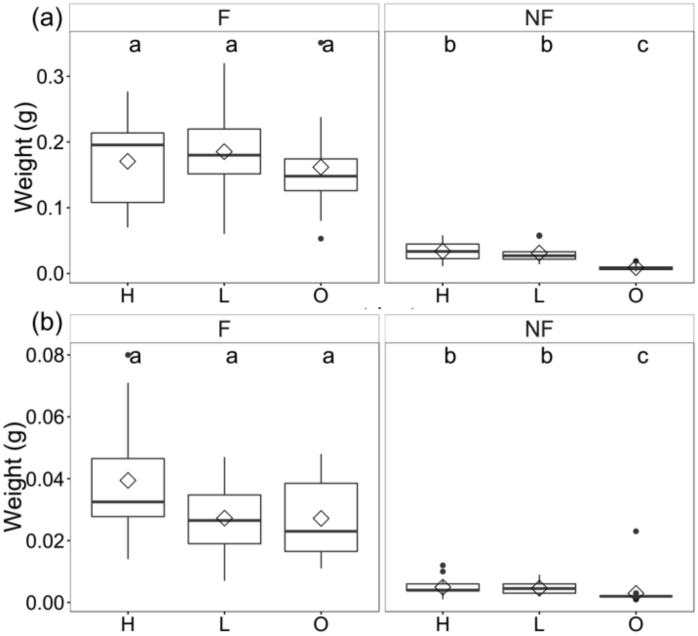


Figure 2 Box-plot of dry biomass of (a) above-ground (shoot) and (b) below-ground (root) parts of *Drosophyllum* plants measured at the end of the nutrient addition experiment as a function of two main treatments and their interactions: feeding with flies (F) or no feeding (NF) and addition of high-strength (H), low-strength (L) nutrient solution or distilled water (O). The combinations of the treatment levels resulted in six treatment groups. Different letters represent significant pairwise differences (Tukey´s HSD, $P < 0.05$) of group means (diamonds) between the six treatment groups. Note that the statistical comparisons were performed on the log-transformed biomass measure to ensure variance homoscedasticity.

On average, the root portion of *Drosophyllum* plants made up 14.7 % (± 0.06 SD) of the total plant dry biomass. This result was similar to previous investigations of root/shoot ratios in *Drosophyllum* (Adamec, 2009). No significant differences in the relative contribution of roots to total plant biomass were found between the six treatment combinations (Kruskal-Wallis $\chi^2 = 9.6$, d.f. = 5, p-value = 0.1).

Table 1 Two-way repeated-measures ANOVA of the effects of insect feeding, soil fertilization, and their interaction on changes in above-ground size through time (days since emergence) of *Drosophyllum* plants.

	df	Mean squares	*F*-ratio	*P*-value
Response: plant size				
Between-group effect: Error (plant ID)				
Insect feeding	1	1.4×10^6	483.0	< 0.01
Soil fertilization	2	1.3×10^4	4.5	0.01
Insect feeding × Soil fertilization	2	6.5×10^3	2.2	0.1
Residuals	113	2.9×10^3		
Within-subject effect				
Days	6	2.8×10^5	668.7	< 0.01
Insect feeding × Days	6	1.6×10^5	395.3	< 0.01
Soil fertilization × Days	12	6.9×10^3	16.3	< 0.01
Insect feeding × Soil fertilization × Days	12	3.4×10^3	8.1	< 0.01
Residuals	678	419		

Table 2 Two-way MANOVA, using the Pillai test statistic, of the effects of "insect feeding" and "soil fertilization" factors and their interaction on above- (shoot) and below-ground (root) dry biomass (g).

	df	Pillai	approx *F*	num df	dem df	*P*-value
Shoot and root dry biomass (g)						
Insect feeding	1	0.865	170.172	2	53	< 0.01
Soil fertilization	2	0.352	5.767	4	108	< 0.01
Insect feeding × Soil fertilization	2	0.369	6.099	4	108	< 0.01
Residuals	53					

Soil-fertilized plants presented significantly higher δ^{15}N signatures in both shoot and root tissues than not fertilized plants, regardless of being supplied with fruit flies on the leaves or not (Fig. 3; Kruskal-Wallis $\chi^2 = 70.0$, d.f. = 5, $p < 0.05$).

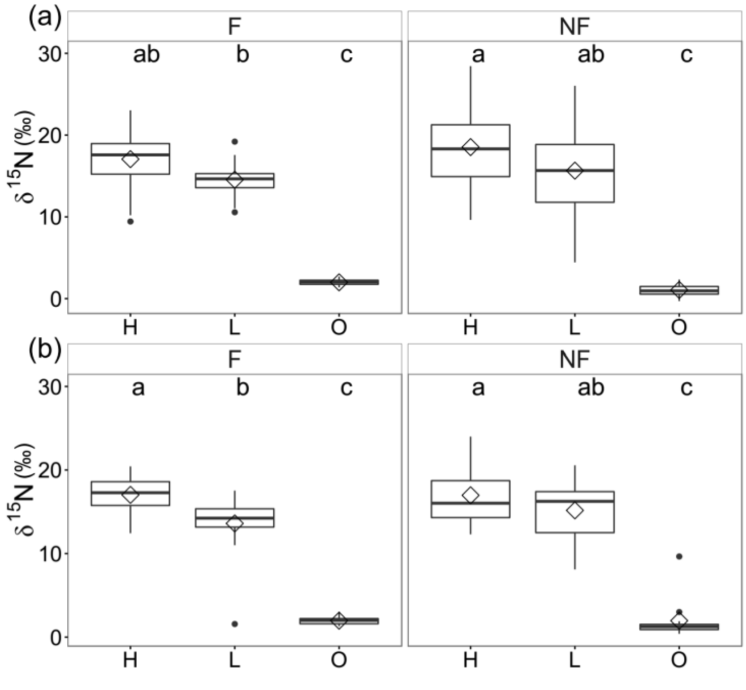


Figure 3 Box-plot of δ^{15}N in (a) shoot and (b) root tissues of *Drosophyllum* plants measured at the end of the nutrient addition experiment as a function of two main treatments and their interactions: feeding with flies (F) or no feeding (NF) and addition of high-strength (H) or low-strength (L) nutrient solution or distilled water (O). Different letters represent significant pairwise differences (Kruskal-Wallis $\chi^2 = 70.0$, d.f. = 5, $p < 0.05$) of group means (diamonds) between the six treatment groups.

Taking into account the high $\delta^{15}N$ values of the nutrient solution used for soil fertilization (see Materials and methods), this result indicates that *Drosophyllum* plants are able to take up and assimilate soil nutrients through the roots. It shall be noted that higher $\delta^{15}N$ signatures were detected in *H* fertilized than in *L* fertilized plants (Fig. 3), although higher fertilization strength did not cause an increase in plant growth (Figs. 1 and 2).

4.5 Discussion

Carnivorous plants are predicted to benefit from prey capture under a specific set of environmental conditions, *i.e.*, nutrient-poor, wet soils and open habitats, which offset the cost of producing trapping structures (Givnish *et al*. 1984; Benzing 1987, 2000). However, our nutrient addition experiment provides the first evidence that a strong carnivorous syndrome may evolve in dry environments. *Drosophyllum* plants invest resources in carnivorous structures as well as in well developed, deep roots (Adlassnig 2005, 2006) that, however, seem to play only a minor role in nutrient acquisition. Plants fed with insects in the greenhouse acquired on average more than five times as much biomass as soil-fertilized plants, with root nutrient uptake showing no additive benefits to plant growth (Fig. 2). These results support the hypothesis that root functionality other than nutrient acquisition (e.g. securing water availability) may be a key factor determining the distribution of carnivorous plants with respect to soil moisture (Brewer *et al*. 2011). Indeed, unlike most other carnivorous plant species, *Drosophyllum* produces large, xeromorphic roots most likely as an adaptation to water uptake in non-waterlogged soils under a Mediterranean climate (Carlquist and Wilson, 1995; Adlassnig *et al*. 2005). Both, the xeromorphic root features (for soil water acquisition) and carnivory (for nutrients) may allow this species to persist in nutrient-poor, dry Mediterranean heathlands.

The strong reliance of *Drosophyllum* on prey-derived nutrients for growth highlighted by our greenhouse study is corroborated by field observations and field experiments showing great efficiency of plants of this species in attracting prey (Darwin 1875; Chapter 3). Individuals produce complex, mucilagenous stalked glands, multicellular and vascularized with both xylem and phloem vessels (Renner and Specht 2011). It shall be emphasized that this species is, together with the part-time carnivorous *Triphyophyllum peltatum*, the only fly-paper carnivorous species whose glandular trichomes have floem vessels (Renner and Specht 2011). This would allow *Drosophyllum* plants to add phloem sap exudates including carbohydrates and volatile organic compounds to the mucilage droplets, increasing their viscosity and hygroscopicity (carbohydrates; Adlassnig *et al*. 2006, 2010) as well as their efficiency in insect attraction (volatile organic compounds; Jurgens *et al*. 2009). As a result, even juvenile *Drosophyllum* individuals, consisting of one rosette with 10 leaves, may contain > 100 prey insects (chapter 3). The strong carnivorous character in *Drosophyllum* stands out compared with *Byblis lamellata* (Byblidaceae), the only morphologically and ecologically similar carnivorous species, found in non-waterlogged, seasonally dry, siliceous sands (Conran *et al.* 2002). Unlike *Drosophyllum*, *B. lamellata* has simple trapping structures and does not have sessile, proteolytic enzyme-producing glands to directly digest prey insects, but may use insect mutualists that feed on trapped prey to gain nutrients by digesting their faeces (Hartmeyer 1998).

Despite the strong reliance on prey for plant growth, our results indicate that *Drosophyllum* is able to take up soil nutrients from the roots, when available, and assimilate them in both root and leaf tissue (Fig. 3), although growth is far from optimal in the absence of insect prey (Figs. 1 and 2). *Drosophyllum* is a post-fire dwelling species (chapter 5) with life-history adaptations to recurrent fires, which include a mass post-fire recruitment from a persistent soil seed-bank (Müller and Deil 2001). Fires release a flush of mineral nutrients to soil, including N and P, which are quickly (within one year) leached away (Certini 2005; Dijkstra and Adams 2015). By being able to assimilate nutrients from the roots, *Drosophyllum* plants might benefit from that transient, post-fire flush at their early seedling stages, when insect capture is unlikely due to small size. They might hence use it to assist plant growth to prey-capture levels. Similar results have been found for another fire-adapted carnivorous plant, *Dionaea muscipula*, and may also indicate adaptations to post-fire nutrient fluctuations (Gao *et al*. 2015). As lateral roots appear to be lost in mature *Drosophyllum* plants (Adamec 2009), nutrient uptake via roots is likely limited to the seedling and juvenile plant stages, but future studies must determine whether mature *Drosophyllum* individuals can also potentially assimilate nutrients from the soil.

In practice, nutrient absorption via roots in adult *Drosophyllum* plants is likely limited as roots lack adaptations, such as microsymbiont associations or cluster roots, for nutrient scavenging in low-fertility soils (Carlquist and Wilson 1995; Adlassnig *et al*. 2005, 2006). On the other hand, virtually all non-carnivorous plant species in heathland habitats show root adaptations for nutrient scavenging (Lambers *et al*. 2006). Carnivory in *Drosophyllum* may therefore be seen as an alternative strategy to acquire nutrients in nutrient-poor, Mediterranean heathlands, with high specialization to leaf prey capture and digestion to compensate for the lack of root adaptations. Such a trade-off or constraint-avoidance solution has been shown in wetland soils, where carnivorous genera produce shallow, low-porosity roots to prevent hypoxia, obtaining nutrients from prey instead (Karlsson and Pate 1992; Brewer *at al*. 2011; Gao *et al*. 2015).

Despite showing little efficiency in nutrient acquisition, roots may be critical in allowing *Drosophyllum* to persist in dry habitats. In many *Drosophyllum* populations, plants consume prey insects throughout the year, even in the dry summer months (Adlassnig *et al*. 2006; MP and FO, pers. obs.). It has been suggested that plants satisfy a large part of their water demand through the highly hygroscopic mucilage of leaf glands that capture water from air moisture (Adlassning *et al*. 2006; Adamec 2009). However, it is unlikely that hygroscopic mucilage is sufficient to maintain the water balance in *Drosophyllum* individuals, particularly in the dry summers where average air humidity does not exceed 66.5 % (± 9.0 SE) (Appendix S2). The xeromorphic features and relatively large size of tap roots in this species, typical of plants adapted to water-limited soils (Carlquist and Wilson, 1995), indicate that, apart from the anchoring role, roots would play an important role in maintaining the water balance in *Drosophyllum* plants.

4.6 Conclusions

Contrary to the prediction of the cost-benefit analysis of the evolution of plant carnivory, we provide evidence that carnivory may evolve in non-waterlogged, dry soils. Therefore, roots, decoupled from nutrient-acquisition functions, may be critical in determining the distribution of carnivorous genera in response to soil moisture. Previous investigation on the nutrition of carnivorous plants has largely focused on a few genera, all found in boggy or waterlogged soils, where the ecological conditions have favored a reduction of the root system (Brewer 2003; Brewer *et al*. 2011) coupled with a maintenance of flexible nutrient acquisition strategies (e.g., Ellison and Gotelli 2002; Millett *et al*. 2012), or even the ability to switch off carnivory under increasing soil nutrients (Ellison *et al*. 2003). Although it is certainly true that a majority of carnivorous plants are found in waterlogged soils and have reduced, shallow roots (Adlassnig *et al*. 2005; Brewer *et al*. 2011), a full understanding of the carnivorous syndrome can only be gained by considering species that have adapted to extremely low soil fertility conditions with no association to boggy habitats. Our study species, *Drosophyllum lusitanicum*, has complex, sticky glands on their fly-paper-trap leaves and is very effective in attracting prey insects (chapter 3). At the same time, the species is also very effective at avoiding water stress, allowing it to persist on dry soils (Adlassnig *et al*. 2006). Using a unique system, our study supports the hypothesis that root functionality coupled with carnivory may explain the distribution of carnivorous plants better than photosynthetic cost and benefits per se. We therefore urge for more studies on underrepresented carnivorous taxa from non-waterlogged habitats, such as *Byblis* spp. in Australia or epiphytes such as *Catopsis berteroniana* (Adamec 2010) to gain a more complete picture of the link between soil properties and the evolution of plant carnivory beyond bogs.

4.7 Acknowledgements

We thank Beatriz Gasalla and José-Ramón Aracama for helping with the rearing of the fruit flies (*Drosophila virilis*). Ceferino Carrera, from the *Servicio de Invernadero* (UCA) for helping with the experiment in the University glasshouse. The Andalusian Consejería de Medio Ambiente provided the necessary permits to work with *Drosophyllum lusitanicum* , an endemic, red-listed species. Funding was provided by project BREATHAL (CGL2011-28759/BOS, Spanish Ministerio de Ciencia e Innovación-MICINN). M.P. was supported by a Spanish FPI grant from the MICINN.

4.8 References

Abbott MJ, Brewer JS. 2016. Competition does not explain the absence of a carnivorous pitcher plant from a nutrient-rich marsh. *Plant and Soil*, 1-10.

Adamec L. 1997. Mineral nutrition of carnivorous plants: a review. *The Botanical Review* **63**:273-299.

Adamec L. 2002. Leaf absorption of mineral nutrients in carnivorous plants stimulates root nutrient uptake. *New Phytologist* **155**:89-100.

Adamec L. 2009. Ecophysiological investigation on *Drosophyllum lusitanicum*: Why doesn't the plant dry out? *Carnivorous Plant Newsletter* **38**:71–74.

Adamec L. 2010. Carnivorous plants: ecophysiological look at plant carnivory. Why are plants carnivorous? In: Seckbach J, Dubinski Z. eds. *All Flesh is Grass. Plant-Animal Interrelationships. Cellular Origin, Life in Extreme habitats and Astrobiology*. Springer: 457–492.

Adlassnig W, Peroutka M, Eder G, Pois W, Lichtscheidl IK. 2006. Ecophysiological observations on *Drosophyllum lusitanicum*. *Ecological Research* **21**:255-62.

Adlassnig W, Peroutka M, Lambers H, Lichtscheidl IK. 2005. The roots of carnivorous plants. *Plant and Soil* **274**:127-140.

Bateman AS, Kelly SD. 2007. Fertilizer nitrogen isotope signatures. *Isotopes in Environmental and Health Studies* **43**:237-247.

Benzing DH. 1987. The origin and rarity of botanical carnivory. *Trends in Ecology and Evolution* **2**:364-369.

Benzing DH. 2000. *Bromeliaceae: Profile of an Adaptive Radiation*. Cambridge University Press.

Brewer JS. 2003. Why don't carnivorous pitcher plants compete with non-carnivorous plants for nutrients? *Ecology* **84**:451-62.

Brewer JS, Baker DJ, Nero AS, *et al*. 2011. Carnivory in plants as a beneficial trait in wetlands. *Aquatic Botany* **94**:62–70.

Butler JL, Ellison AM. 2007. Nitrogen cycling dynamics in the carnivorous northern pitcher plant, *Sarracenia purpurea*. *Functional Ecology* **21**:835-843.

Carlquist S, Wilson EJ. 1995. Wood anatomy of *Drosophyllum* (Droseraceae): ecological and phylogenetic considerations. *Bulletin of the Torrey Botanical Club* **122**:185-189.

Certini G. 2005. Effects of fire on properties of forest soils: a review. *Oecologia* **143**:1-10.

Conran JG, Lowrie A, Moyle-Croft J. 2002. A revision of *Byblis* (Byblidaceae) in southwestern Australia. *Nuytsia* **15**:11-19.

Correia E, Freitas H. 2002. *Drosophyllum lusitanicum*, an endangered West Mediterranean endemic carnivorous plant: threats and its ability to control available resources. *Botanical Journal of the Linnaean Society* **140**:383–390.

Darwin C. 1875. *Insectivorous plants*. London, UK: John Murray.

Darwin F. 1878. Experiments on the nutrition of *Drosera rotundifolia*. *Journal of the Linnean Society, Botany (London)* **17**:17–23.

Dijkstra FA, Adams MA. 2015. Fire Eases Imbalances of Nitrogen and Phosphorus in Woody Plants. *Ecosystems* **18**:769-779.

Ellison AM. 2006. Nutrient limitation and stoichiometry of carnivorous plants. *Plant Biology* **8**:740-747.

Ellison AM, Gotelli NJ. 2001. Evolutionary ecology of carnivorous plants. *Trends in Ecology* and *Evolution* **16**:623-629.

Ellison AM, Gotelli NJ. 2002. Nitrogen availability alters the expression of carnivory in the northern pitcher plant *Sarracenia purpurea*. *Proceedings of the National Academy of Sciences* **99**:4409–4412.

Ellison AM, Gotelli NJ. 2009. Energetics and the evolution of carnivorous plants—Darwin's 'most wonderful plants in the world'. *Journal of Experimental Botany* **60**:19-42.

Ellison AM, Gotelli NJ, Brewer JS, Cochran-Stafira DL, Kneitel JM, Miller TE, Worley AC, Zamora R. 2003. The evolutionary ecology of carnivorous plants. *Advances in Ecological Research* **33**:1-74.

Farnsworth EJ, Ellison AM. 2008. Prey availability directly affects physiology, growth, nutrient allocation and scaling relationships among leaf traits in 10 carnivorous plant species. *Journal of Ecology* **96**:213-221.

Gao P, Loeffler TS, Honsel A, Kruse J, Krol E, Scherzer S, Kreuzer I, Bemm F, Buegger F, Burzlaff T, Hedrich R. 2015. Integration of trap and root derived nitrogen nutrition of carnivorous *Dionaea muscipula*. *New Phytologist* **205**:1320-1329.

Garrido B, Hampe A, Maranon T, Arroyo J. 2003. Regional differences in land use affect population performance of the threatened insectivorous plant *Drosophyllum lusitanicum* (Droseraceae). *Diversity and Distributions* **9**:335–350.

Givnish TJ. 2015. New evidence on the origin of carnivorous plants. *Proceedings of the National Academy of Sciences* **112**:10-11.

Givnish TJ, Burkhardt EL, Happel RE, Weintraub JD. 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. *American Naturalist* **124**:479-497.

Gotelli NJ, Ellison AM. 2002. Nitrogen deposition and extinction risk in the northern pitcher plant, *Sarracenia purpurea*. *Ecology* **83**:2758-2765.

Hanslin HM, Karlsson PS. 1996. Nitrogen uptake from prey and substrate as affected by prey capture level and plant reproductive status in four carnivorous plant species. *Oecologia* **106**:370-375.

Hartmeyer S. 1998. Carnivory in *Byblis* revisited II: the phenomenon of symbiosis on insect trapping plants. *Carnivorous Plant Newsletter* **27**:110-113.

Heubl G, Bringmann G, Meimberg H. 2006. Molecular phylogeny and character evolution of carnivorous plant families in Caryophyllales—revisited. *Plant Biology* **8**:821-830.

Karlsson PS, Carlsson B. 1984. Why does *Pinguicula vulgaris* L. trap insects? *New Phytologist* **97**: 25-30.

Karlsson PS, Nordell KO, Carlsson BÅ, Svensson BM. 1991. The effect of soil nutrient status on prey utilization in four carnivorous plants. *Oecologia* **86**:1-7.

Karlsson PS, Pate JS. 1992. Contrasting effects of supplementary feeding of insects or mineral nutrients on the growth and nitrogen and phosphorous economy of pygmy species of *Drosera*. *Oecologia* **92**:8-13.

Król E, Płachno BJ, Adamec L, Stolarz M, Dziubińska H, Trębacz K. 2012. Quite a few reasons for calling carnivores 'the most wonderful plants in the world'. *Annals of Botany* **109**:47-64.

Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ. 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany* **98**:693-713.

Millett J, Svensson BM, Newton J, Rydin H. 2012. Reliance on prey-derived nitrogen by the carnivorous plant *Drosera rotundifolia* decreases with increasing nitrogen deposition. *New Phytologist* **195**:182-188.

Moran JA, Merbach MA, Livingston NJ, Clarke CM, Booth WE. 2001. Termite prey specialization in the pitcher plant *Nepenthes albomarginata*—evidence from stable isotope analysis. *Annals of Botany* **88**:307-311.

Müller J, Deil U. 2001. Ecology and structure of *Drosophyllum lusitanicum* (l.) Link populations in the south-west of the Iberian Peninsula. *Acta Botanica Malacitana* **26**:47-68.

Ojeda F, Pausas JG, Verdú M. 2010. Soil shapes community structure through fire. *Oecologia* **163**:729-735.

Pavlovič A, Saganová M. 2015. A novel insight into the cost–benefit model for the evolution of botanical carnivory. *Annals of Botany*, doi:10.1093/aob/mcv050.

R Core Team. 2015. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna: URL http://www.R-project.org/.

Renner T, Specht CD. 2011. A sticky situation: assessing adaptations for plant carnivory in the Caryophyllales by means of stochastic character mapping. *International Journal of Plant Sciences* **172**:889–901.

Schulze W, Schulze ED, Pate JS, Gillison AN. 1997. The nitrogen supply from soils and insects during growth of the pitcher plants *Nepenthes mirabilis*, *Cephalotus follicularis* and *Darlingtonia californica*. *Oecologia* **112**:464-71.

Schulze W, Schulze ED, Schulze I, Oren R. 2001. Quantification of insect nitrogen utilization by the venus fly trap *Dionaea muscipula* catching prey with highly variable isotope signatures. *Journal of Experimental Botany* **52**:1041-1049.

Thorén LM, Tuomi J, Kämäräinen T, Laine K. 2003. Resource availability affects investment in carnivory in *Drosera rotundifolia*. *New Phytologist* **159**:507-511.

4.9 Appendix S1 - Analysis of whole-plant biomass

In the main text, we assess, using MANOVA, whether root and shoot biomass of *Drosophyllum lusitanicum* plants differ between the two treatments used in our study. The treatments consisted of feeding (F) flies to plants or not (NF); and adding high-strength (H), low-strength (L) nutrient solution, or distilled water (O) to the soil. Here, we present results of a two-factor ANOVA measuring differences in whole-plant (roots + shoot) biomass as a function of the two treatments and their interaction:

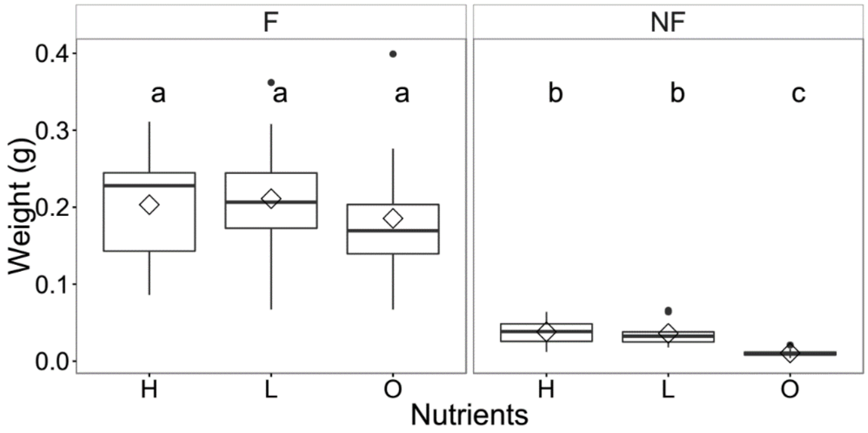


Figure S1.1 Box-plot of biomass of *Drosophyllum* plants measured at the end of the nutrient addition experiment as a function of two main treatments and their interactions: feeding with flies (F) or no feeding (NF) and addition of high-strength (H), low-strength (L) nutrient solution or distilled water (O). The combinations of the treatment levels resulted in six treatment groups. Different letters represent significant pairwise differences (Tukey´s HSD, $P < 0.05$) of group means (diamonds) between the six treatment groups. Note that the statistical comparisons were performed on the log-transformed biomass measure to ensure variance homoscedasticity.

4.10 Appendix S2 - Analysis of whole-plant biomass

We measured climatic variables from January 2013-December 2015 in 10 *Drosophyllum* populations in southwestern Spain. To do so, we positioned HOBO data loggers (Onset Computer Corporation, 2013) 1 m above ground within each population and recorded relative humidity (%), among other variables, in hourly intervals. Figure S2.1 shows average seasonal values. The overall average relative humidity across sites and years = 66.5 % (± 9.0 SE).

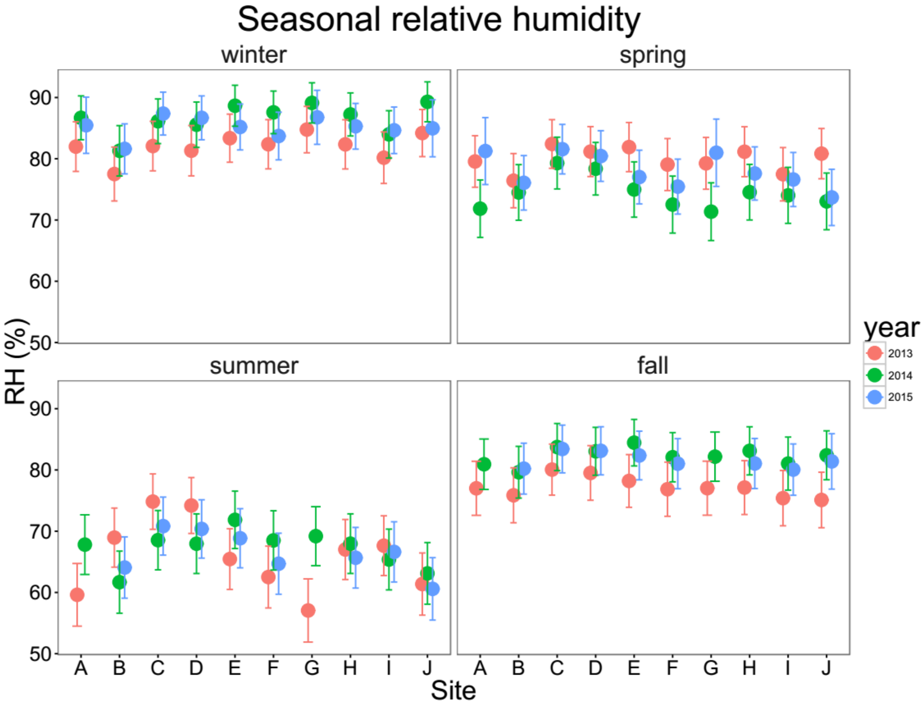
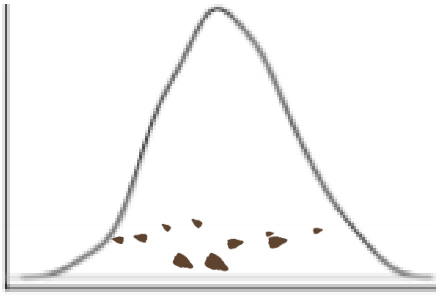


Figure S2.1 Mean (± S.E.) of seasonal relative humidity measured with HOBO data loggers at the 10 *Drosophyllum* across three years. Note that climate data were not available for the summer and fall of 2015 at some sites.



CHAPTER 5

Accounting for uncertainty in dormant life stages in stochastic demographic models

CHAPTER 5

Accounting for uncertainty in dormant life stages in stochastic demographic models

Maria Paniw, Pedro F. Quintana-Ascencio, Fernando Ojeda, and Roberto Salguero-Gómez

This chapter has been accepted for publication in *Oikos*.

5.1 Abstract

Dormant life stages are often critical for population viability in stochastic environments, but accurate field data characterizing them are difficult to collect. Such limitations may translate into uncertainties in demographic parameters describing these stages, which then may propagate errors in the examination of population-level responses to environmental variation. Expanding on current methods, we (i) apply data-driven approaches to estimate parameter uncertainty in vital rates of dormant life stages and (ii) test whether such estimates provide more robust inferences about population dynamics. We built integral projection models (IPMs) for a fire-adapted, carnivorous plant species using a Bayesian framework to estimate uncertainty in parameters of three vital rates of dormant seeds – seed-bank ingression, stasis and egression. We used stochastic population projections and elasticity analyses to quantify the relative sensitivity of the stochastic population growth rate (log λ_s) to changes in these vital rates at different fire return intervals. We then ran stochastic projections of log λ_s for 1000 posterior samples of the three seed-bank vital rates and assessed how strongly their parameter uncertainty propagated into uncertainty in estimates of log λ_s and the probability of quasi-extinction, $P_q(t)$. Elasticity analyses indicated that changes in seed-bank stasis and egression had large effects on log λ_s across fire return intervals. In turn, uncertainty in the estimates of these two vital rates explained > 50 % of the variation in log λ_s estimates at several fire-return intervals. Inferences about population viability became less certain as the time between fires widened, with estimates of $P_q(t)$ potentially > 20% higher when considering parameter uncertainty. Our results suggest that, for species with dormant stages, where data is often limited, failing to account for parameter uncertainty in population models may result in incorrect interpretations of population viability.

Keywords: Bayesian statistics; *Drosophyllum lusitanicum*, environmental stochasticity, hierarchical models, integral projection models (IPMs), parameter uncertainty, plant population dynamics, quasi-extinction, stochastic population growth rate (log λ_s)

5.2 Introduction

Variation is the rule, rather than the exception, in natural settings (Tuljapurkar 1990; Boyce *et al*. 2006; Morris *et al*. 2008; Ehrlén *et al*. 2016). Selection pressures have emerged that shape strategies maximizing the passing on of genes to the next generation in the light of such a variation (Benton and Grant 1996; Smallegange and Coulson 2013). Dormancy is a life history strategy allowing organisms to avoid stress (Grime 1977) via sporulation in microorganisms (Pozzi *et al.* 2015), diapause in some animals (Schiesari and O'Connor 2013) or persistent seed banks in many plants (Doak *et al.* 2002). In the latter, seeds delay germination under environmental unpredictability, compensating for the risk of mortality associated with other stages through time (Cohen 1966; Venable 2007).

Persistent seed banks play a crucial role in the viability of many plant populations (Baskin and Baskin 1998; Doak *et al.* 2002), including in rare and invasive species (Adams *et al.* 2005; Gioria *et al.* 2012). Seed dormancy typically evolves in habitats where important events, such as precipitation (Gremer and Venable 2014) or fires (Quintana-Ascencio *et al.* 2003; Menges and Quintana-Ascencio 2004), are unpredictable. In these habitats, plant species with high temporal variation in reproductive output and high risk of reproductive failure with increasing environmental stochasticity typically produce dormant seeds (Baskin and Baskin 1998; Venable 2007; Tielbörger *et al.* 2012). The resulting seed banks may buffer against environmental stochasticity (Cohen 1966; Rees *et al.* 2006) and, in turn, against genetic drift (e.g., Honnay *et al.* 2008). Therefore, seed banks are of great interest in ecological and evolutionary processes because they may provide an important link between environmental stochasticity and population viability.

Understanding how seed banks influence population dynamics in stochastic environments is crucial to accurately project population trends (Menges 2000; Adams *et al.* 2005). Plant species with persistent seed banks may optimize fitness in stochastic environments by decoupling two key demographic processes: reproduction and survival-dependent growth (Doak *et al.* 2002). On the one hand, seeds that enter (*ingress*) and remain dormant (*stasis*) in the seed bank do not contribute to immediate germination and aboveground growth, therefore lowering *apparent* short-term population growth rate estimates ($\widehat{\lambda'}$). On the other hand, germination (*egression*) from the seed bank can be triggered by environmental cues at a later time (Venable 2007), thus increasing $\hat{\lambda}'$. Failing to accurately describe seed-bank transitions and the uncertainty around related parameters may therefore result in flawed estimates of projected population growth rates and extinction probability (Higgins *et al.* 2000; Doak *et al.* 2002).

Parameter uncertainty in general has been shown to contribute substantially to uncertainty in stochastic population models including dormant stages. However, the specific contributions to this uncertainty of vital-rate parameters describing seed-bank transitions remain little explored (Evans *et al.* 2010; Elderd and Miller 2016). In part, this is due to the difficulty of obtaining data for such vital rates, which results in models omitting, using simplified, or using latent (unobserved) parameters (Doak *et al.* 2002; Evans *et al.* 2010). Obtaining long-term seed-bank data is challenging for two reasons: (i) seeds may persist in the soil for periods far exceeding our own lifespans (e.g., Shen-Miller *et al.* 1995), and (ii) due to their typically small size, tracking the fates of individual seeds in natural habitats without disrupting the soil is currently a nearly impossible task (Baskin and Baskin 1998; Navarra and Quintana-Ascencio 2012). Consequently, even if data on seed banks are collected, researchers usually extrapolate their long-term fates (Fig. 1) from short-term field experiments or models (Menges 2000). These approaches are sensitive to parameter uncertainty due to relatively small sample sizes and must account for this uncertainty when estimating population dynamics.

Here we show that in population models incorporating limited data on critical vital rates describing seed-bank transitions, the related parameter uncertainty alone (independent of other vital rates) may contribute greatly to the uncertainty around estimates of stochastic population dynamics. Therefore, incorporating parameter uncertainty into stochastic simulations will significantly improve demographic interpretations. Using the fire-adapted carnivorous *Drosophyllum lusitanicum* (Drosophyllaceae) as a case study, we quantified how parameter uncertainty in seed-bank dynamics affected the potential interpretation of population-level responses to changes in fire regimes. The role of the seed bank is not well known for the study species, but we expected related vital rates to play a critical role in the estimation of viability as has been shown for other fire-adapted species (Menges and Quintana-Ascencio 2004; Adams *et al.* 2005). Building on existing approaches to separate sources of variation (Evans *et al.* 2010; Elderd and Miller 2016), we developed Bayesian stochastic integral projection models (IPMs; Easterling *et al.* 2000; Ellner and Rees 2006) using census data for above-ground and limited experimental data for the seed-bank transitions. We used elasticity analyses to determine the relative sensitivity of the stochastic population growth rate, log λ_s, to changes in the mean of the three seed-bank vital rates. We then used stochastic simulations of different fire return intervals and IPMs built from parameter samples of seed-bank vital rates to estimate nested levels of variation in log λ_s and assess the variation (uncertainty) in estimates of the probability of quasi-extinction, $P_q(t)$, among parameter samples. We provide *R* scripts to apply the models and simulations (Appendix S1). Our results have important implications for the use of models to understand complex life cycles (e.g. those including diapause, vegetative dormancy, or migration) where vital rate quantification from data may contain high uncertainty.

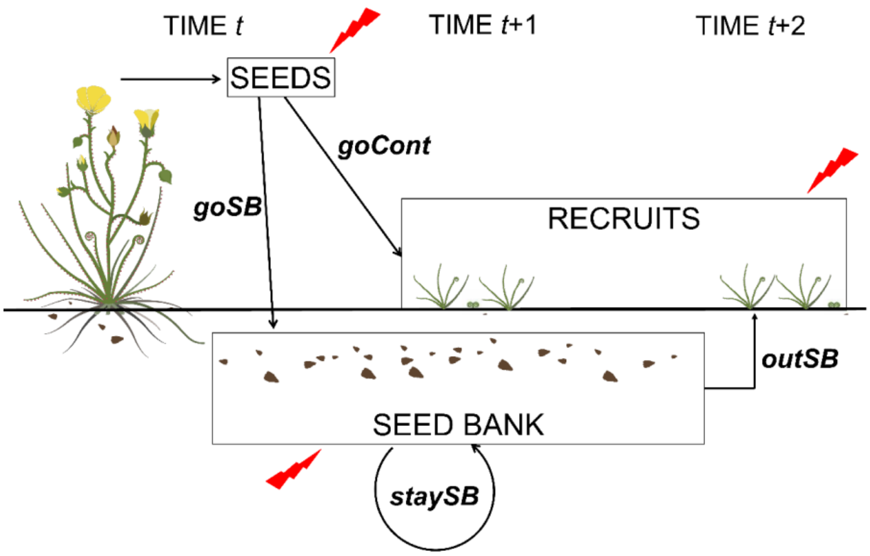


Figure 1 Possible fates of seeds after maturation at time *t* in the studied species *Drosophyllum lusitanicum.* Mature seeds either germinate and become established as recruits (*goCont*) the growing season following maturation in *t+1* or enter the permanent seed bank (*goSB*). Once in the seed bank, seeds may either survive another year without germinating (*staySB*) or germinate (*outSB*) at *t+2*, or in later years. Mortality of seeds or seedlings before establishment is indicated by red lightning bolts and was only estimated from data for seeds before they reach soil.

5.3 Materials and methods

5.3.1 Study species

We used data from natural populations of the fire-adapted, carnivorous short-lived subshrub *Drosophyllum lusitanicum* (L.) Link (Drosophyllaceae) (*Drosophyllum*, hereafter) to build IPMs. This species is endemic to the SW Iberian Peninsula and N Morocco and is associated with fire-prone Mediterranean heathlands (chapter 6). Natural heathlands burn every 40 years on average, but may burn as early as 10 years after fire or remain unburned for > 70 years (Ojeda 2009; Plan INFOCA 2012). Burned stands recover to mature vegetation within 3-5 years following fires (Calvo *et al.* 2002), and shrubs outcompete above-ground *Drosophyllum* individuals (chapter 8). Plants flower in the second year after germination and produce hard-coated, pear-shaped seeds (2.48 cm ± 0.1 SE in length; chapter 2). Most seeds remain dormant in the soil, and mass germination occurs after fire (Correia and Freitas 2002; Appendix S2). Reproductive plants produce 9.1 (± 2.6) viable seeds per fruit and up to 66 fruits (6.0 ± 0.3) per plant. Although viable dormant seeds can accumulate in vast numbers (chapter 2), and populations persist largely as seeds in the soil in between fires (Paniw unpubl.), little is known about the importance of seed fates for population dynamics of this rare carnivorous species.

5.3.2 Demographic data

We parameterized integral projection models (IPMs) with census and experimental field and laboratory data. We estimated vital rates of individuals with above-ground biomass from five annual censuses (2011-2015) comprising a total of 1,371 individuals from five populations spanning the distribution range of *Drosophyllum* in SW Spain. The populations differed with respect to the time since last fire of the habitat (*TSF*, hereafter), being two, four, six, 10, and 29 years in 2015 (see Table A2.1 in Appendix A2 for details on TSF for all site-year combinations). Vital rates included survival (σ), growth (γ), probability of flowering (φ_0), number of flowering stalks (φ_1), number of flowers per stalk (φ_2), number of seeds per flower (φ_3), and seedling size distribution the next year (φ_4) (Appendix S2). The IPM's state variable for its continuous component was *size* = log(# of leaves × length of longest leaf (cm)), after model selection for σ, γ, φ_0 and φ_1. We also quantified above-ground seed survival from the demographic census data in each population and year as $\sigma_S = 1$ – flower damage (Appendix S2). We then used this parameter to modify vital rates describing seed production (φ_0, φ_1, φ_2, φ_3, and φ_4).

We performed two 3-year field seed burial experiments and a greenhouse germination trial, overall using > 5,100 seeds, to quantify the possible fates of seeds – including seeds in the seed bank, the discrete component in the IPM (Fig. 1). Details on all experiments can be found in Appendix A2. Both field experiments were initiated in September 2012 and 2013, when reproductive *Drosophyllum* individuals release seeds. In one experiment, we randomly collected seeds from five populations and buried mesh bags containing 20 seeds each in recently burned and adjacent unburned heathland patches. We then dug out mesh bags 18 months after burial to estimate seed seed-bank stasis (*staySB*), which consisted of two probabilities: surviving and not germinating from the seed bank (Fig. 1). We assumed that the proportion of viable seeds encountered after 18 months corresponded to stasis within one time interval in the IPMs (one year), ensuring that seed-bank dynamics were at the same time scale as the rest of the species' life cycle modeled (Appendix S2). In a separate experiment, using the same design as in the mesh-bag burial experiment, we sowed 50 seeds < 1 cm below the soil surface. We recorded germination 6 and 18 months after sowing to estimate, respectively, immediate seedling establishment, *i.e.*, the probability of establishment in the spring following seed dispersal (*goCont*), and the probability of establishment, or egression, from the seed bank at least two springs after dispersal (*outSB*; Fig. 1). The vital rate *outSB* consisted of two probabilities that we could not separate: seedling emergence and survival to establishment (Fig. 1). We defined the proportion of seeds entering the seed bank (*goSB*) as 1- *goCont* - ω_S, where ω_S = seedling mortality prior to the census, *i.e.*, seedlings that emerged 4 months after sowing but failed to establish (Appendix S2). Lastly, in greenhouse trials, we exposed seeds to heat and smoke treatments and quantified germination, which we used as a proxy for seed-bank egression after fires (*outSB* in TSF_0, see below). Similarly, seed-bank stasis after fire was estimated from an examination of *Drosophyllum* seeds in soil samples from recently burned patches (Appendix S2).

5.3.3 Model parameterization

We used a Bayesian framework to fit all vital-rate models because of its advantages over frequentist approaches, including straightforward inclusion of spatial and temporal variation and ease of uncertainty simulation (Evans *et al.* 2010; Elderd and Miller 2016).

We modeled the above-ground vital rates as functions of *size* using generalized linear mixed models (GLMMs). To account for environmental variability in vital rates, we included *TSF* as a covariate in all vital-rate models. As heathland habitats >3 years after fire do not change significantly in species composition and structure, we organized *TSF* into a categorical variable consisting of 1, 2, 3, or >3 years since fire. IPMs for TSF_0 (burning) consisted of stasis in and germination from the seed bank, with 0 transition probabilities elsewhere, reflecting the death of above-ground individuals by fire. Using the deviance information criterion (DIC), we chose the most plausible model for each vital rate (Table 1; see Appendix S3 for all candidate models). The best-fit models describing growth (γ) and probability of flowering (φ_0) were defined as

$$g(\mu_{(i)}) = \alpha_0 + \alpha_{j(i)} + \beta_c \times \mathrm{size}_{(i)} + \beta_{jc} \times \mathrm{size}_{(i)} + \alpha_{s(i)} \qquad (1)$$

where $g()$ is the link function applied to the likelihood distribution of the response μ for each individual i; α_0 is the model intercept; α_j is the mean response at each *TSF* level j, compared with the model intercept; β_c is the overall slope for *size*; β_{jc} is the change of the *size* slope at each *TSF* level j; and α_s is the random effect on the model intercept for each site s (Table 1). Ideally, both random temporal and spatial variation should have been included, but our data did not offer enough degrees of freedom, as year × site interactions are confounded with *TSF* effects. In our models we used only spatial variation. Sites were chosen to span the topographic gradient for our species (Appendix S2). *Size* × *TSF* interactions (β_{jc}) were not significant for survival (σ), the number of stalks (φ_1), and number of flowers per stalk (φ_2), so these models contain only additive effects (Table 1). No data were available to link seedling size in time t+1 to parent size in t, and we therefore excluded the β parameters, keeping all other aspects of the general model design (eqn. 1; Table 1). Number of seeds per flower (φ_3) was treated as a constant in all models as it did not depend on size or *TSF* (Likelihood ratio test, D = 1.4, *d.f.* = 1).

Table 1 Parameterization of the models used to describe vital rates of *Drosophyllum lusitanicum*. The models shown described the data best among several candidate models. Superscripts indicate the names of parameters in the R scripts (Appendix S1). The distributions B, $\aleph$, and NB correspond to the Bernoulli, normal, and negative binomial distribution, respectively. *TSF* – time since last fire. *PFS* – post-fire habitat status. ΔDIC indicate the difference in values between the chosen model and the second-best model with fewer parameters, which could be a – intercept-only; b – *size* only; c – *size* + *TSF*. See main text and Appendix S3 for detail.

Vital-rate model	Parameters	Link function	Likelihood distribution	ΔDIC
Survival (σ)	$\mu^{surv} = \alpha_0^{surv} + \alpha_j^{surv}[TSF] + \beta_c^{surv} \times size + \alpha_s^{surv}[site]$	logit(σ)	$\sigma \sim B(\mu^{surv})$	-432.0[b]
Growth (γ)	$\mu^{gr} = \alpha_0^{gr} + \alpha_j^{gr}[TSF] + (\beta_c^{gr} + \beta_{jc}^{gr}) \times size + \alpha_s^{gr}[site]$	none	$\gamma \sim \aleph(\mu^{gr}, \tau^{gr})$	-5.0[c]
Probability of flowering (φ_0)	$\mu^{fl} = \alpha_0^{fl} + \alpha_j^{fl}[TSF] + (\beta_c^{fl} + \beta_{jc}^{fl}) \times size + \alpha_s^{fl}[site]$	logit(φ_0)	$\varphi_0 \sim B(\mu^{fl})$	-9.0[c]
Number of flowering stalks (φ_1)	$\mu^{fs} = \alpha_0^{fs} + \alpha_j^{fs}[TSF] + \beta_c^{fs} \times size + \alpha_s^{fs}[site]$	log(φ_1)	$\varphi_1 \sim NB(\rho^{fs}, \mu^{fs})$	-7.0[b]
Number of flowers per stalk (φ_2)	$\mu^{fps} = \alpha_0^{fps} + \alpha_j^{fps}[TSF] + \beta_c^{fps} \times size + \alpha_s^{fps}[site]$	log(φ_2)	$\varphi_2 \sim NB(\rho^{fps}, \mu^{fps})$	-5.0[b]
Seedling size (φ_4)	$\mu^{sds} = \alpha_0^{sds} + \alpha_j^{sds}[TSF] + \alpha_s^{sds}[site]$	none	$\varphi_3 \sim \aleph(\mu^{sds}, \tau^{sds})$	-20.0[a]
Immediate germination (*goCont*)	$\mu^{goCont} = \alpha_0^{goCont} + \alpha_p^{goCont}[PFS] + \alpha_b^{goCont}[block]$	logit(*goCont*)	*goCont* ~ $B(\mu^{goCont})$	-38.2[a]
Stasis is seed bank (*staySB*)	$\mu^{staySB} = \alpha_0^{staySB} + \alpha_p^{staySB}[PFS] + \alpha_b^{staySB}[block]$	logit(*staySB*)	*staySB* ~ $B(\mu^{staySB})$	-6.8[a]
Egression from seed bank (*outSB*)	$\mu^{outSB} = \alpha_0^{outSB} + \alpha_p^{outSB}[PFS] + \alpha_b^{staySB}[block]$	logit(*outSB*)	*outSB* ~ $B(\mu^{outSB})$	-206.0[a]

Table 2 Extrapolation of seed-related vital rates calculated from field experiments to time since fire (*TSF*) categories used to build integral projection models (IPMs) for *Drosophyllum lusitanicum*. The four vital rates estimated in *burned* (B) and *unburned* (U) heathland patches (see methods) were modeled as binomial functions (Table 1); Constant values (†) of vital rates in some TSF categories were obtained from soil seed bank cencuses (*staySB* in $TSF_{0,1}$), a greenhouse germination trial (*outSB* in TSF_0), measurements of seedling mortality (*goSB* in $TSF_{2,3,>3}$), or censuses of actual field germination (c; see Appendix S2 for details); σ_S is seed survival in $TSF_{2,3,>3}$ (see main text).

	TSF_0	TSF_1	TSF_2	TSF_3	$TSF_{>3}$
Immediate germination (*goCont*)	0	0	σ_{S2} × *goCont*_U× c†	σ_{S3} × *goCont*_U× c†	$\sigma_{S>3}$ × *goCont*_U × c†
Ingression into seed bank (*goSB*)	0	0	σ_{S2} × (1-*goCont*_U-0.03†)	σ_{S3} × (1-*goCont*_U-0.03†)	$\sigma_{S>3}$ × (1-*goCont*_U-0.03†)
Stasis in seed bank (*staySB*)	0.1†	0.05†	*staySB*_B	*staySB*_U	*staySB*_U
Egression from seed bank (*outSB*)	0.81†	*outSB*_B	*outSB*_B × c†	*outSB*_U × c†	*outSB*_U × c†

Vital rates related to seed-bank transitions (*goSB*, *staySB*, and *outSB*, Table 1) were defined as binomial functions of the post-fire status of experimental patches (arranged as blocks), $\alpha_{\mathrm{p(r)}}$, for each replicate *r*, where *p* could be either burned or unburned, and a random block effect, α_{b}:

$$g(\mu_{(r)}) = \alpha_0 + \alpha_{p(r)} + \alpha_{b(r)} \tag{2}$$

The predictions obtained from eqn. 2 were then associated with different *TSF* categories, assuming that the fixed effects of models for the three seed-bank vital rates in burned and unburned patches represented dynamics in $\mathrm{TSF}_{1,2}$ and $\mathrm{TSF}_{3,>3}$, respectively (Table 2).

5.3.4 IPM construction

In order to associate environmental (post-fire) states with vital rates in stochastic simulations (see below), we built IPMs for each combination of *TSF* and site-effect estimates. The IPMs consisted of two coupled equations integrated over $L = 0$ and $U = 9.6$ sizes *x* at *t* to give a vector of sizes *y* at *t*+1. The lower and upper integration limits corresponded to the minimum observed size (individual with one, 1-cm long leaf) and 1.1 × maximum observed size, respectively. The first of the two equations describes the composition of the seed bank (*S*) at *t* + 1 through the contribution of seeds produced by above-ground individuals (*goSB*) and dormant seeds remaining in the seed bank (*staySB*) at *t*:

$$S(t+1) = S(t)\mathrm{staySB} + \int_L^U \varphi_0(x)\,\varphi_1(x)\varphi_2(x)\varphi_3\sigma_S \mathrm{goSB}\, n(x,t)\,dx \tag{3}$$

The second equation describes the dynamics of above-ground individuals through emergence and establishment of seedlings from the seedbank, survival of established individuals, and contributions of seedlings by reproductive individuals the previous year:

$$n(y,t+1) = S(t)\mathrm{outSB}\varphi_3(y) + \int_L^U [\sigma(x)\gamma(y,x) + \varphi_0(x)\varphi_1(x)\varphi_2(x)\varphi_3\sigma_S \mathrm{goCont}\,\varphi_4(y)]\,n(x,t)\,dx \tag{4}$$

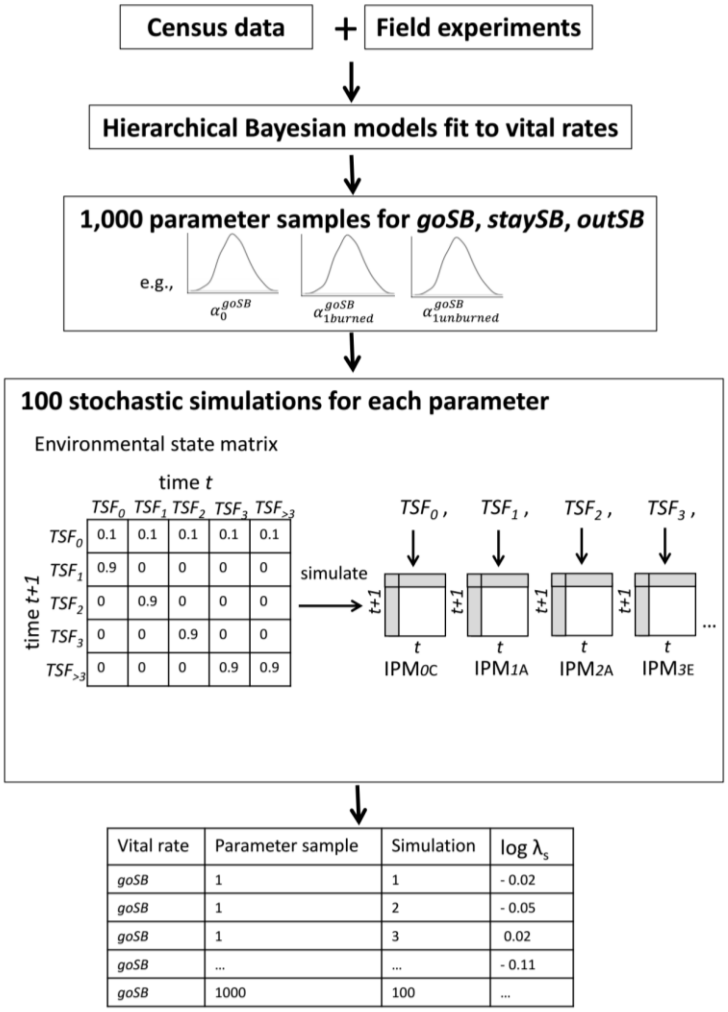
5.3.5 Parameter uncertainty

We used MCMC sampling to estimate the distributions of all 99 model parameters quantifying vital rates. In all models, we used normal (μ = 0; $1/\theta^2 = 1\times10^{-06}$) or uniform uninformative priors for most fixed factors. The posterior sampling was based on 100,000 iterations, after a burn-in of 100,000 steps, using four chains and subsampling every 400th simulated value (see Appendix S3 for details on all priors and MCMC sampling procedures). We therefore obtained the parameter distributions for *goSB*, *staySB*, and *outSB* from 1,000 samples of the joint posterior distribution of the parameters α_0 and $\alpha_{\mathrm{p(r)}}$ (eqn. 2). The full Bayesian models and application of MCMC convergence diagnostics can be found in the R script `BayModel.R` in the Appendix S1. We ran all MCMC simulations in OpenBUGS v. 3.2.3 using the R package BRugs to create an R interface to OpenBUGS (Lunn *et al.* 2009).

5.3.6 Stochastic simulations of population dynamics

We built the *TSF*-site specific IPMs for each posterior parameter sample (n = 1,000) associated with the vital rates describing seed-bank transitions: ingression into (*goSB*), stasis (*staySB*), egression from the seed bank (*outSB*), and both *staySB* and *outSB*. We sampled parameters for seed-bank stasis and egression independently because seeds that do not stay in the seed bank may die before successful establishment, *i.e.*, $outSB \neq 1 - staySB$ (Fig. 1). We kept the remaining vital rate parameters at their average posterior values to assess effects of parameter uncertainty on estimates of population viability of seed-bank related vital rates only (see `makeIPM.R` in Appendix S1).

For each parameter sample, we ran 100 simulations of stochastic population projections to assess population viability under a naturally occurring range of fire return intervals for the study region (Ojeda 2009): 10 to 100 years at 10-year increments (Fig. 2). At each fire return interval, we defined *TSF* transitions as a Markov-chain process with states corresponding to the five *TSF* categories: 0, 1, 2, 3, and > 3 years after fire and transitions between states corresponding to fire probability = 1/fire return interval (Fig. 2). Each of 100 simulations for a given fire return interval initiated with an IPM depicting TSF_0, and population dynamics were projected for t = 4,000 years after discarding the initial 500 iterations (Fig. 2). At each iteration, one of five site IPMs at a given *TSF* state was randomly chosen, while the sequence of TSF states during the iterations was determined by the Markov-chain process (Fig. 2). For each simulation, we calculated the stochastic population growth rate, log λ_s (Caswell 2001, eq. 14.61). Scripts for the simulations of population viability are available in `sLambdaSimul.R` and `sLambdaRmpi.R` for implementation using parallel processing.



	TSF_0	TSF_1	TSF_2	TSF_3	$TSF_{>3}$
TSF_0	0.1	0.1	0.1	0.1	0.1
TSF_1	0.9	0	0	0	0
TSF_2	0	0.9	0	0	0
TSF_3	0	0	0.9	0	0
$TSF_{>3}$	0	0	0	0.9	0.9

Vital rate	Parameter sample	Simulation	log λ_s
goSB	1	1	- 0.02
goSB	1	2	- 0.05
goSB	1	3	0.02
goSB	...	...	- 0.11
goSB	1000	100	...

Figure 2 Hierarchical structure of simulations of the stochastic population growth rate, log λ_s, incorporating parameter uncertainty of three vital rates: seed-bank ingression (*goSB*), stasis (*staySB*), and egression (*outSB*; Fig. 1). Bayesian posterior distributions were sampled to obtain 1,000 parameters for each vital rate. For each parameter, log λ_s were simulated from 100 stochastic projections, each run over 4,000 discrete time steps t using Markov chain transitions between five time-since-fire (TSF) environments (0, 1, 2, 3, >3). The transitions depended on 10 fire return intervals (here 0.1 probability of burning corresponds to 1 fire in 10 years). Each environmental TSF state was associated with five IPMs, one for each site (A-E) modeled in the study. The first row and column of IPMs (grey) depict seed-bank transitions. Differences in log λ_s estimates among the 1,000 parameter samples and 100 stochastic projections depicted parameter uncertainty and environmental variability, respectively.

Our simulations therefore produced two nested levels of log λ_s estimates obtained from (i) 1,000 samples of parameters, and (ii) 100 simulations of population projections within each parameter sample (Fig. 2). Differences in log λ_s among parameters represented parameter uncertainty while differences among the 100 simulations represented environmental variability. The latter variability consisted of both between-state (picking IPMs corresponding to different TSF categories at each iteration) and within-state (picking a site from the random effect estimates at each iteration) variability. We quantified the contribution of parameter uncertainty to variation in log λ_s by fitting a GLMM to the estimates of log λ_s at each fire return interval treating the posterior parameters as a random effect (see Evans *et al.* 2010). Lastly, we compared the distribution of log λ_s estimates when incorporating parameter uncertainty to estimates based on environmental variability only. We obtained the latter by calculating log λ_s for 100 simulations using IPMs built from average parameter samples for each TSF category (`makeIPM.R` in Appendix S1).

From the mean and variance of the 100 log λ_s estimates at each posterior parameter sample and fire return interval, we analytically obtained the probability of quasi-extinction $P_q(t)$ at t = 50 and 100 years as described in Trotter *et al.* (2013). We chose the extinction threshold to be 0.01, *i.e.*, populations were considered extinct when population sizes (including seeds in the seed bank) fell to 1 % of current population sizes (see Quintana-Ascencio *et al.* 2003).

In order to compare the effects of changes in *goSB*, *staySB*, and *outSB* on log λ_s, relative to other vital rates, at different fire-return intervals, we perturbed each vital rate used to compose the IPMs by its mean, μ, and standard deviation, σ, across all environmental states (see `perturbVR.R` in Appendix S1). We then used the chain rule to calculate (i) how these perturbations affected the IPM kernels, and (ii) how the latter in turn affected log λ_s. These calculations provided us with elasticities, E^{μ} and E^{σ}, of log λ_s to changes in the mean and variance of vital rates, respectively (Tuljapurkar *et al.* 2003; Haridas and Tuljapurkar 2005; Appendix S4). Unlike deterministic elasticities however, E^{μ} and E^{σ} do not sum to one and thus do not provide a measure of relative contribution (Haridas and Tuljapurkar 2005). To calculate the relative elasticities focusing on changes in the mean of each vital rate, we therefore divided the E^{μ} for each vital rate, for example *staySB*, summed over all affected IPM kernel entries, *j*, by the total E, summed over E^{μ} and E^{σ} for all vital rates, *vr*, (see Morris *et al.* 2008):

$$\sum_j E^{\mu}_{staySBj} \Big/ \sum_i \left(E^{\mu}_{vri} + E^{\sigma}_{vri}\right) \tag{5}$$

We used mean parameter values and a subset of five fire return intervals, 10, 30, 50, 80, and 100 years, to calculate the elasticities. As *Drosophyllum* is a post-fire dwelling, short-lived species with vital-rate variation governed by post-fire habitat succession, we didn't consider intrinsic demographic trade-offs, for example between reproduction and growth (Miller *et al.* 2012), in the elasticity calculations.

5.4 Results

5.4.1 Importance of seed-bank vital rates for stochastic population dynamics

Seed-bank stasis (*staySB*) and egression (*outSB*; Fig. 1) had the largest relative effects on the stochastic growth rate, log λ_s, of *Drosophyllum* populations across fire return intervals (Fig. 3). In particular, changing the average of *staySB* produced the highest relative elasticities, E^{μ}, among all vital rates (0.5 at fire return interval of 100 years), followed by *outSB*. For both vital rates, relative E^{μ} increased with fire return interval (Fig. 3). Ingression into the seed-bank, *goSB*, had relatively low E^{μ}, remaining approx. 0.05 across the five fire return intervals simulated.

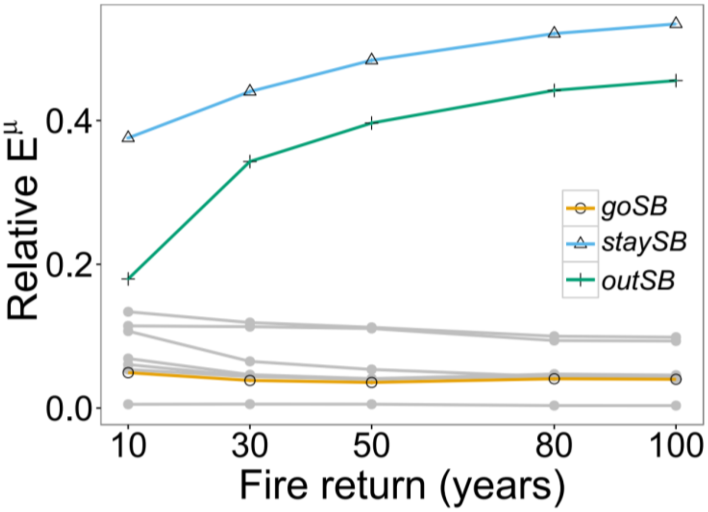


Figure 3 Seed-bank vital rates govern the population dynamics of *Drosophylum*, regardless of fire return interval. Elasticities of the stochastic population growth rate, log λ_s, to changes in the mean, E^{μ}, of seed-bank stasis (*staySB*) and egression (*outSB*) are higher compared with other vital rates (filled grey points and lines) at five simulated fire return intervals: 10, 30, 50, 80, and 100 years. SE around the relative E^{μ} obtained from 100 simulations were < 1×10^{-03}.

5.4.2 Influence of parameter uncertainty on estimation of population growth and extinction

In all simulations, average log λ_s decreased monotonically with increasing fire return interval (Spearman's $\rho = -1$), while $P_q(t)$ increased with increasing fire return interval (Figs. 4 and 5). In simulations using mean parameter values, log λ_s variance decreased with increasing fire return interval because fewer TSF states (largely $\text{TSF}_{<3}$) were sampled at each iteration with burning becoming less likely (Fig. 4). However, when uncertainties in *staySB*, *outSB*, or both were incorporated into simulations, estimates of log λ_s were more variable compared to simulations based on mean parameters, and their variation increased with increasing fire return (Fig. 4). Accordingly, the proportion of variation among the 100,000 log λ_s estimates attributed to parameter uncertainty varied across fire return intervals and vital rates sampled, being < 0.01 for *goSB* and increasing from > 0.1 at 10 years to > 0.7 at 100 years return interval for *staySB* and *outSB* (Fig. 4; Table S4.2). The largest contribution of parameter uncertainty was obtained when including samples of both *staySB* and *outSB* into simulations (Table S4.2).

The high uncertainty in the estimates of log λ_s at increasing fire return intervals influenced potential inferences about population viability. Whereas the 100 projections of log λ_s based on environmental variability alone (grey boxplots in Fig. 4) showed a clear decline in viability at a fire return interval of ≥ 50 years, high uncertainty associated with these projections meant that the certainty in the threshold of 50 years (fire return interval) was relatively low (Fig. 4). In fact, uncertainty in $P_q(t)$ markedly increased when accounting for parameter uncertainty in *staySB* and *outSB*. Compared with estimates based on mean parameter values, $P_q(t)$ could be > 20 percentage points higher or lower under particular combinations of *staySB* and *outSB* (Fig. 5). The strongest effects of parameter uncertainty appeared at $t = 100$ years, where $P_q(t)$ as high as 0.77 cannot be ruled out at a fire return interval of 100 years (Fig. 5b).

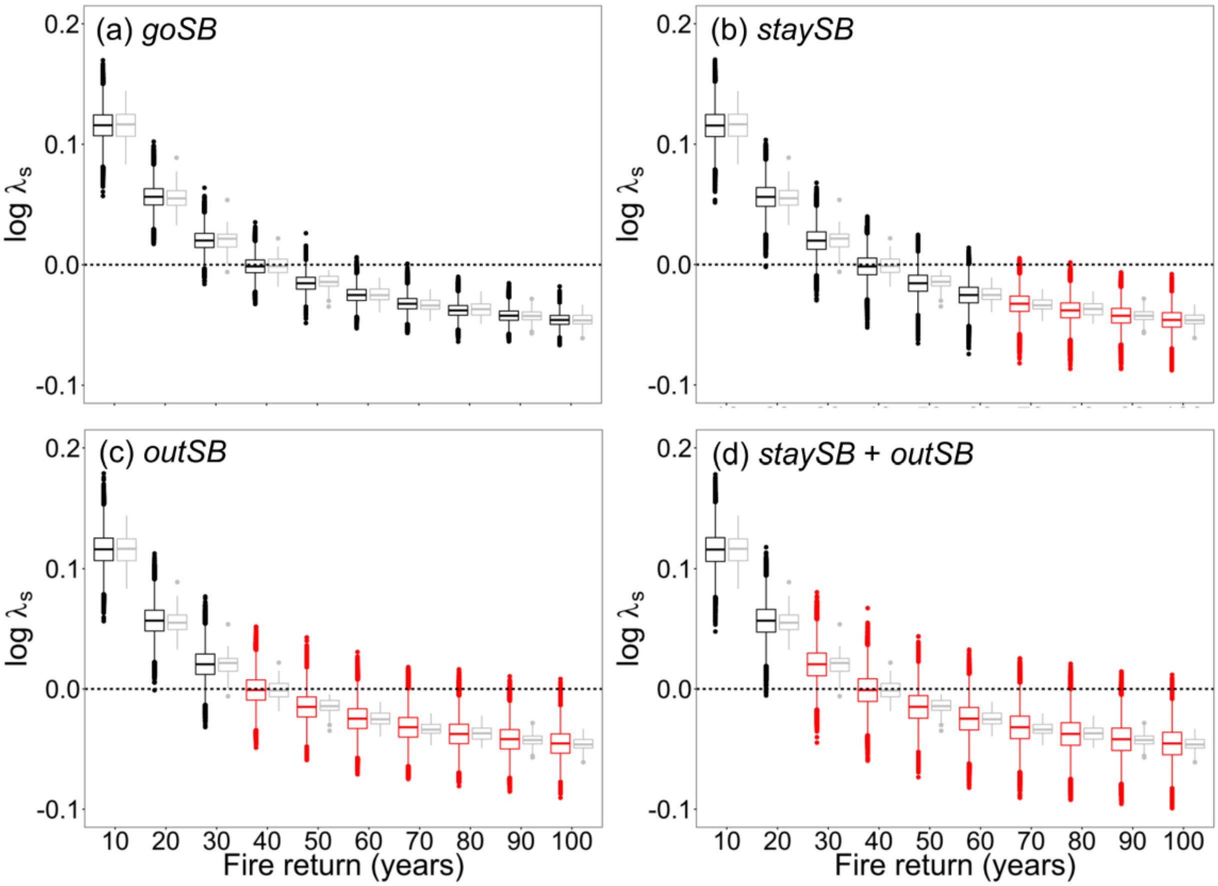


Figure 4 Parameter uncertainty contributes significantly to variation of simulated stochastic population growth rate estimates (log λ_s). Box-and-whisker plots display log λ_s as function of fire return interval (*x*-axis). At each fire return interval, the black and red box plots summarize the variation among 100,000 log λ_s obtained from 100 stochastic projections of log λ_s for each of 1000 posterior parameter samples describing (a) seed-bank ingression (*goSB*), (b) stasis (*staySB*), (c) egression (*outSB*), and (d) both *staySB* and *outSB*. Red box plots indicate a proportional contribution of parameter uncertainty to the variation in log $\lambda_s > 50$ %. Grey box plots in a-d summarize variation in log λ_s estimates from 100 stochastic simulations using mean parameter values for all vital rates. Black horizontal dashed lines indicate stable population sizes.

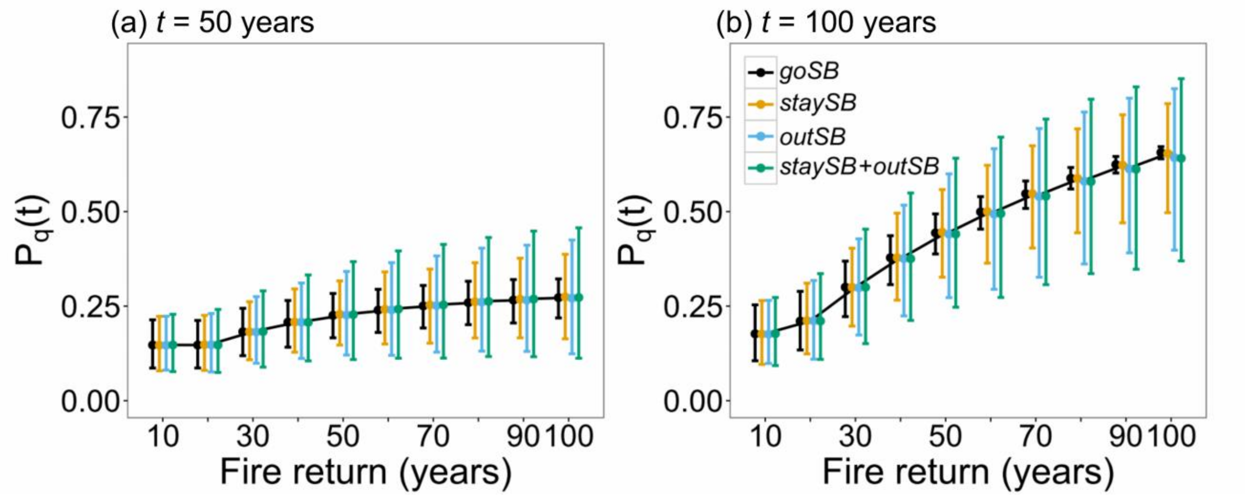


Figure 5 Increases in the probability of quasi-extinction, $P_q(t)$, at t = 50 or 100 years as function of fire return interval. The extinction threshold was assumed to be 0.01. At each fire return, $P_q(t)$ was calculated from the mean and variance of 100 stochastic growth rates obtained for each of 1000 posterior parameter samples describing seed-bank ingression (*goSB*), stasis (*staySB*), egression (*outSB*), and both *staySB* and *outSB* (different colors in plot). Points represent $P_q(t)$ averaged over the 1000 parameter samples. Error bars show ± 95 % non-parametric quantile CI (2.5 and 97.5 quantile) obtained from the $P_q(t)$ for each of the 1000 parameter samples.

5.5 Discussion

Dormant life stages such as larvae in diapause, some spores, or seeds in permanent seed banks are believed to play key roles in the adaption of species to environmental stochasticity (Benton and Grant 1996; Smallegange and Coulson 2013). Demographic information on these life stages, however, is often limited (Doak *et al.* 2002). When incorporating such data in population models, the parameter uncertainty in vital rates describing dormant life stages must be quantified in order to separate sources of variability for measures such as extinction or invasion risk or the stochastic population growth rate, log λ_s (Ellner and Fieberg 2003; Evans *et al.* 2010; Lee *et al.* 2015). Here we provide evidence that uncertainty around vital-rate parameters describing critical seed-bank transitions of a fire-adapted plant may translate into large uncertainty in the estimates of population-level parameters, and omitting it can seriously bias interpretation of population performance. The Bayesian framework we employed to quantify parameter uncertainty was developed by Evans *et al.* (2010) for matrix population models and recently extended to IPMs by Elderd and Miller (2016). Our study provides an important extension to the work by Elderd and Miller – the explicit consideration of discrete, dormant stages and categorical covariates (*TSF*) when constructing Bayesian IPMs, simulating stochastic environmental transitions, and quantifying contributions of parameter uncertainty to population dynamics.

5.5.1 The role of the seed bank for population dynamics

Our results showed that life-cycle transitions related to the seed bank (Fig. 1) strongly influence population dynamics of the fire-adapted *Drosophyllum lusitanicum*. Seed-bank stasis can ensure population persistence when above-ground individuals cannot survive in long-unburned habitats (Menges and Quintana-Ascencio 2004; Adams *et al.* 2005); while large egression events after fires and periodic egression into favorable microhabitats in unburned stands result in growth of above-ground individuals, which replenish the seed bank (Quintana-Ascencio *et al.* 2003; chapter 6). Our elasticity analyses suggested that increases in both seed-bank stasis and egression would strongly, positively affect the stochastic population growth rate (Fig. 3). However, these two vital rates are negatively correlated, implying that seed-bank stasis can only be optimized at the expense of egression and vice versa (Benton and Grant 1996). At long fire return intervals, an increase in the importance of seed-bank stasis has been shown in other studies (Quintana-Ascencio *et al.* 2003; Menges and Quintana-Ascencio 2004) and would likely be more critical for *Drosophyllum* populations than egression. This is because egression is highly dependent on open microhabitats being created in unburned habitats, which occurs irregularly and on a small scale in natural heathlands (Paniw unpubl.). On the other hand, changes in seed-bank ingression affected population growth far less than either seed-bank stasis or egression. This vital rate varied little across time-since-fire habitats (Appendix S2), and vital rates related to above-ground fecundity have a stronger effect on population dynamics, which has been demonstrated for a number of disturbance-adapted, early colonizing species (Silvertown *et al.* 1996; Smith *et al.* 2005).

5.5.2 Parameter uncertainty in dormant life stages and inference about population dynamics

Quantifying parameter uncertainty of vital rates with strong effects on population growth can help researchers to account for the uncertainty in the effect of environmental processes on stochastic population dynamics (Evans *et al.* 2010). For *Drosophyllum*, parameter uncertainty related to seed-bank stasis and egression explained up to 79 % of log λ_s variation among our 100,000 simulations. Overall, the uncertainty around estimates of both log λ_s and $P_q(t)$ increased with fire-return interval modeled. This occurred mainly because seed-bank dynamics become more important for persistence of a fire-adapted species in the absence of fires (Quintana-Ascencio *et al.* 2003), with uncertainty in their estimates increasingly affecting the accuracy with which population dynamics can be assessed. Therefore, with limited data on seed-bank dynamics in the case of *Drosophyllum* and many other species (Baskin and Baskin 1998), a robust interpretation of viability analyses in long unburned populations relies primarily on the incorporation of parameter uncertainty into population analyses. Meanwhile, interpretations about the role of environmental processes themselves (e.g., fire regimes) become increasingly uncertain when projecting data-limited population dynamics into the future (Boyce *et al.* 2006).

With high potential for errors in the estimates of population dynamics for species with limited demographic data, uncertainty analyses can become critical when defining management strategies (Hunter *et al.* 2010). Fire is of vital importance for *Drosophyllum*, a species that reaches full reproductive potential within the first 2-4 post-fire years in natural Mediterranean heathlands (Correira and Freitas 2002) and then mostly persists in the seed bank until the next fire or disturbance occurs (chapter 6). However, current fire return intervals in the Mediterranean have increased due to fire suppression (Ojeda 2009; Turco *et al.* 2016), threatening population viability (chapter 6). At fire return intervals of ≥ 50 years, which is still within the upper range of natural fire regimes across Mediterranean heathlands (Ojeda 2009; Plan INFOCA 2012), the mean estimates of $\log \lambda_s < 0$, implying population decline. However, the variation around this mean attributed to parameter uncertainty in *staySB* and *outSB* indicates that *Drosophyllum* populations may be able to persist with a fire return interval of about 60 years, and some even with a fire return interval of up to 70 years. For conservation management of this species, which may include prescribed burning or controlling for factors that may jeopardize survival of dormant seeds in the seed bank (chapter 6), the accurate estimation of parameter uncertainty may directly define the heathlands considered for management depending on time since last fire. As population growth of *Drosophyllum* showed non-zero elasticities to changes in the remaining vital rates (Fig. 4) and given the large number of parameters estimated in our models, including samples of all parameters into the simulations would further increase the uncertainty of $\log \lambda_s$ and $P_q(t)$ estimates (Evans *et al.* 2010; Appendix S4). However, our aim here was to emphasize that interpretations of long-term stochastic population dynamics may strongly depend on quantification of a few critical vital rates and their uncertainties.

5.5.3 Implications of uncertainty for other life histories

Studies of other species with adaptations to buffer environmental stochasticity may also benefit from a better understanding of different sources of uncertainty, particularly under the emerging threats of climate change. In plants, vegetative dormancy may be as difficult to estimate as seed dormancy (Lesica and Crone 2007) but can play a critical role in buffering populations from stress, either physical (Shefferson *et al.* 2005) or climatic (Salguero-Gómez *et al.* 2012). Likewise, in many insects, prolonged diapause can spread adult survival over several years but may be difficult to estimate (Solbreck and Widenfalk, 2012). Whether and how such strategies may continue to buffer populations under human-induced disturbance and climatic changes is an emerging question (Boyce *et al.* 2006; Morris *et al.* 2008). An equally important question may be how to account for the inherent uncertainty due data-limited vital rates when assessing the significance of climatic variables on changes in population dynamics (Elderd and Miller 2016).

Within a given life cycle, the quantification of parameter uncertainty may also be important for the estimates of correlated vital rates. Uncertainties in egression of seeds from the seed bank may for example influence estimates of recruitment (Eager *et al.* 2014). In *Drosophyllum,* recruitment is dependent on the environment and not so much on plant density. However, many species with persistent seed banks may exhibit a negative density dependence of seedling establishment (Eager *et al.* 2014). Here, uncertainty in the number of recruits from the seed bank may propagate to uncertainties in above-ground vital rates. In other organisms, responses to stress such as vegetative dormancy may have future consequences on fitness, e.g., lower growth as above-ground individual (Gremer *et al.* 2012). As such, large variation in the estimates of dormancy may directly influence the estimates of several other vital rates once individuals emerge above-ground. Studies of population dynamics encounter many types of covariation in vital rates (Tuljapurkar 1990; Morris *et al.* 2008), and the potential propagation of uncertainty throughout different vital rates has received little attention in plant demography as opposed to animal demography (Hunter *et al.* 2010; Lee *et al.* 2015).

5.5.4 Conclusions

Increasingly sophisticated methods are being used to address ecological and evolutionary questions regarding environmental stochasticity (Salguero-Gómez and de Kroon 2010; Low-

Décarie *et al.* 2014). Population models have also gained complexity and realism in the last decades, allowing for more reliable analysis of population dynamics by accounting for different sources of variation in underlying vital-rate regressions (Evans *et al.* 2010; Merow *et al.* 2014; Tye *et al.* 2016). Here, we have contributed to this important body of literature by showing that, when dealing with dormant life-cycle stages with limited field data, stochastic models may gain robustness in the interpretation of projected population dynamics by including parameter uncertainty around vital rate means. An exhaustive sensitivity analysis to parameter uncertainty may strongly influence conservation management decisions, and we encourage population ecologists to explicitly address such uncertainties in their modeling approaches.

5.6 Acknowledgements

We are grateful to M. Gil-López, M. Scott, and M. Collado-Aliaño for logistical support during the data collection, to V. González-Ortiz *Drosophyllum* illustrations, and A. Jiménez's CITI team (UCA) for access to parallel processing. This study has been financed by project BREATHAL (CGL2011-28759/BOS; Spanish *Ministerio de Economía y Competitividad*) to F.O and R.S.G. M.P. was supported by a FPI scholarship granted by the Spanish *Ministerio de Economía y Competitividad*, R.S.-G. by the Australian Research Council (DE140100505), the Max Planck Institute for Demographic Research and a NERC IRF NE/M018458/1, and P.F.Q.-A. by NSF-DEB 1347247 and NSF-DEB 0812753.

5.7 References

Adams VM, Marsh DM, Knox JS. Importance of the seed bank for population viability and population monitoring in a threatened wetland herb. 2005. *Biological Conservation* **124**:425-436.

Baskin CC, Baskin JM 1998. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press.

Benton TG, Grant A. 1996. How to keep fit in the real world : Elasticity analyses and selection pressures on life histories in a variable environment. *The American Naturalist* **147**:115–139.

Boyce MS, Haridas CV, Lee CT, NCEAS Stochastic Demography Working Group. 2006. Demography in an increasingly variable world. *Trends in Ecology and Evolution* **21**:141-148.

Calvo L, Tárrega R, De Luis E. 2002. The dynamics of Mediterranean shrubs species over 12 years following perturbations. *Plant Ecology* **160**:25-42.

Caswell H. 2001. *Matrix Population Models: Construction, Analysis and Interpretation* (2nd ed.). Sinauer Associates.

Cohen D. 1966. Optimizing reproduction in a randomly varying environment. *Journal of Theoretical Biology* **12**:119-129.

Correia E, Freitas H. 2002. *Drosophyllum lusitanicum*, an endangered West Mediterranean endemic carnivorous plant: threats and its ability to control available resources. *Botanical Journal of the Linnean Society* **140**: 383-390.

Doak DF, Thomson D, Jules ES. 2002. Population viability analysis for plants: understanding the demographic consequences of seed banks for population health. In: Beissinger, S. R. and McCullough, D. R. (eds.), *Population Viability Analysis*. Univ. Chicago Press, pp. 312-337.

Eager EA, Rebarber R, Tenhumberg B. 2014. Modeling and analysis of a density-dependent stochastic integral projection model for a disturbance specialist plant and its seed bank. *Bulletin of Mathematical Biology* **76**:1809-1834.

Easterling MR, Ellner SP, Dixon PM. 2000. Size-specific sensitivity: applying a new structured population model. *Ecology* **81**:694–708.

Ehrlén J, Morris WF, Euler T, Dahlgren JP. 2016. Advancing environmentally explicit structured population models of plants. *Journal of Ecology* **104**:292-305.

Elderd BD, Miller TE. 2016. Quantifying demographic uncertainty: Bayesian methods for Integral Projection Models (IPMs). *Ecological Monographs* **86**:125-144.

Ellner SP, Fieberg J. 2003. Using PVA for management despite uncertainty: effects of habitat, hatcheries, and harvest on salmon. *Ecology* **84**:1359-1369.

Ellner SP, Rees M. 2006. Integral projection models for species with complex demography. *The American Naturalist* **167**:410-428.

Evans ME, Holsinger KE, Menges ES. 2010. Fire, vital rates, and population viability: a hierarchical Bayesian analysis of the endangered Florida scrub mint. *Ecological Monographs* **80**:627-649.

Gioria M, Pyšek P, Moravcová L. 2012. Soil seed banks in plant invasions: promoting species invasiveness and long-term impact on plant community dynamics. *Preslia* **84**:327-350.

Gremer JR, Crone EE, Lesica P. 2012. Are dormant plants hedging their bets? Demographic consequences of prolonged dormancy in variable environments. *The American Naturalist* **179**:315-327.

Gremer JR, Venable DL. 2014. Bet hedging in desert winter annual plants: optimal germination strategies in a variable environment. *Ecology Letters* **17**:380-387.

Grime JP. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist* **111**:1169-1194.

Haridas CV, Tuljapurkar S. 2005. Elasticities in variable environments: properties and implications. *The American Naturalist* **166**:481–495.

Higgins SI, Pickett ST, Bond WJ. 2000. Predicting extinction risks for plants: environmental stochasticity can save declining populations. *Trends in Ecology and Evolution* **15**:516-520.

Honnay O, Bossuyt B, Jacquemyn H, Shimono A, Uchiyama K. 2008. Can a seed bank maintain the genetic variation in the above ground plant population? *Oikos* **117**:1–5.

Hunter CM, Caswell H, Runge MC, Regehr EV, Amstrup SC, Stirling I. 2010. Climate change threatens polar bear populations: a stochastic demographic analysis. *Ecology* **91**:2883-2897.

Lee AM, Bjørkvoll EM, Hansen BB, Albon SD, Stien A, Sæther BE, Engen S, Veiberg V, Loe LE, Grøtan V. 2015. An integrated population model for a long-lived ungulate: more efficient data use with Bayesian methods. *Oikos* **124**:806-816.

Low-Décarie E, Chivers C, Granados M. 2014. Rising complexity and falling explanatory power in ecology. *Frontiers in Ecology and the Environment* **12**:412-418.

Lunn D, Spiegelhalter D, Thomas A, Best N. 2009. The BUGS project: Evolution, critique and future directions (with discussion). *Statistics in Medicine* **28**:3049--3082.

Menges ES 2000. Population viability analyses in plants: challenges and opportunities. *Trends in Ecology and Evolution* **15**:51-56.

Menges ES, Quintana-Ascencio, PF. 2004. Population viability with fire in *Eryngium cuneifolium*: deciphering a decade of demographic data. *Ecological Monographs* **74**:79-99.

Merow C, Dahlgren JP, Metcalf CJ, Childs DZ, Evans ME, Jongejans E, Record S, Rees M, Salguero-Gómez R, McMahon SM. 2014. Advancing population ecology with integral projection models: a practical guide. *Methods in Ecology and Evolution* **5**:99-110.

Miller TE, Williams JL, Jongejans E, Brys R, Jacquemyn H. 2012. Evolutionary demography of iteroparous plants: incorporating non-lethal costs of reproduction into integral projection models. *Proceedings of the Royal Society of London B: Biological Sciences*: rspb20120326.

Morris WF, Pfister CA, Tuliapurkar S, Haridas CV, Boggs CL, Boyce MS, Bruna EM, Church DR, Coulson T, Doak DF, Forsyth S. 2008. Longevity can buffer plant and animal populations against changing climatic variability. Ecology **89**:19–25.

Navarra JJ, Quintana-Ascencio PF. 2012. Spatial pattern and composition of the Florida scrub seed bank and vegetation along an anthropogenic disturbance gradient. *Applied Vegetation Science* **15**:349-358.

Ojeda F. 2009. 4030 Brezales secos europeos. In: VV. AA. (eds.), *Bases Ecológicas Preliminares para la Conservación de los Tipos de Hábitat de Interés Comunitario en España*. Ministerio de Medio Ambiente, y Medio Rural y Marino, pp. 1-66.

Plan INFOCA. 2012. *Incedios forestales*. Junta de Andalucia: Consejería de Medioambiente y Ordenación del Territorio. <http://www.juntadeandalucia.es/medioambiente>

Pozzi AC, Bautista-Guerrero HH, Nouioui I, Cotin-Galvan L, Pepin R, Fournier P, Menu F, Fernandez MP, Herrera-Belaroussi A. 2015. In-planta sporulation phenotype: a major life history trait to understand the evolution of Alnus-infective Frankia strains. *Environmental Microbiology* **17**:3125-3138.

Quintana-Ascencio PF, Menges ES, Weekley CV, 2003. A fire-explicit population viability analysis of *Hypericum cumulicola* in Florida rosemary scrub. *Conservation Biology* **17**:433–449.

Radchuk V, Turlure C, Schtickzelle N. 2013. Each life stage matters: the importance of assessing the response to climate change over the complete life cycle in butterflies. *Journal of Animal Ecology* **82**:275-285.

Rees M, Childs DZ, Metcalf JC, Rose KE, Sheppard AW, Grubb PJ. 2006. Seed dormancy and delayed flowering in monocarpic plants: selective interactions in a stochastic environment. *The American Naturalist* **168**:E53-E71.

Salguero-Gómez R, De Kroon H. 2010. Matrix projection models meet variation in the real world. *Journal of Ecology* **98**:250-254.

Salguero-Gómez R, Siewert W, Casper BB, Tielbörger K. 2012. A demographic approach to study effects of climate change in desert plants. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **367**:3100-3114.

Schiesari L, O'Connor MB. 2013. Diapause: delaying the developmental clock in response to a changing environment. *Current Topics in Developmental Biology* **105**:213–246.

Shefferson RP, Kull T, Tali K. 2005. Adult whole-plant dormancy induced by stress in long-lived orchids. - *Ecology* **86**:3099-3104.

Shen-Miller J, Mudgett MB, Schopf JW, Clarke S, Berger R. 1995. Exceptional seed longevity and robust growth: ancient sacred lotus from China. *American Journal of Botanty* **82**:1367-1380.

Silvertown J, Franco M, Menges E. 1996. Interpretation of elasticity matrices as an aid to the managament of plant populattions for conservation. *Conservation Biology* **10**:591–597.

Smallegange IM, Coulson T. 2013. Towards a general, population-level understanding of eco-evolutionary change. *Trends in Ecology and Evolution* **28**:143-148.

Smith M, Caswell H, Mettler-Cherry P. 2005. Stochastic flood and precipitation regimes and the population dynamics of a threatened floodplain plant. *Ecological Applications* **15**:1036–1052

Solbreck C, Widenfalk O. 2012. Very long diapause and extreme resistance to population disturbance in a galling insect. *Ecological Entomology* **37**:51-55.

Tielbörger K, Petrů M, Lampei C. 2012. Bet-hedging germination in annual plants: a sound empirical test of the theoretical foundations. *Oikos* **121**:1860-1868.

Trotter MV, Krishna-Kumar S, Tuljapurkar S. 2013. Beyond the mean: sensitivities of the variance of population growth. *Methods in Ecology and Evolution* **4**:290-298.

Tuljapurkar S. 1990. *Population Dynamics in Variable Environments*. Springer.

Tuljapurkar S, Horvitz CC, Pascarella JB. 2003. The many growth rates and elasticities of populations in random environments. *The American Naturalist* **162**:489-502.

Turco M, Bedia J, Di Liberto F, Fiorucci P, von Hardenberg J, Koutsias N, Llasat MC, Xystrakis F, Provenzale A. 2016. Decreasing fires in Mediterranean Europe. *PLoS One* **16**:e0150663.

Tye MR, Menges ES, Weekley C, Quintana-Ascencio PF, Salguero-Gómez R. 2016. A demographic ménage à trois: interactions between disturbances both amplify and dampen population dynamics of an endemic plant. *Journal of Ecology*: in press.

Venable DL. 2007. Bet hedging in a guild of desert annuals. *Ecology* **88**:1086-1090.

5.8 Appendix S1 - Overview of the R code

Here, we provide an overview of the .R and data files to accompany the main text. The .R files should be opened with an R editor (e.g., R Studio). The R code is fully commented and intended to replicate the analyses used in the manuscript. To understand variable names, the reader is referred to the main text itself. All files can be found on a CD attached to the back cover and at Dryad (doi:10.5061/dryad.rq7t3).

Data files:

dataDroso.csv: Demographic transitions of *Drosophyllum lusitanicum* populations recorded in annual censuses (from 2011to 2015) in five populations. These data are used to quantify vital rates of above-ground individuals.

dataDrosoSB.csv: Seed fates (in a binary format) inferred from two experiments. These data are used to quantify the transitions related to the seed bank and associated parameter uncertainties.

In case the reader wishes to forego the step of fitting the Bayesian models, we provided a *mcmcOUT.csv* file with 1000 posterior parameter values for each of the parameters estimated with Bayesian models using uninformative priors.

R code:

`BayModel.R`: Executes and saves the results of a Bayesian model quantifying all vital rates; illustrates basic diagnostics that can be run on the results of an MCMC run (i.e., the posterior parameter distribution) to check for model convergence and autocorrelation of the posterior samples.

`makeIPM.R`: Demonstrates how to construct IPMs including continuous and discrete (seed bank) transitions for (A) mean parameter values and (B) from the parameter distributions of the Bayesian models; saves IPMs for all parameters related to seed-bank ingression, stasis, and ingression. The code is based on the supporting material in Ellner and Rees (2006), *Am. Nat.*, 167, 410-428.

`perturbVR.R`: Demonstrates how to construct IPMs from perturbed vital rates. Each IPM is obtained by (a) perturbing a vital rate by its mean or standard deviation (see `makeVRmu.R` on constructing mean vital-rate kernels) and (b) constructing a new IPM kernel incorporating the perturbed vital rate.

`makeIPMmu.R` and `makeVRmu.R`: functions to constructs IPMs and vital-rate kernels, respectively, for average environments.

`sLambdaSimul.R`: Runs simulations, based on different fire return intervals, of the stochastic population growth rate using IPMs constructed (A) from mean parameter values, (B) from perturbed vital rates, and (C) for each posterior sample of the parameters describing seed-bank ingression (*goSB*), stasis (*staySB*) and egression (*outSB*); calculates the stochastic population growth rate, its elasticities, and the probability of quasi-extinction at time *t*. The structure of the code is based on Tuljapurkar et al. (2003), *Am. Nat.*, 162, 489-502 and Trotter et al. (2013), *Methods Ecol. Evol.*, 4, 290-298.

`sLambdaRmpi.R`: Implements the simulations of the stochastic population growth rate using parallel processing, where simulations are split into different processors of a supercomputer to greatly speed up computational time.

5.9 Appendix S2 - Details on demographic censuses and field experiments on *Drosophyllum lusitanicum* (L.), Link (Drosophyllaceae)

Here, we provide details on the study species, sites, demographic censuses, and field experiments that were the sources of the vital-rate data used in the manuscript.

5.9.1 Study species

Drosophyllum lusitanicum (L.) Link. (Drosophyllaceae) is a geographically and taxonomically rare carnivorous subshrub, endemic to Mediterranean heathland habitats in SW Iberian Peninsula and N Morocco (Garrido *et al*. 2003). This species is distinguished from the majority of carnivorous plants by its xeromorphic woody anatomy and unique realized niche in dry, nutrient-poor, acidic soils and harsh environments marked by prolonged summer drought (Adlassnig *et al*. 2006; Adamec 2009).

Drosophyllum is a short-lived subshrub (mean life expectancy of 2.1 years ± 1.6 S.D.). Individuals range in size between 1-17 rosettes, and each rosette contains ~ 14 leaves (11.8 ± 5.6 cm in leaf length) but can contain up to 45 leaves. Rosettes are produced each growing season, at the onset of the rainy season (Adlassnig *et al*. 2006; Adamec 2009). The leaves bear sticky mucilage on stalked glands to capture and digest small insects of mostly Diptera, Coleoptera, and Lepidoptera (Adlassnig *et al*. 2006; Chapter 3). The species is predominantly autogamous (Ortega-Olivencia *et al*. 1995; 1998) and produces hard-coated seeds that can remain dormant in the soil for at least 10 years (chapter 6). Figure S2.1 provides a simplified life-cycle graph of the species.

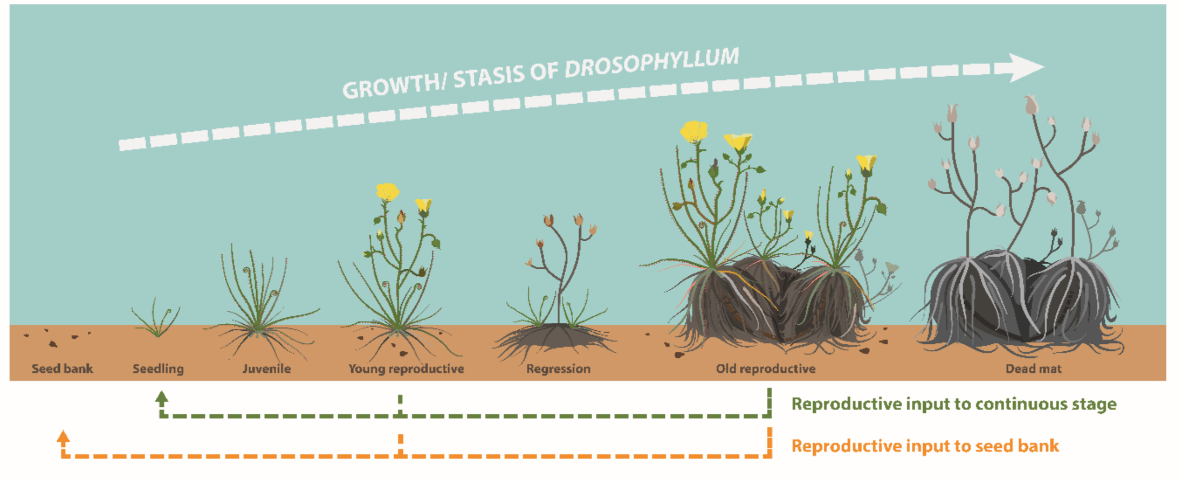


Figure S2.1 Life cycle of *Drosophyllum lusitanicum*

5.9.2 Study area and environmental data

The demographic censuses were conducted in the northern Strait of Gibraltar (SW Spain; Fig. S2.2; Table S2.1). This region is characterized by a mild Mediterranean climate with a rainy season in winter and spring followed by summer drought somewhat alleviated by coastal moist winds (Ojeda *et al*. 2000). The altitude at the sites ranged from 200 to 450 m a.s.l. The heathland communities in the study area are dominated by species of the Ericaceae (*i.e.*, *Calluna vulgaris*, *Erica australis*, *E. umbellata*, and *E. scoparia*) and Fabaceae (*i.e.*, *Stauracanthus boivini* and *Genista tridens*; Garrido *et al*. 2003). These communities occur on sandstone soils which are acidic (pH of 4.46 ± 0.41) and highly infertile (Adamec 2009).

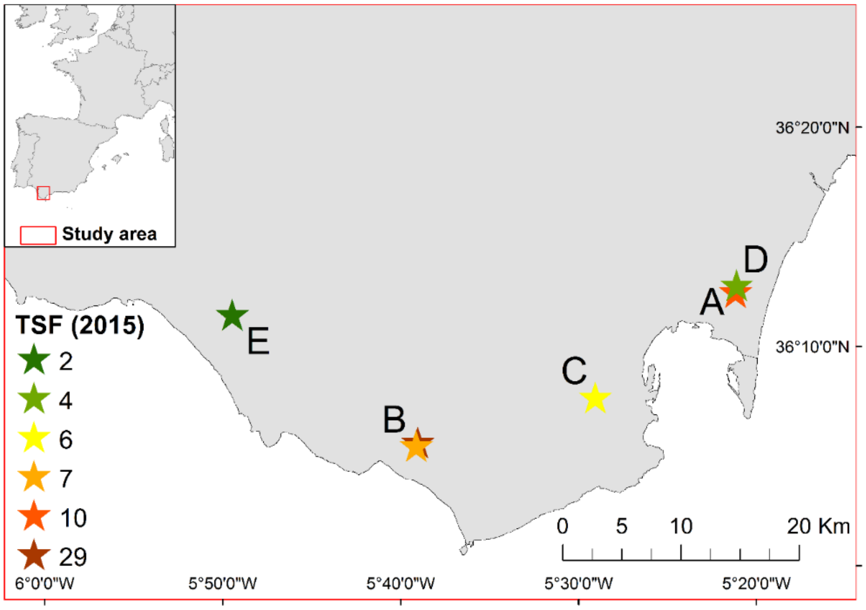


Figure S2.2 Locations in SW Spain (study area) where demographic censuses of *Drosophyllum lusitanicum* were conducted (2011-2015); TSF - time since last fire; A-E site identification as can be found in the data file *dataDroso.csv* (note that for modeling two adjacent populations (100-m distance) were pooled into one site (B).

Table S2.1 Time-since-fire (TSF) of the five populations monitored at each site-year combination. Overall, we censused nine TSF$_{>3}$, three TSF$_3$, two TSF$_2$, and two TSF$_1$ states. A balanced design (equal numbers of TSF states) could not be achieved as we could not perform prescribed burns and only few populations of our rare study species persist in natural heathland habitats.

	Site A	Site B	Site C	Site D	Site E
2011	**TSF$_{>3}$**	**TSF$_3$**	**TSF$_2$**	**burned**	**NA**
2012	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_3$**	**TSF$_1$**	**NA**
2013	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_2$**	**burned**
2014	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_3$**	**TSF$_1$**
2015	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_2$**

The heathlands in this geographic area represent the most important natural habitats for *Drosophyllum* (Garrido *et al.* 2003). They have not experienced large-scale anthropogenic pressures (e.g., infrastructure development and afforestation) common in Morocco and Portugal (Garrido *et al.* 2003). We positioned HOBO data loggers (Onset Computer Corporation 2013) 1 m above ground within each of our five study populations to record temperature (°C) and relative humidity (%) in hourly intervals from January 2013-December 2015. The data loggers confirmed that the microclimate did not differ significantly between the sites and years (Fig. S2.3).

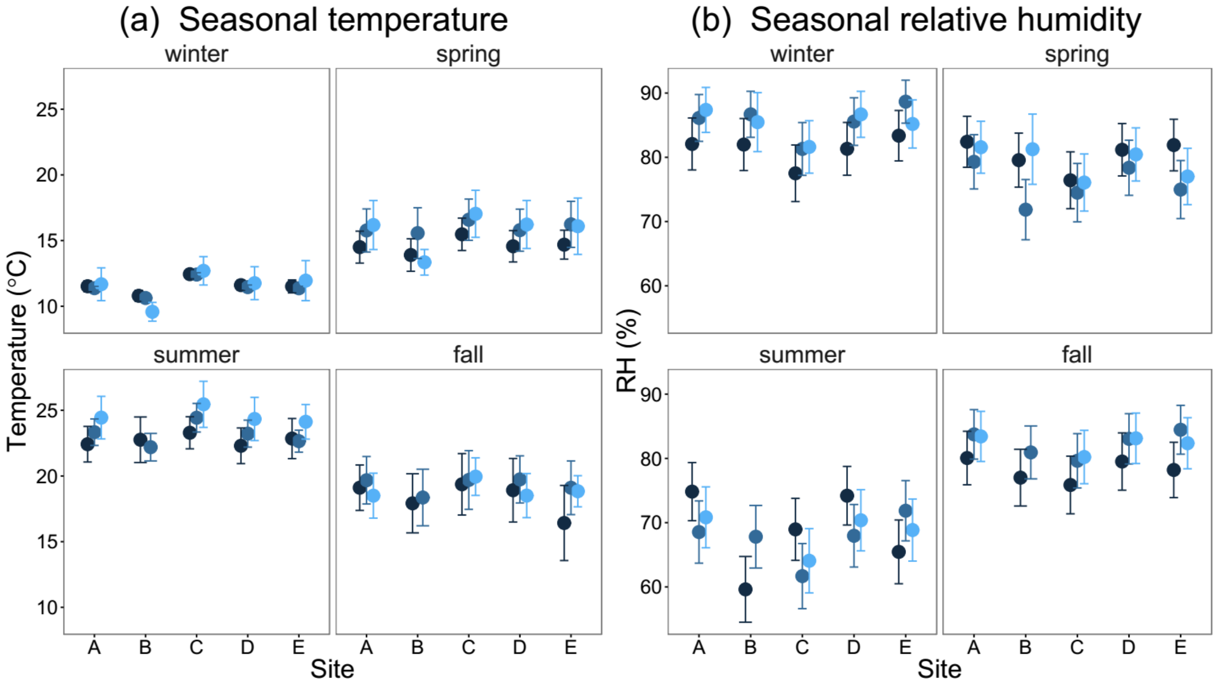


Figure S2.3 Mean (± S.E.) of seasonal temperature (a) and relative humidity (b) measured with HOBO data loggers at the five study sites (site B is pooled across two populations) in 2013(dark blue), 2014 (medium blue) and 2015 (light blue). The two populations pooled in B were relatively close together (100 m apart), and climate values there were measured with the same HOBO. Note that climate data were not available for the summer and fall of 2015 at site B.

5.9.3 Demographic data: above-ground vital rates

The transitions between continuous stages were based on individual-level data collected in annual censuses between April 2011 and April 2015. In each study population, we established ten 1 × 1-m^2 quadrats along four horizontal line transects of 10 m each, resulting in 40 quadrats. The four transects were located 3 m from each other and perpendicular to the main slope. We quantified the size of all tagged individuals by measuring the sum of rosettes, number of leaves per rosette, and the length of the longest leaf. We defined new recruits (as opposed to plants not identified during the previous census due to observation error, 1 %) as plants with < 9 leaves and a maximum leaf length of 11 cm.

We quantified the reproductive output of individuals as the product of the number of flowering stalks per plant, number of flowers per stalk, and number of seeds per flower. In order to estimate the production of viable seeds per flower, we randomly collected 100 individual fruits from reproductive *Drosophyllum* individuals across all populations in which plants flowered in August 2012 and July 2014. We estimated the average number of seeds per fruit to be 9.1 (± 2.6 SE). We also quantified the number of damaged flower heads (mainly from herbivory or wind) in each population and year (Fig. S2.4).

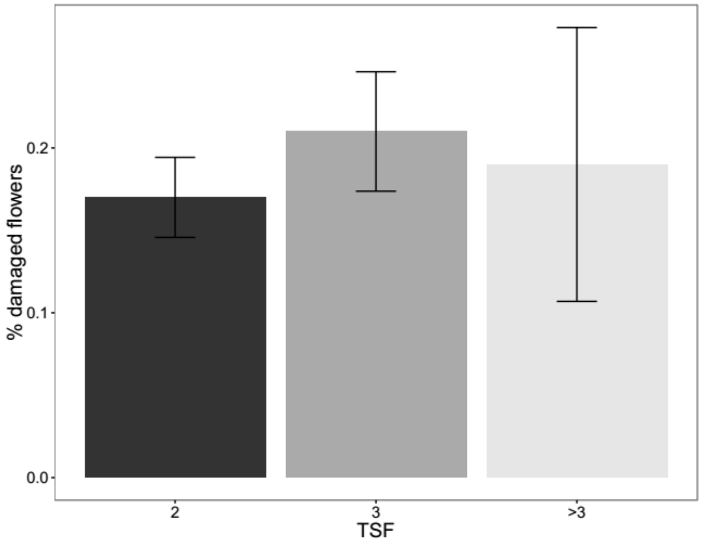


Figure S2.4 Mean (± S.E.) damaged flowers in *Drosophyllum* populations across time-since-fire categories.

5.9.4 Seed fates

In the following paragraphs, you will find a detailed description of how seed-bank vital rates were derived and linked to TSF categories. Table S2.2 summarizes the derivation of these vital rates.

Table S2.2 Extrapolation of seed fates from field experiments to time since fire (TSF) categories in the integral projection models (IPMs) based on field data. *R* is the total number of experimental replicates within *burned* (B) and *unburned* (U) heathland patches; † constant in models; σ_S is seed survival. Vital rates *goCont*, *staySB,* and *outSB* are described in the text below.

	TSF$_0$	TSF$_1$	TSF$_2$	TSF$_3$	TSF$_{>3}$
% of seeds immediately germinating (*goCont*)	0	0	σ_S × *goCont*_U× 0.18 = 0.008 R = 14	σ_S × *goCont*_U × 0.18 = 0.007 R = 14	σ_S × *goCont*_U × 0.18 = 0.007 R = 14
% seeds entering seed bank (*goSB*)	0	0	σ_S × (1-*goCont*_U-0.03) = 0.76 R = 14	σ_S × (1-*goCont*_U-0.03) = 0.77 R = 14	σ_S × (1-*goCont*_U-0.03) = 0.76 R = 14
% seeds staying in seed bank (*staySB*)	0.1†	0.05†	*staySB*_B = 0.6 R = 49	*staySB*_U = 0.85 R = 105	*staySB*_U = 0.85 R = 105
% seeds germinating from seed bank (*outSB*)	0.85 × 0.84 = 0.68	*outSB*_B × 0.84 = 0.05 R = 21	*outSB*_B × 0.18 = 0.01 R = 21	*outSB*_U × 0.18 = 0.005 R = 21	*outSB*_U × 0.18 = 0.005 R = 21

5.9.5 Immediate recruitment and recruitment from the seed bank

We quantified immediate seedling recruitment (*goCont* in the main text) and recruitment from the seed bank (*outSB* in the main text) using a field experiment. This experiment was initially conducted at a natural-heathland site (Sierra Carbonera; 36° 12' 16" N, 5° 21' 39" W) in August 2012 and repeated in August 2013. Care was taken to choose a study site that was potentially (edaphically and climatically) suitable for *Drosophyllum* but with the closest natural *Drosophyllum* population at least 200 m away (site D, Fig. S2.1). In this experiment, we established seven randomized paired blocks perpendicular to an elevation gradient (Fig. S2.5). Each block consisted of adjacent burned (last fire in the summer of 2011) and unburned (last fire in the summer of 2005) patches. We sowed 50 seeds, randomly collected from > 80 individuals across five *Drosophyllum* populations, in squares (20 × 20 cm, 0.5-1 cm deep) in the burned and unburned patches. In three blocks, we created a control treatment by digging up soil in 20 cm^2 without sowing seeds to control for potential germination of naturally occurring seeds by mechanical disturbance. We recorded the number of seedlings in each square in the spring (April) following sowing, from which we determined *goCont*. We also recorded seedling emergence in winter (February), in order to estimate the mortality of newly emerged seedlings from emergence to April. On average, 3 % of sown seeds died as seedlings (Fig. S2.6). Input into the seed bank was then defined as 1-*goCont*-0.03. Seeds that germinated two growing seasons after sowing were considered germination out of the seed bank.

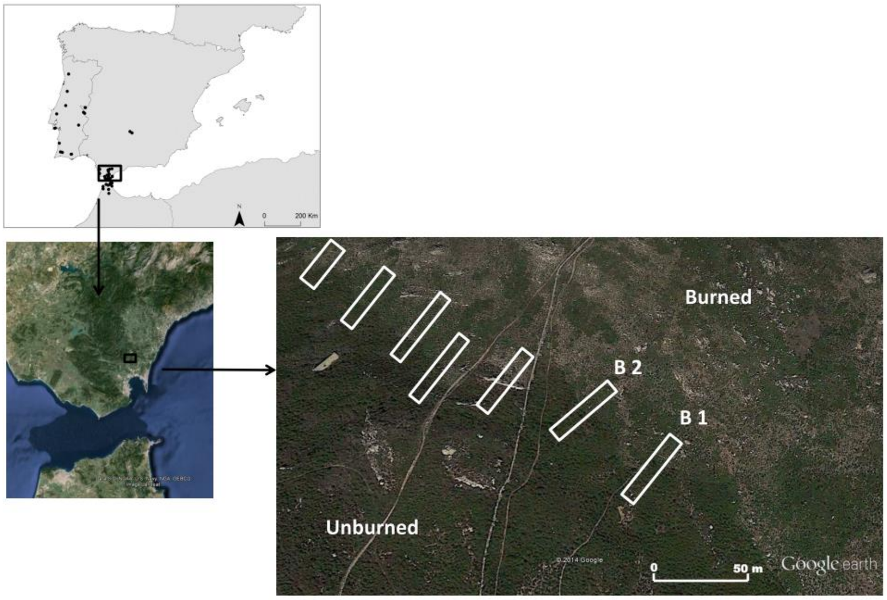


Figure S2.5 Location in SW Spain of the site where seed-addition and seed-bank experiments were performed. At the site, the experiments were performed in seven blocks in burned and unburned patches. The back dots in the upper map identify *Drosophyllum* presence locations across the range of the species.

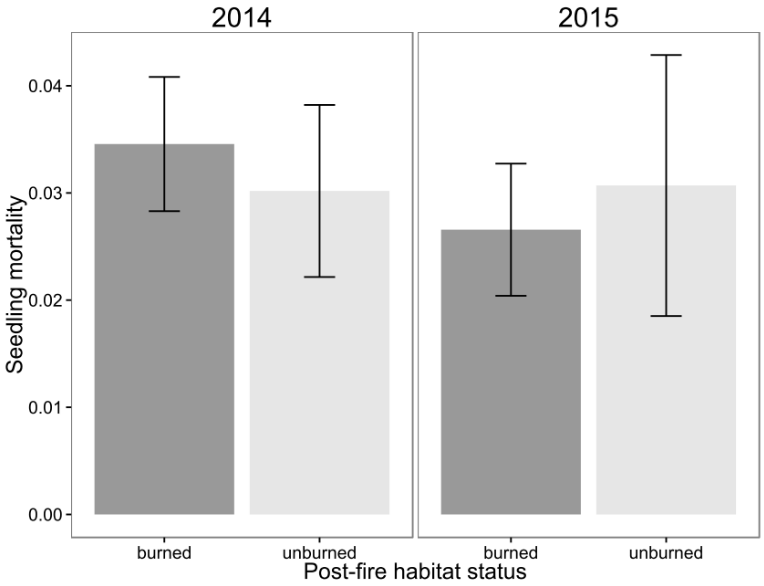


Figure S2.6 Mean (± S.E.) mortality of 50 sewn seed as seedlings as function of burned and unburned habitat patches and two years in which mortality was measured.

In addition, we estimated germination from the seed-bank after fire in a growth-chamber experiment. We applied three *fire* treatments to *Drosophyllum* seeds: (a) heating seeds at 100 ºC for 5 minutes; (b) incubating seeds in a smoke solution (see Jäger *et al*. 1996) for 24 h; and (c) combining treatments (a) and (b). Treated seeds along with dry (unmanipulated) and wet (incubation in distilled water) controls were incubated for 3 months at 20 (± 2) ºC in darkness. We quantified germination from the seed bank after fire (TSF_0, see main text) as 81 %, which corresponded to germination results from the *fire* × *heat* treatment (Fig. S2.7), multiplied by the probability of seedling establishment (84 %), which corresponded to survival of emerged seedlings from February to April in burned habitat patches from the experiment detailed above (data not shown).

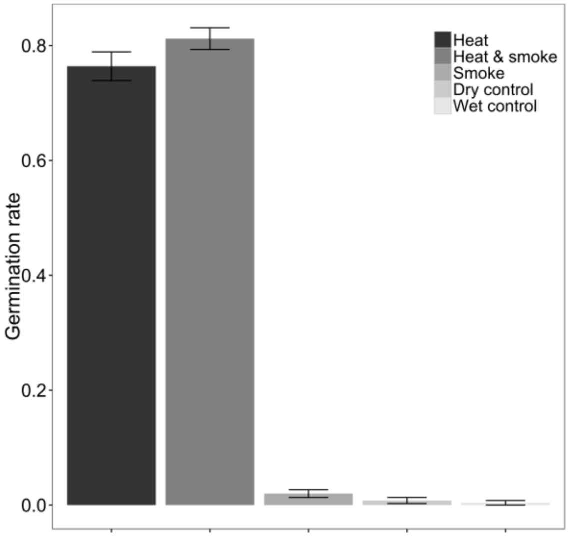


Figure S2.7 Mean (± S.E.) germination in a growth-chamber experiment as a function of different seed treatments.

Additional seed-bank analyses conducted in the spring of 2012, in which we collected 20 random soil samples from the five study populations and an additional three populations from the study area and counted viable *Drosophyllum* seeds in the samples, indicated depleted seed banks in early post-fire habitats (Fig. S2.8). We therefore assumed that only a small proportion of seeds (0.05-0.1) survives in the seed bank after fire.

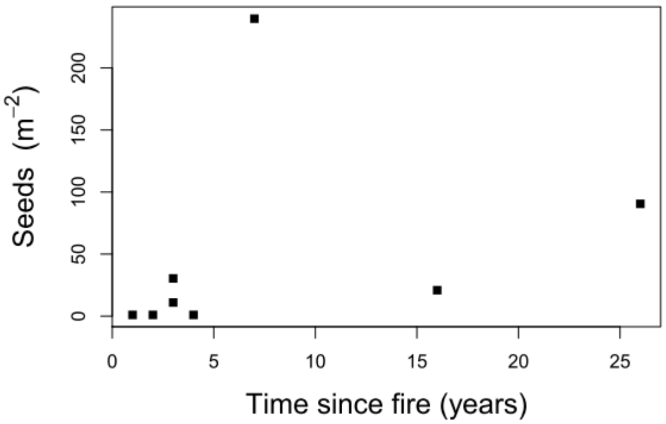


Figure S2.8 Number of seeds per m^2 (extrapolated from 20 samples in 4 × 4 cm cores) in *Drosophyllum* populations characterized by different time since fire.

Our estimates of germination, both *goCont* and *outSB*, were likely elevated compared to germination in natural populations in unburned habitats. This is because we had to perturb soil in order to bury seeds and therefore removed potential sources that may otherwise have inhibited germination, e.g., herbaceous vegetation cover, roots of shrubs, or plant litter. Using our census data, we therefore calculated the ratio of recruits at *t*+1 to seeds available at *t* in all five populations. We estimated from the seed addition experiments detailed above that approximately 65 % of recruits at *t*+1 came from seeds produced at *t* and 35 % from the seed bank. Using this information, we estimated the average *real* immediate germination to be approx. 0.06 % in heathlands with 3 or >3 years since fire (Fig. S2.9). This corresponded to $goCont_{\mathrm{unburned}}$ × 0.18 and $goCont_{\mathrm{unburned}}$ × 0.18. Assuming that we biased *goCont* and *outSB* equally in the seed addition experiment, we multiplied both vital rates by 0.18 in $\mathrm{TSF}_{3,>3}$ to get a more realistic estimate of germination proportions in long unburned heathlands.

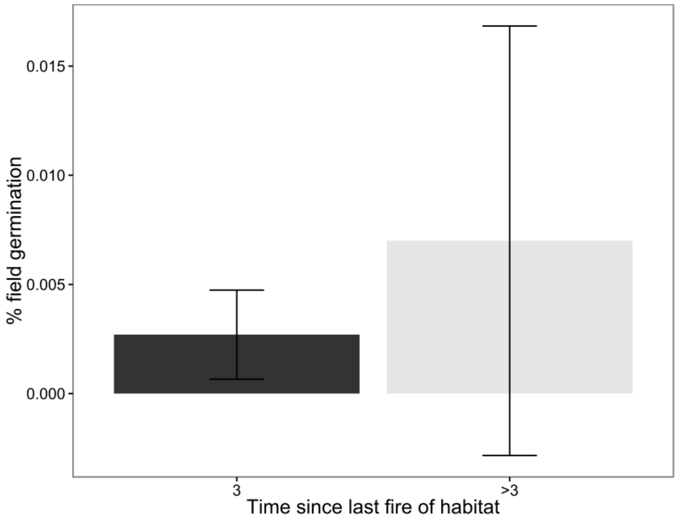


Figure S2.9 Mean (± S.E.) estimated immediate germination in the field as a function of time since fire of the habitat.

5.9.6 Seed-bank stasis

Lastly, we estimated long-term seed-bank dynamics (*staySB* in main text) from an *in-situ* experiment identical to the germination experiment in design and location (Fig. S2.4) and initiated in September 2012 (repeated in 2013 and 2014). We randomly buried (5-8 cm depth) four mesh bags (4 × 4 cm^2, 1 mm mesh size), each containing 20 seeds collected across five *Drosophyllum* populations, in burned and unburned patches. We removed one bag each spring (April) in 2013 and 2014, and the remaining bags in April 2015, and counted the number of viable seeds. Seed-bank survival was measured from bags buried for 1.5 years in the soil, *i.e.*, bags buried in September 2012 and 2013 and retrieved in April 2013 and 2015, respectively. This is because seed mortality in seed bags dug up six months after burial (in April 2013 and 2014) was very low (< 1%); seeds that did not remain in the mesh bags had germinated, as evidenced by the presence of seedlings or empty seed coats with characteristic germination signs. We therefore assumed that measuring stasis after 1.5 years in soil corresponds to what we would have measured by keeping seeds in the soil April-April. We also pooled the data across the two years since we did not have enough data to model year as a random effect.

5.9.7 References

Adamec L. 2009. Ecophysiological investigation on *Drosophyllum lusitanicum*: Why doesn't the plant dry out? *Carnivorous Plant Newsletter* **38**:71–74.

Adlassnig W, Peroutka M, Eder G, Pois W, and Lichtscheidl, I.K. 2006. Ecophysiological observations on *Drosophyllum lusitanicum*. *Ecological Research* **21**:255–262.

Garrido B, Hampe A, Marañón T, Arroyo J. 2003. Regional differences in land use affect population performance of the threatened insectivorous plant *Drosophyllum lusitanicum* (Droseraceae). *Diversity and Distributions* **9**:335–350.

Jäger AK, Light ME, Van Staden J. 1996. Effects of source of plant material and temperature on the production of smoke extracts that promote germination of light-sensitive lettuce seeds. *Environmental and Experimental Botany* **36**:421-429.

Ojeda F, Marañón T, Arroyo J. 2000. Plant diversity in the Aljibe Mountains (S. Spain): a comprehensive account. *Biodiversity and Conservation* **9**:1323–1343.

Onset Computer Corporation. 2013. HOBO Data Loggers. Madrid.

Ortega-Olivencia A, Carrasco Claver JP, Devesa Alcaraz JA. 1995. Floral and reproductive biology of *Drosophyllum lusitanicum* (L.) Link (Droseraceae). *Botanical Journal of the Linnean Society* **118**: 331–351.

Ortega-Olivencia A, Paredes JA, Rodriguez-Riano T, Devesa JA. 1998. Modes of self-pollination and absence of cryptic self-incompatibility in *Drosophyllum lusitanicum* (Droseraceae). *Botanica Acta* **111**:474-480.

5.10 Appendix S3 - Additional information on the modeling processes used quantify variation in the stochastic growth rate and elasticities in stochastic simulations

In this appendix, we provide (a) the statistics we used to determine the best-fit model for a vital rate; (b) an overview and Bayesian diagnostics of the best-fit models (vital rate regressions) parameterized; and (c) A detailed description of elasticity calculations.

5.10.1 DIC model selection

From the census and experimental data on *Drosophyllum*, we chose the best-fit model for each vital rate (see Table S3.1) by comparing several candidate models using DIC. The DIC is a hierarchical modeling equivalent of the AIC measure used in frequentist statistics (Burnham *et al.*, 2011). It is defined as

$$DIC = \overline{D} + 2(\overline{D} - D(\bar{\theta}))$$

where $\overline{D}$ is the posterior mean deviance (-2 × log likelihood of the model) evaluated from deviance measures at each step of the simulation chain, $2(\overline{D} - D(\bar{\theta}))$ is the effective number of parameters, and $D(\bar{\theta})$ is deviance calculated at the posterior mean of the parameters (Spiegelhalter *et al*. 2002). As in AIC model comparison, lower values of DIC imply higher model plausibility. Differences of $\geq$ -5 suggest substantially better fit of the best model compared to the second best model with fewer parameters.

For above-ground vital rates (main text), our candidate models were (a) an intercept-only model, (b) a model including only *size* as predictor, (c) a model including *size* + *TSF* as predictors, and (d) a model including *size* × *TSF* as predictors. *TSF*, or time since fire, was a categorical variable consisting of *j* = 4 levels (1, 2, 3, >3 years since fire). One exception was seedling size (φ_4) where candidate models were either an intercept-only model or a model including *TSF* as predictor of seedling size. Similarly for below-ground, seed-related vital rates, candidate models were (a) an intercept-only model and (b) a model including post-fire habitat state, *PFS*, as predictor, consisting of two levels *p*, burned and unburned. The random *site* (for *s* = 5 sites) and *block* (for *b* = 7 blocks) effects were included in all candidate models describing above- and below-ground vital rates, respectively. All models within the Bayesian framework were parameterized as described below for the best-fit models.

5.10.2 Overview of Bayesian model parameterization

Here, we intend to facilitate the understanding of the R script that runs the Bayesian approaches via OpenBUGS (*i.e.*, `BayModel.R`). For example, using the inverse logit, the likelihood function of probability of flowering within the Bayesian models is described as:

```
for ( f in 1:NtotalFL ) {

fl[f] ~ dbern( mu.fl[f] )

mu.fl[f] <- 1/(1+exp(-( a0.fl + a1.fl[TSF.fl[f]] +
aS.fl[site.flf]] + ( bc.fl + bcTSF.fl[TSF.fl[f]]) * size.fl[f]
)))

}
```

This means that for each data record, *f*, where flowering (either 0 or 1) is known for `NtotalFL` individuals, probability of survival, `fl[f]`, is estimated as a Bernoulli distribution, `dbern`. The parameter describing the shape of the distribution, `mu.fl[f]`, is a function, with associated parameters, of the categorical variable time since fire, `a1.fl[TSF.fl[f]]`, the categorical variable site, `aS.fl[site.fl[f]]`, the continuous variable *size*, `bc.fl * size.fl[f]`, and the interaction of *TSF* and *size*, `bcTSF.fl[TSF.fl[f]] * size.fl[f]`. The parameter `a0.surv` describes the overall mean of the survival data.

We used normal uninformative priors ($\mu = 0$; $1/\theta^2 = 1\times10^{-6}$) for most fixed factors and for the Gamma-distributed rate parameters, ρ, of the Poisson-Gamma mixture models for the number of flowering stalks (φ_1) and number of flowers per stalk (φ_2). (Fig. S3.1). The τ parameters describing the standard deviation in the growth (γ) and seedling-size (φ_4) likelihood functions were associated with uniform priors. We used *hyperpriors* for the random site and block effects (Fig. S3.1). The *hyperpriors* were defined as a normal distribution $N(0,\tau)$ in which the precision, τ to be estimated using the prior $\sigma \sim unif(0,100)$ for the linear and $\sigma \sim unif(0,20)$ for the logistic regressions, respectively. Details on the priors we used can be found in the R file `BayModel.R`.

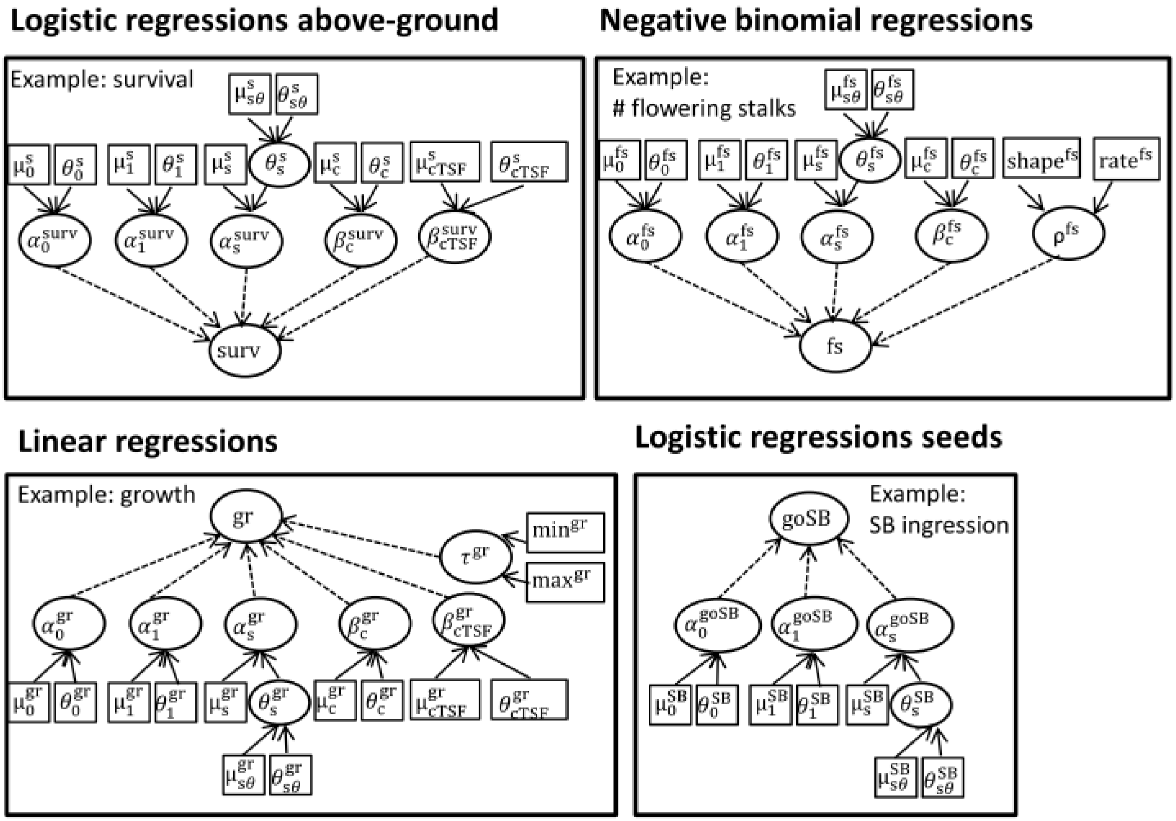


Figure S3.1 Hierarchical structure of the Bayesian modeling process used in the study to simulate log λ_s as a function of parameter uncertainty. Regression models (black boxes) within the Bayesian framework describe the vital rates of the study species. Within the model boxes, circles and rectangles represent parameters to be estimated and fixed data points/prior definitions, respectively (see main text). Hierarchical estimation of parameters occurred via *hyperpriors*.

The posterior sampling of parameters was based on 100,000 iterations, after a burn-in of 100,000 steps, using four chains and subsampling every 400$^{\mathrm{th}}$ simulated value. We initialized the four chains starting with different initial parameter values, which were smaller (by a magnitude of 0.6 or 0.8) or bigger (by 1.2 and 1.5) the then ones obtained from mixed-effect models fitted to the full *Drosophyllum* dataset. We used trace plots and the Gelman-Rubin-Brooks diagnostic of convergence (Brooks and Gelman 1998). In addition, to make the model identifiable, we used the sum-to-zero constraint (Kaufman and Sain 2010) on all categorical variables. For each vital rate *x*, this constrains the difference between the model mean, α_0^{x}, and the parameters at each level of a categorical variable, e.g., $\alpha_{\mathrm{j[TSF]}}^{\mathrm{x}}$, to sum to zero.

We assessed convergence of the chains within the Bayesian framework in several ways: using trace plots and the Gelman-Rubin-Brooks diagnostic of convergence (Brooks and Gelman 1998), plotting priors vs. posteriors, and performing posterior predictive checks as described in Kéry (2010, p. 247). The results can be found in Appendix S4.

5.10.3 Elasticity analyses of lower-level vital rates

Elasticities of *a* = log λ_s to IPM kernel transitions *ij* can be calculated using the formula derived by Tuljapurkar (1990) and Tuljapurkar *et al*. (2003):

$$E_{aij} = \lim_{T\to\infty}\left(\frac{1}{T}\right)\sum_{t=1}^{T-1}\frac{\mathrm{V}_i(t)'C_{ij}(t)\mathrm{U}_j(t-1)}{\lambda(t)\langle\mathbf{V}(t)'\mathbf{U}(t)\rangle} \qquad \text{(F.1)}$$

where **V** and **U** are the left and right eigenvectors associated with λ at each iteration *t* and *t*-1, respectively, and $C_{ij}(t)$ denotes the IPM of proportional changes in entries *ij*. Elasticities to changes in mean transitions can be calculated by defining $C_{ij}(t) = \mu_{ij}$, where μ_{ij} is the average transition *ij* across a sequence of environmental states during simulations. Similarly, elasticities to changes in the standard deviation of transitions can be calculated by defining $C_{ij}(t) = K_{ij}(t) - \mu_{ij}$, where K_{ij} (t) is the IPM kernel values for transition *ij* at time *t*.

The perturbation kernel $C(t)$ can also be derived from lower-level vital rates but requires the application of the chain rule (Haridas and Tuljapurkar 2005).

Elasticities of log λ_s to changes in the mean of the vital rates defined for *Drosophyllum* (see main text) were calculated by perturbing a vital rate *j* VR_j(t) to VR_j(t) + $\overline{VR_j(t)}$, where $\overline{VR_j(t)}$ is the average of VR_j across environmental states. This is equivalent to perturbing the entire vital-rate functions (Rees and Ellner 2009). The resulting changes in the kernel $C(t)$ were substituted into equation F1. For elasticities of log λ_s to changes in the standard deviation of a vital rate, the vital rate *j* would be perturbed from VR_j(t) to VR_j(t) + VR_j(t) - $\overline{VR_j(t)}$. The script `lamdaSimul.R` in Appendix S1 shows how to perform the elasticity calculations for the lower vital rates.

5.10.4 References

Brooks SP, Gelman A. 1998. General methods for monitoring convergence of iterative simulations. *Journal of Computational and Graphical Statistics* **7**:434–455.

Burnham KP, Anderson DR, Huyvaert KP. 2011. AIC model selection and multimodel inference in behavioral ecology : some background , observations , and comparisons. *Behavioral Ecological Sociobiology* **65**:23–35.

Kaufman CG, Sain SR. 2010. Bayesian functional ANOVA modeling using gaussian process prior distributions. *Bayesian Analysis* **5**:123-149.

Kéry M. 2010. *Introduction to WinBUGS for Ecologists: A Bayesian Approach to Regression, ANOVA, Mixed Models and Related Analyses*. Academic Press..

Rees M, Ellner, SP. 2009. Integral projection models for populations in temporally varying environments. *Ecological Monographs* **79**:575-594.

Spiegelhalter DJ, Best NG, Carlin BP, Van Der Linde A. 2002. Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* **64**:583-639.

5.11 Appendix S4 - Additional modeling results

Here, we present (a) a graphical representation of the fit of models quantifying vital rates; (b) results from our diagnostic analyses aimed to test performance of the vital-rate models fitted in the Bayesian framework; (c) contribution of parameter uncertainty to the variance of the stochastic growth rate at different fire return intervals; and (d) contribution of uncertainty of the remaining (not seed bank) model parameters to overall variance.

5.11.1 Visualization of model fit

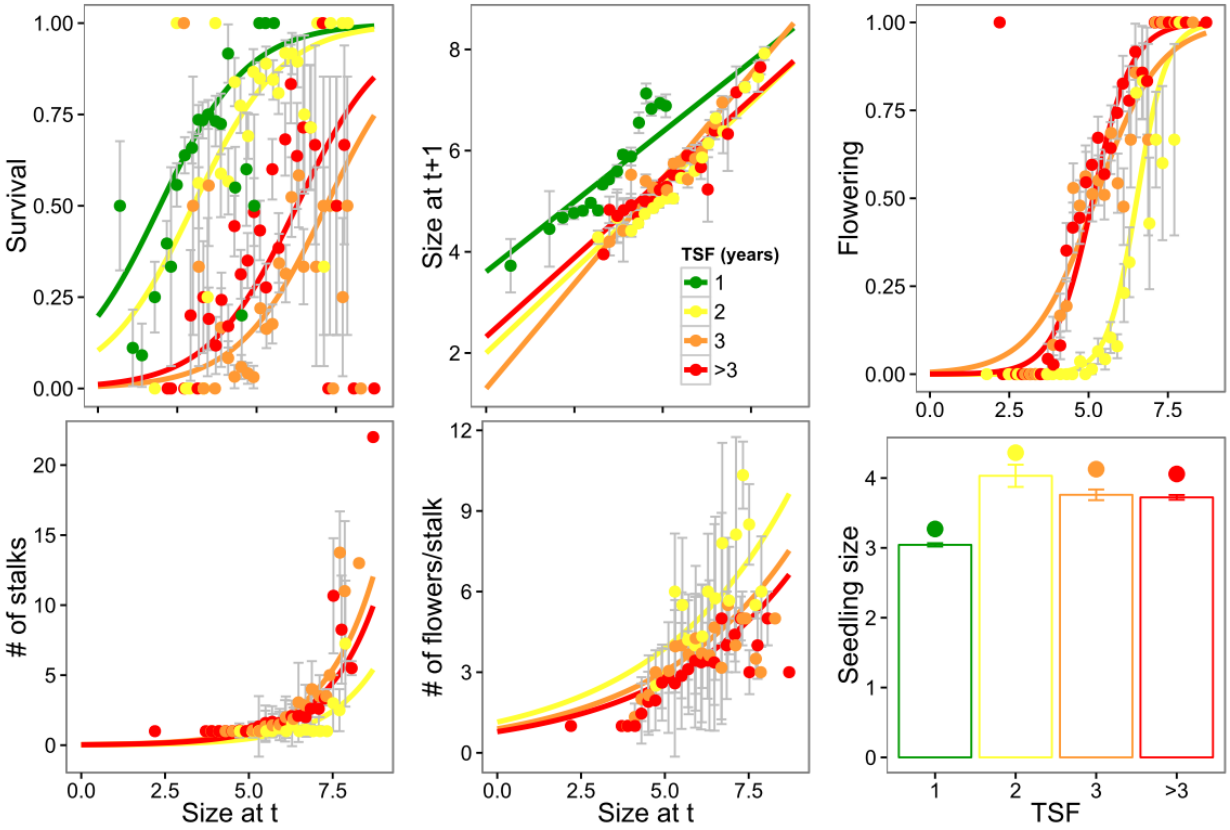


Figure S4.1 Predictions of vital rates (lines**;** points for seedling size) describing population dynamics of the continuous state (above-ground individuals) in the life cycle of the carnivorous plant *Drosophyllum* as a function of time-since-fire (TSF) categories, depicted by different colors. Predictions were obtained with Bayesian models. The points (except for seedling size) represent mean observed values (± S.E.) of the response variables for each TSF category within certain size ranges (0.1–9.0 at interval of 0.1) at *t* for display purposes.

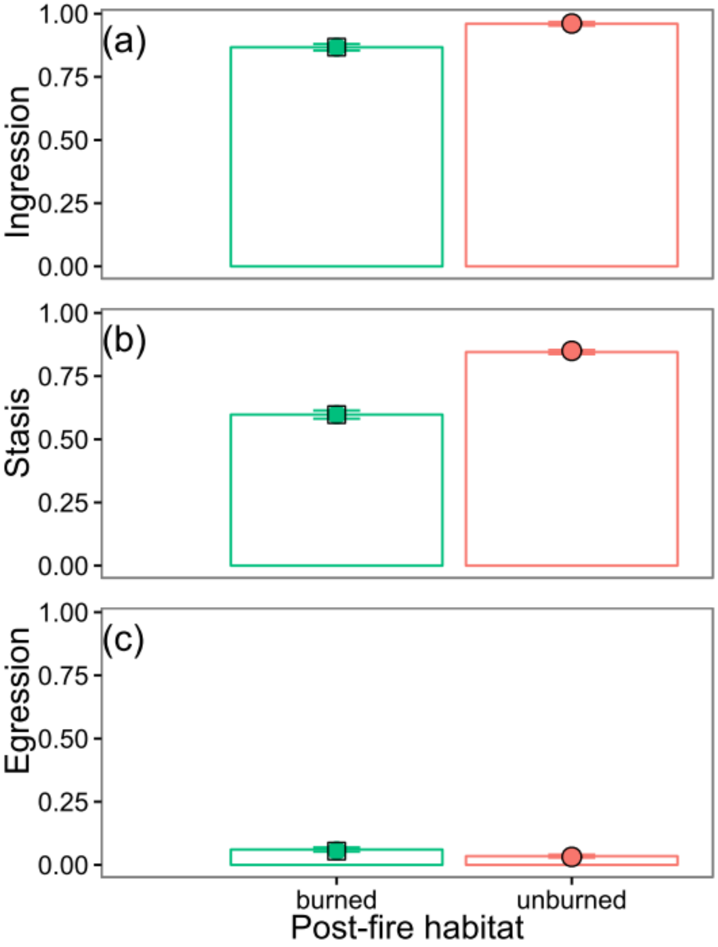


Figure S4.2 Predictions of vital rates (points) describing seed-bank trnasitions in burned and unburned patches where seed addition experiments were performed. Predictions were obtained with Bayesian (point shape) models. The bars represent mean observed values (± S.E.). The parameters depict (a) ingression into (*goSB*), (b) stasis in (*staySB*), and (c) egression out of the seed bank (*outSB*).

5.11.2 Diagnostics of model convergence within Bayesian framework

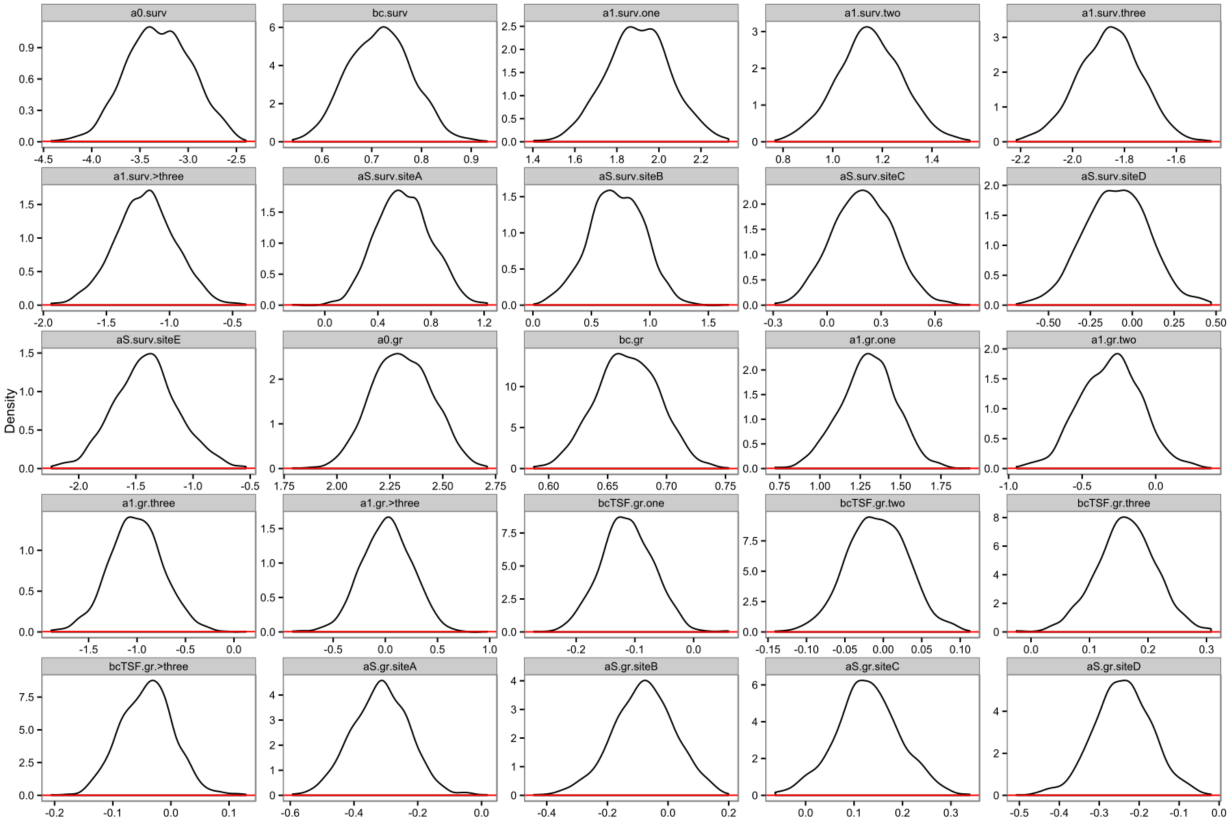


Figure S4.3 Prior (red) vs. posterior (black) density plots for 25 parameters representing above-ground transitions(all priors are non-informative). The remaining parameters had similar shapes.

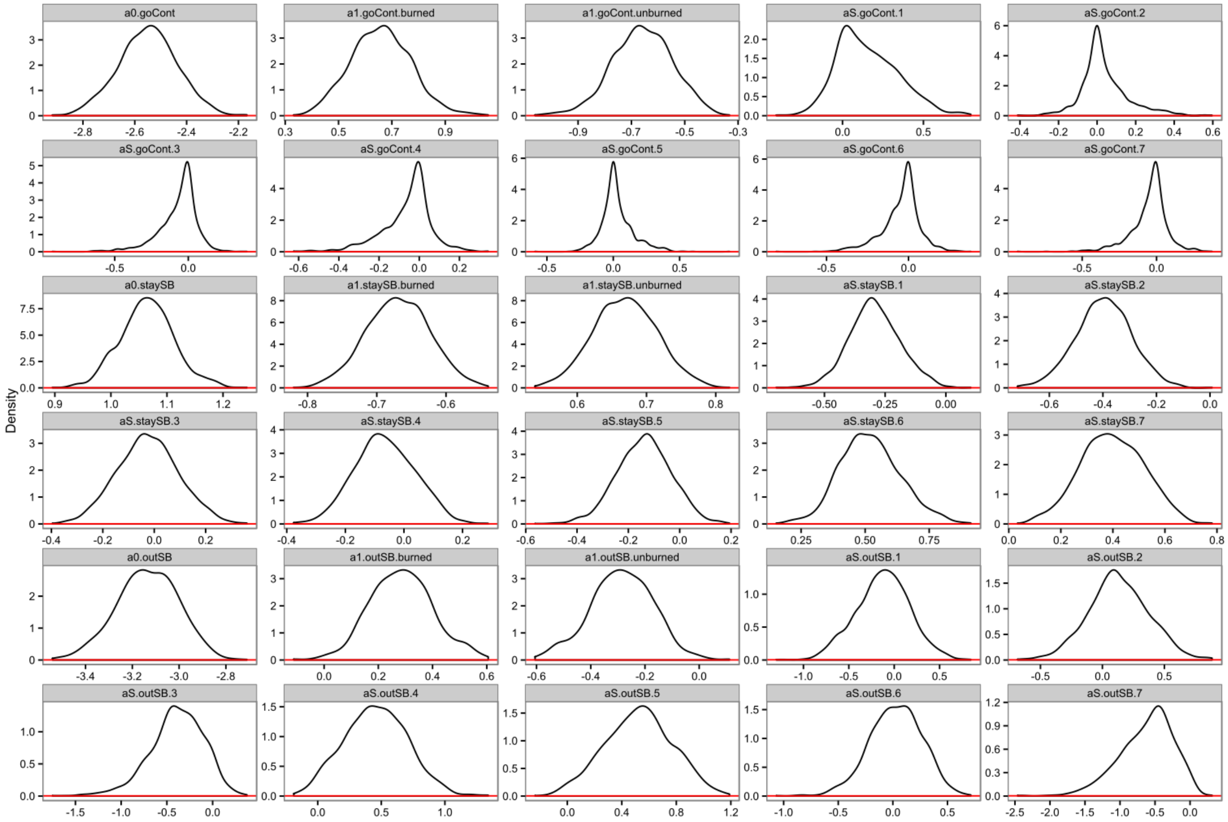


Figure S4.4 Prior (red) vs. posterior (black) density plots for parameters related to seed-bank transitions (ingression – 1-*goCont*; stasis – *staySB*; and egression – *outSB*).

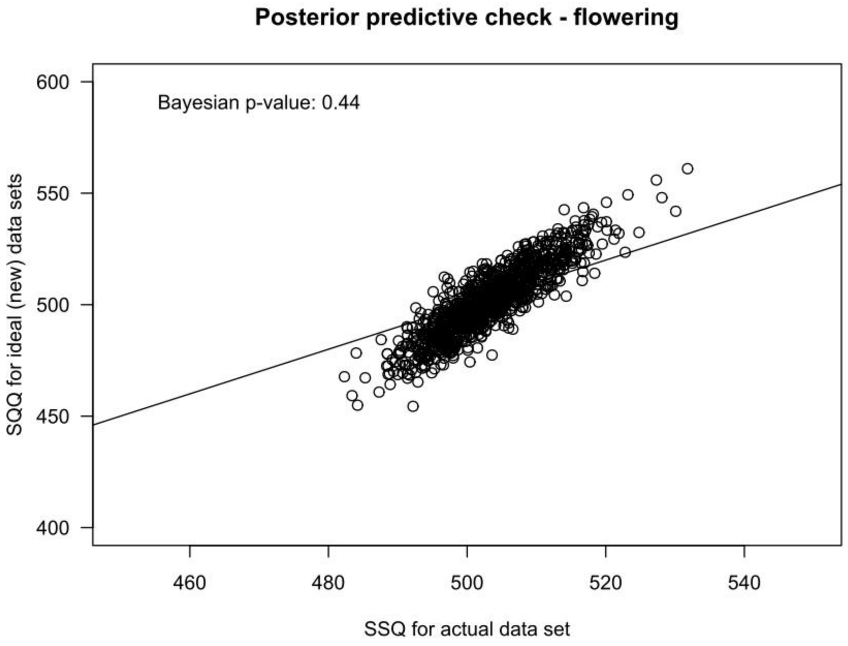


Figure S4.5 Graphical posterior predictive check of the model adequacy for the vital rate probability of flowering (φ_0). The Bayesian *p*-value is equal to the proportion of symbols above the 1:1 line.

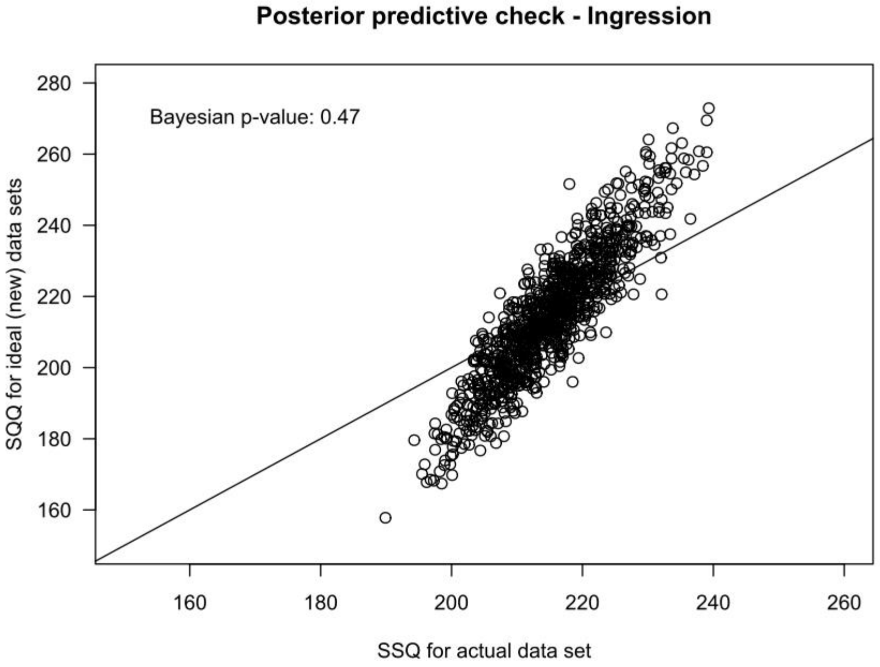


Figure S4.6 Graphical posterior predictive check of the model adequacy for the vital rate ingression (1-*goCont*). The Bayesian *p*-value is equal to the proportion of symbols above the 1:1 line.

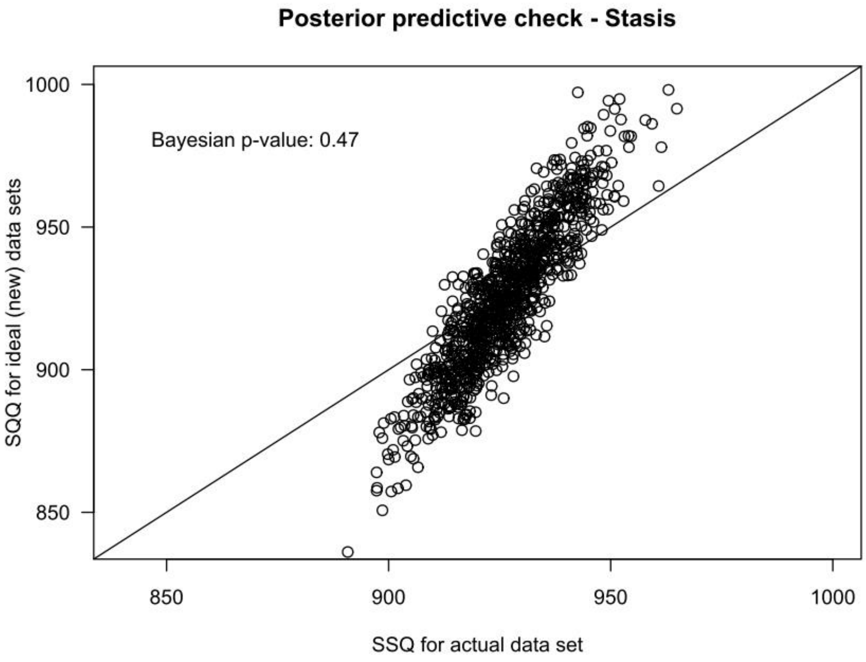


Figure S4.7 Graphical posterior predictive check of the model adequacy for the vital rate stasis (*staySB*). The Bayesian *p*-value is equal to the proportion of symbols above the 1:1 line.

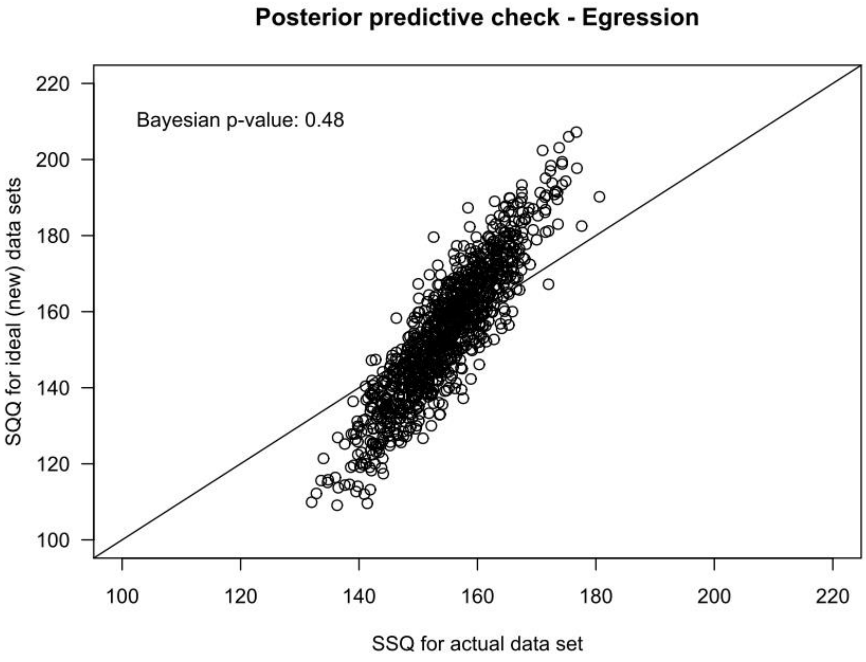


Figure S4.8 Graphical posterior predictive check of the model adequacy for the vital rate egression (*outSB*). The Bayesian *p*-value is equal to the proportion of symbols above the 1:1 line.

5.11.3 Parameter uncertainty

Table S4.1 Differences in proportional contribution of parameter uncertainty to the variation in the stochastic population growth rate of *Drosophyllum lusitanicum* as a function of vital-rate parameters sampled in stochastic simulations. The simulations were defined by varying fire return intervals, and the vital rates sampled corresponded to ingression, *goSB*, stasis, *staySB*, and egression, *outSB* (Figure 1 in main text). Proportional contributions of > 0.5 are highlighted in bold.

Vital rate	Fire return interval (years)									
	10	20	30	40	50	60	70	80	90	100
goSB	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	< 0.01	< 0.01
staySB	0.12	0.25	0.34	0.41	0.46	0.50	**0.54**	**0.57**	**0.60**	**0.62**
outSB	0.14	0.32	0.44	**0.51**	**0.57**	**0.62**	**0.66**	**0.68**	**0.71**	**0.73**
staySB+outSB	0.17	0.40	**0.51**	**0.60**	**0.64**	**0.69**	**0.72**	**0.75**	**0.77**	**0.79**

5.11.4 Parameter uncertainty in all vital rates

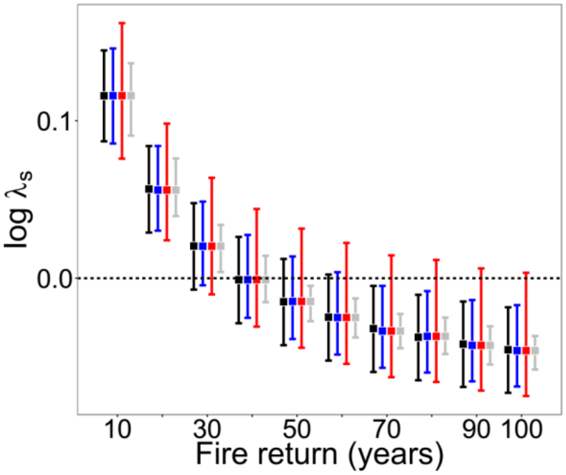


Figure S4.9 Plots display average log λ_s (points) and 2.5 and 97.5 quantiles (vertical lines) as function of fire return interval (x-axis). At each fire return interval, the quantiles were calculated from log λ_s estimates obtained from four different approaches: running 100 stochastic projections of log λ_s for each of 1,000 posterior parameter samples describing either seed-bank ingression and stasis (black; six parameter); or model parameters describing above-ground survival (blue; 13 parameters); or all parameters related to above-ground dynamics (red; 93 parameters); or running 100 stochastic simulations using mean parameter values for all vital rates (grey). Black horizontal dashed lines indicate stable population sizes.

CHAPTER 6

Local-scale disturbances can benefit an endangered, fire-adapted plant species in Western Mediterranean heathlands in the absence of fire

CHAPTER 6

Local-scale disturbances can benefit an endangered, fire-adapted plant species in Western Mediterranean heathlands in the absence of fire

Maria Paniw, Roberto Salguero-Gómez, and Fernando Ojeda

This chapter was published in *Biological Conservation*, 187 (2015), 74–81.

6.1 Abstract

Fire-prone Mediterranean heathlands are biodiversity hotspots and home to a multitude of fire-dependent species. Fire-suppression policies worldwide have increased the risk of extinction of such species or have delegated relative prominence to other disturbances. This study explores how such a substitution of disturbances can alter the realized niche and population structure of a post-fire specialist. Using multi-model inference, we evaluate the relative magnitude and directionality (positive or negative) in the effect of large-scale (entire range) and local-scale (southern Spain) anthropogenic disturbances on the occurrence and abundance of *Drosophyllum lusitanicum*. This rare carnivorous plant is endemic to heathlands in the Southwestern Iberian Peninsula and northwestern Morocco managed under strict fire suppression policies. An increase in regional-scale anthropogenic disturbances did not affect regional-scale occurrence but significantly decreased abundance of *Drosophyllum*. On the other hand, local-scale anthropogenic disturbances positively affected both population occurrence and abundance. We demonstrate that non-fire anthropogenic disturbances can drive the population structure and distribution of an endemic post-fire specialist in Mediterranean heathlands, but their impacts change with their spatial scale. Although large-scale disturbances may threaten populations, small-scale disturbances may be more significant for species occurrence, and positively affect the distribution and abundances of pyrophytic species. In heathlands where fire suppression is a common practice, applying such disturbance regimes can be crucial for the preservation of fire-adapted species.

Keywords: anthropogenic disturbances, carnivorous plants, *Drosophyllum lusitanicum*, fire suppression, post-fire specialists, pyrophytic species

6.2 Introduction

Mediterranean heathlands, characterized by dense shrub vegetation on acidic, nutrient-poor soils, constitute a singular biome worldwide owing to their remarkable plant diversity and endemism levels (Cowling *et al.* 1996; Médail and Quézel 1999; Ojeda *et al.* 2001). The five Mediterranean-climate regions in which heathlands occur occupy less than 5% of the global area and host about 20% of the world's vascular plants, many of them endemics (Cowling *et al.* 1996). The diverse flora of heathlands has provided humans with important resources such as fuel or animal feed for millennia (Hobbs *et al.* 1995). In part, the high levels of endemism in Mediterranean heathlands are due to recurrent wildfires, natural disturbances that have promoted unique adaptations such as fire-induced germination or post-fire resprouting (Ojeda 2001; Ojeda *et al.* 2010; Keeley *et al.* 2012).

Effective post-fire recruitment from a soil-stored persistent seed bank is one of the key adaptations in Mediterranean heathlands (Keeley *et al.* 2011). Fire-induced cues for germination can be direct (*i.e.*, pyrogenic), such as heat shock and/or smoke-derived compounds (Keeley and Bond 1997; Moreira *et al.* 2010), or indirect, such as marked increases in light levels (Keeley 1987; Ooi *et al.* 2014) or drastic reductions of allelochemical inhibitors following the removal of aboveground vegetation and litter by fire (Preston and Baldwin, 1999). Many short-lived perennial plants are killed by fire and only recruit after fire from a seed bank. After a fire-triggered recruitment event, their mostly even-aged populations reach highest above-ground densities in the first few years after fire, and then decline, disappearing aboveground into the mature community vegetation, but persisting in the seed bank (e.g., Quintana-Ascencio *et al.* 1998, 2003). Many of these post-fire dwelling species are hence highly sensitive to alterations in fire frequency (Quintana-Ascencio *et al*. 2003; Lawson *et al.* 2009).

In the western Mediterranean Basin, fire suppression and habitat degradation have altered fire frequency in heathlands in the last centuries, which poses a threat to post-fire specialists (e.g., Bartolomé *et al.* 2005). Fire suppression has become a common goal in current management policies (Bartolomé *et al.* 2005; Keeley *et al.* 2012; Fernandes *et al.* 2013), which has led to the loss of heathlands by oak (*Quercus* spp.) or pine (*Pinus* spp.) forest encroachment (Bartolomé *et al.* 2005). The transformation of heathlands into forests is also the result of active afforestation campaigns (Andres and Ojeda 2002). The combination of fire suppression and anthropogenic disturbances should increase local extinction risk of pyrophytic species (Bartolomé *et al.* 2005; Lawson *et al.* 2010; Fagúndez 2013). Nonetheless, species responding to indirect fire-related germination cues, such as light increase from vegetation removal, may still find refuges from local extinction in habitats where disturbances such as shrub slashing mimic the fire effect of elimination of the aboveground vegetation (Pavlovic 1994; Quintana-Ascencio *et al.* 2007). At the same time, anthropogenic disturbances that prevent natural habitat succession (e.g., vegetation clearance) and therefore allow for multiple recruitment events of post-fire specialists may affect not only the distribution but also the age structure of otherwise cohort-pulsed populations (Quintana-Ascencio *et al.* 2007).

Here, we explore how anthropogenic disturbances occurring at different spatial scales affect the regional and local occurrence and population structure of the endemic, post-fire dwelling carnivorous plant *Drosophyllum lusitanicum* (L.) Link. (Drosophyllaceae; hereafter *Drosophyllum*). This pyrophytic species is threatened by large-scale anthropogenic disturbances such as afforestation and fire suppression (Correia and Freitas 2002) but profits from small-scale vegetation clearances (Garrido *et al.* 2003). In order to predict the current distribution and population structure of *Drosophyllum*, we fitted both responses to explanatory variables related to non-fire disturbances at two spatial scales. As regional-scale disturbances are associated with permanent habitat loss (Correia and Freitas 2002) while local-scale disturbances may increase germination and above-ground survival (Quintana-Ascencio *et al.* 2007), we tested the following hypothesis: anthropogenic disturbances will have significant effects on the distribution/abundance of a post-fire specialist with indirect germination cues, but the effect will be negative at a regional and positive at a local spatial scale. We used generalized linear models to predict occurrence and abundance of various stages in the life cycle of *Drosophyllum* and model inference to quantify the relative importance of explanatory variables.

6.3 Materials and methods

6.3.1 Study species

Drosophyllum lusitanicum is a geographically and phylogenetically rare carnivorous subshrub, endemic to heathlands in the southwestern Iberian Peninsula and northwestern tip of Morocco (Garrido *et al.* 2003; Heubl *et al.* 2006). The species is a post-fire specialist on nutrient-poor, acid soils (Correia and Freitas 2002). Individuals are outcompeted by surrounding shrubs in mature post-fire stands, and populations persist in soil seed banks until the appropriate fire cues (presumably heat shock and light from above-ground vegetation removal) trigger their germination (Correia and Freitas 2002). Individuals grow in rosettes, and size in this species is a good proxy for age, with plants initially reproducing in the second year after emergence and gaining 1-2 rosettes each growing season (Ortega-Olivencia *et al.* 1995; Garrido *et al.* 2003). The maximum observed lifespan of individuals is approximately 10 years (Juniper *et al.* 1989). However, a mean life expectancy of 1.6 years (M. Paniw, unpubl.), as approximated through integral projection models (Easterling *et al.* 2000; Caswell 2001; Metcalf *et al.* 2013) highlights a greatly skewed distribution of mortality early on in life.

The species is in stark decline due to heathland afforestation (Andrés and Ojeda 2002) and fire suppression. However, some populations in non-fire habitats persist due to anthropogenic disturbances that clear vegetation and create open patches (e.g. fire-break lines, sandstone quarries; Garrido *et al.* 2003). These open patches may expose seeds to direct sunlight and may eliminate putative allelopathic compounds that inhibit germination (Preston and Baldwin 1999). As non-fire disturbances may permanently eliminate vegetation (e.g. quarries, mechanical uprooting) or are maintained by periodic vegetation slashing (e.g. firebreak lines), open space is either long lasting or continuously created, respectively. In either case, the permanent availability of open space promotes annual seed germination and may contribute to the prevalence of mixed-aged populations of *Drosophyllum* (Garrido *et al.* 2003).

In order to model the effects of anthropogenic disturbances and climate on the population structure of *Drosophyllum*, we divided the life cycle of the species into three life-cycle classes: (i) juveniles, defined as individuals of 1-2 years consisting of one rosette without a flowering stalk; (ii) young reproductive individuals (2-3 years old), characterized by 1-2 rosettes with at least one flowering stalk; and (iii) old reproductive individuals (> 3 years old), characterized by at least three rosettes and at least one flowering stalk. The mean number of flowers per stalk is 5 ± 2 (SD), according to Ortega-Olivencia *et al.* (1995). We sampled populations in February-June 2013 and 2014, and chose to exclude newly emerged seedlings from our analyses as fluctuations in the abundance of this life-cycle class is strongly affected by the month of sampling (Garrido *et al.* 2003; M. Paniw, pers. obs.).

6.3.2 Study sites, design, and sampling

We aimed to model the distribution and population structure of *Drosophyllum* at a regional and local scale in order to analyze scale-specific effects of non-fire disturbances. We therefore recorded species occurrence and abundance of the aforementioned three life-cycle classes across the entire range of the species (regional scale) and in southern Spain (local scale). The three regions that comprise the geographical range of *Drosophyllum*, i.e. southern Spain, northern Morocco, and western Portugal, differ in the type, frequency, and intensity of anthropogenic disturbances in heathlands (Garrido *et al.* 2003; Fig. 1d). While *Drosophyllum* is protected in southern Spain (BOJA 1994) and its heathland communities are relatively undisturbed (but see Andrés and Ojeda 2002), these habitats have been severely altered in Portugal by settlement construction and intense aforestation with gum trees (*Eucalyptus globulus* and *E. diversicolor*) and pines (*Pinus pinaster*). Moroccan heathlands have not been altered to the same extent as Portuguese ones, but they experience intense levels of goat browsing and hand-slashing (Ojeda *et al.* 1996; Garrido *et al.* 2003).

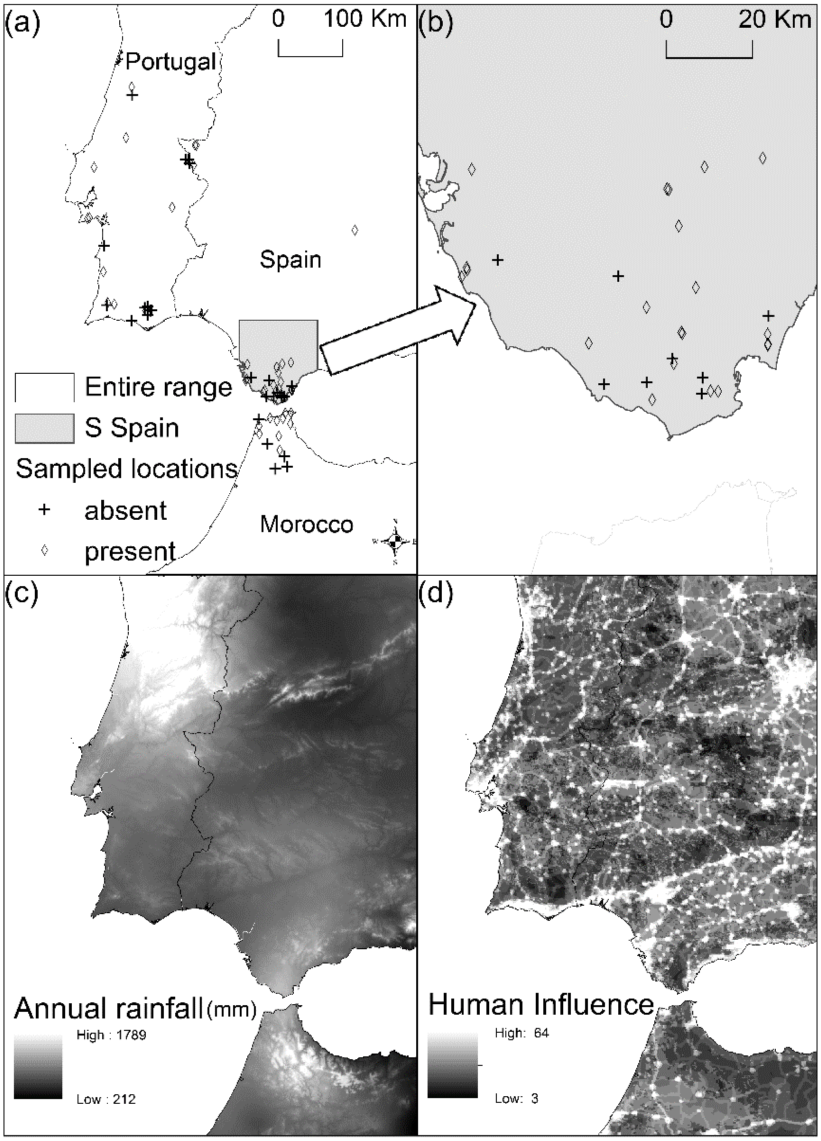


Figure 1 Coordinates of sampled locations indicating presence or absence of *Drosophyllum lusitanicum* populations at the study region (a). Close-up of sampled locations in S. Spain used for local-scale modeling (b); subsampled locations for climatic models marked as stars. Distribution of rainfall (mm) (c) and human activities (no unit) (d).

Across the species' range, we surveyed 74 sites characterized by nutrient-poor soils and different degrees of disturbances in heathlands (Fig. 1a; Appendix S1). We used publications (Correira and Freitas 2002; Garrido *et al.* 2003), species presence records from public online databases (www.flora-on.pt for Portugal and www.anthos.es for Spain), and expert knowledge (see Acknowledgements) to identify sites where *Drosophyllum* populations have been recorded. We then thoroughly surveyed all sites across the species range to authenticate presence of *Drosophyllum*. Sites that suffered a natural fire < 10 years ago were excluded from the regional-scale survey in order to evaluate only effects of non-fire disturbances on the occurrence of populations. We used satellite imagery (Arino *et al.* 2011), public records (Junta de Andalucía 2013), and visual clues (*i.e.*, fire scars on mature scrubs) to determine the post-fire stage of each site. Climatic features were consistent between sites, dominated by oceanic-Mediterranean conditions (Fig. 1c). We took a subset of 30 out of the 74 locations in southern Spain to fit models for anthropogenic disturbances at the local scale (Fig. 1b). Here, the sampled locations represented (i) a mixture of mature Mediterranean heathlands characterized by dense, low scrub cover (Ojeda *et al.* 2000; Garrido *et al.* 2003); (ii) marginal habitats in clearances such as firebreaks, road sides, or on steep slopes characterized by sparse shrub cover; and (iii) managed habitats (e.g., sparsely planted pine plantations on heathlands; Appendix S1).

At both scales, regional and local, abundance of *Drosophyllum* was recorded in sixty 1 m^2 quadrats along six linear transects of 10 m each. Each transect was randomly started at the edge of a *Drosophyllum* population and extended perpendicular to the main slope of the site towards the center (i.e., highest abundance of individuals) of the population. We measured the abundance of different life-cycle classes in each quadrat (juveniles, young reproductive, and old reproductive individuals). Total and class-specific abundances were then used as response variables for the disturbance and climatic analyses at the local scale (below). At the regional scale, the abundances at 60 m^2 were extrapolated to 1 km^2 in order to standardize the abundance estimates to account for the variable size of the sampling area (Appendix S1). We derived three responses from our measurements for the abundance models: total abundance; abundance of juveniles and young reproductive individuals, and abundance of old reproductive individuals. We used three responses because disturbance variables may have different effects on abundance depending on life-cycle stage, indicating whether mixed-aged populations are favored by disturbances.

6.3.3 Anthropogenic disturbances

To model the occurrence and abundance of *Drosophyllum* at two spatial scales, we selected, based on ecological relevance for the distribution of the species, a total of 15 candidate variables (ten and five for regional- and local-scale analysis, respectively) related to non-fire disturbances. All candidate variables were either available as – or converted to — GIS raster layers, and were aggregated to a resolution of 1 km for regional-scale and 30 m for local-scale modeling.

We derived candidate variables approximating anthropogenic disturbances from CORINE land cover (250-m resolution; European Topic Centre on Spatial Information and Analysis, 2013) for regional-scale and a high-resolution (5 m) land-use map (Junta de Andalucía 2007) for local-scale modeling. Candidate variables included sum of and mean log distance to pixels classified as managed, forested (pine plantations) or natural (shrub) areas (regional scale), or distance to roads/firebreaks and percent bare soil (local scale). At the regional scale, we also included the Human Influence Index (HII; Wildlife Conservation Society and Columbia University 2005), which uses data on population density, infrastructure, and land use to classify areas ranging from completely free of human influence (1) to highly disturbed by humans (64). To control for the effect of natural disturbances on *Drosophyllum* populations in the absence of human activities, we calculated three measures of terrain roughness. We obtained all three measures from digital elevation models (NASA LP DAAC 2001) at 90 and 30 m resolution for regional and local analyses, respectively. The measures included slope, slope variability and standard deviation of elevation - calculated for each grid cell based on a 3 × 3 grid-cell neighborhood (Wilson and Gallant 2000).

To reduce colinearity of explanatory variables when deriving relative predictor importance, we included into the final multi-model framework only those explanatory variables which were not strongly correlated (Pearson's $r < 0.4$) with other variables (Thuiller *et al.* 2007). Table 1 summarizes the explanatory variables chosen for statistical analyses at each spatial scale.

Table 1 Description of the explanatory variables used to model occurrence and abundance of *Drosophyllum lusitanicum* (L.) Link. at the regional and local scale.

	Category	Variable	Values	Source
Regional (1 km^2 grid resolution)	Anthropogenic disturbance	(a) Log distance from needle-leaved evergreen forests (m);	(a) Euclidean distance from pixels classified as “needle-leaved evergreen tree cover” aggregated (mean) from 250-m to 1-km resolution;	(a) European Topic Centre, 2010; http://www.eea.europa.eu/data-and-maps
		(b) Human Influence Index	(b) Values obtained from maps classifying human activities & infrastructure and range from 1 (least influenced by humans) to 64 (heavily influenced by humans)	(b) LTW-2, 2005; http://sedac.ciesin.columbia.edu/wildareas/
	Natural disturbance	Terrain roughness (degrees)	Difference between maximum and minimum slope (calculated from a DEM) in a 3 × 3-cell neighborhood of each 90-m grid cell aggregated (mean) to 1-km resolution	LP DAAC, 2001; https://lpdaac.usgs.gov/get_data
Local (30 m^2 grid resolution)	Anthropogenic disturbance	(a) Distance to roads or firebreaks (m)	(a) Euclidean distance from pixels classified as “roads” or “firebreak” aggregated from 5-m (mean) to 30-m resolution	(a) Junta de Andalucia, 2007; http://www.juntadeandalucia.es/
		(b) Bare soil cover (%)	(b) Mean % soil over a 30-m grid calculated from a 5-m resolution land-use map	(b) Junta de Andalucia, 2007
	Natural disturbance	Terrain roughness (degrees)	Difference between maximum and minimum slope (calculated from a DEM) in a 3 × 3-cell neighborhood of each 30-m grid cell	LP DAAC, 2001; https://lpdaac.usgs.gov/get_data

Values for the variables were derived from original datasets using ESRI ArcGIS 10.

6.3.4 Statistical analyses

We determined the relative importance and positive vs. negative effects of the selected explanatory variables on the occurrence and abundance of *Drosophyllum* populations using model averaging based on $\mathrm{AIC_c}$ estimates (Burnham *et al.* 2011). Before fitting models, all explanatory variables were z-transformed (mean = 0, SD = 1) in order to standardize the effect of the variables. At the regional scale, models included additive and interaction effects between all explanatory variables. At the local scale, models included only additive effects due to the small sample size ($n \leq 30$) and because preliminary results testing pairwise interactions between explanatory variables showed no significance of interactions. We fitted generalized linear models with a binomial error distribution to the occurrence data. To fit the abundance models, we chose generalized linear models with a negative binomial error distribution over simpler Poisson models as the latter was overdispersed, while the former provided a better model fit (Ver Hoef and Boveng 2007; Güthlin *et al.* 2013; Zipkin *et al.* 2014). At both spatial scales, we estimated the importance of each explanatory variable in explaining the response variable as the sum of the $\mathrm{AIC_c}$ weights – comparing model $\mathrm{AIC_c}$ to lowest overall $\mathrm{AIC_c}$ – over all models in which variable *i* occurred (Burnham *et al.* 2011). We calculated the strength of the weights by permuting variable *i* 1000 times while keeping all the other variables unchanged and subtracting the median of the 1000 permuted, summed $\mathrm{AIC_c}$ from the original summed weights for variable *i*. We therefore created an absolute weight of evidence (Thuiller *et al.* 2007). We considered only variables with a positive absolute weight to be significant drivers of *Drosophyllum* occurrence or abundance. We determined significant positive vs. negative effects of explanatory variables as the model-averaged mean coefficient values weighted by the $\mathrm{AIC_c}$ weight of the models containing variable *i*. Uncertainty around coefficient estimates was calculated as the unconditional standard error of parameter estimates across models, as described elsewhere (McAlpine *et al.* 2008). We performed all $\mathrm{AIC_c}$ analysis using the R package *MuMIn* (Barton 2014).

6.4 Results

6.4.1 Occurrence and abundance at the regional scale

For regional analyses, $\mathrm{AIC_c}$ weights revealed that terrain roughness, approximated by slope variability, had the highest rank in the model measuring the occurrence of *Drosophyllum*, although the absolute weight for this variable was low (0.15). Predictors approximating anthropogenic disturbances did not have significant effects on the occurrence of *Drosophyllum*, but were significant in all abundance models (Fig. 2).

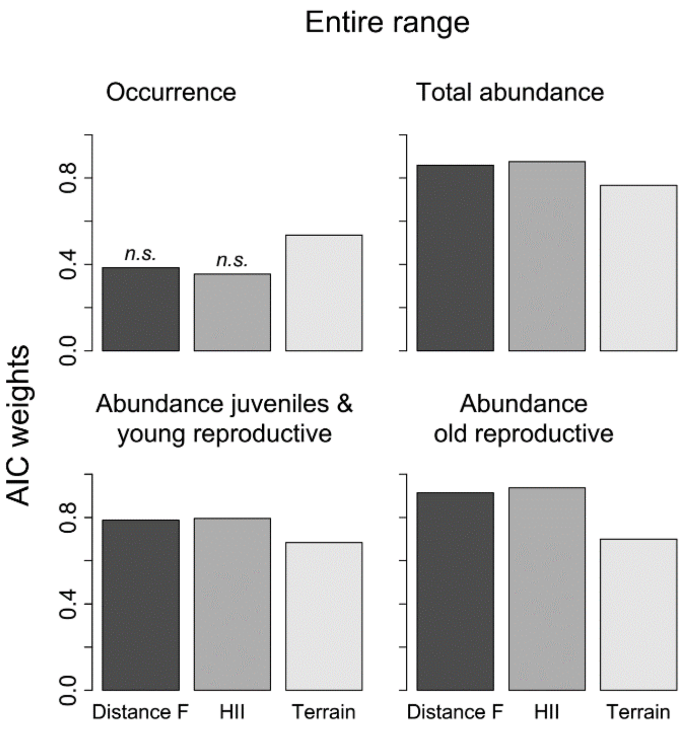


Figure 2 Akaike weights for each explanatory variable used to quantify the occurrence, total abundance, and abundance of different life-cycle classes of *Drosophyllum* in the entire range (including additive and interaction effects). Predictors depict log distance (m) to needle-leaved forests (Distance F), the Human Influence Index (HII), and slope (in degrees) variability (terrain roughness). Variables marked as *n.s.* did not provide a significant fit, i.e. absolute AIC_c weights < 0.

The magnitude and direction of the parameter effects overall showed a negative effect of anthropogenic disturbances (Fig. 3). All abundance measures increased with increasing distance to pine forests and decreased with increasing human influence (HII) (Fig. 3b). Terrain roughness had a negative mean effect in occurrence models although this effect was not consistently significant across models producing large standard errors (Fig. 3a). On the other hand, the interaction between terrain roughness and HII had a strong positive effect in abundance models indicating that for increasing values of HII, population abundances increase in response to terrain roughness (Fig. 3b). Distance to forest showed the strongest positive parameter effect in all abundance models and particularly for abundance of old reproductive individuals. For the latter response, the interaction between distance to forests and HII showed a strong negative parameter effect, although S.E. estimates were also large (Fig. 3b).

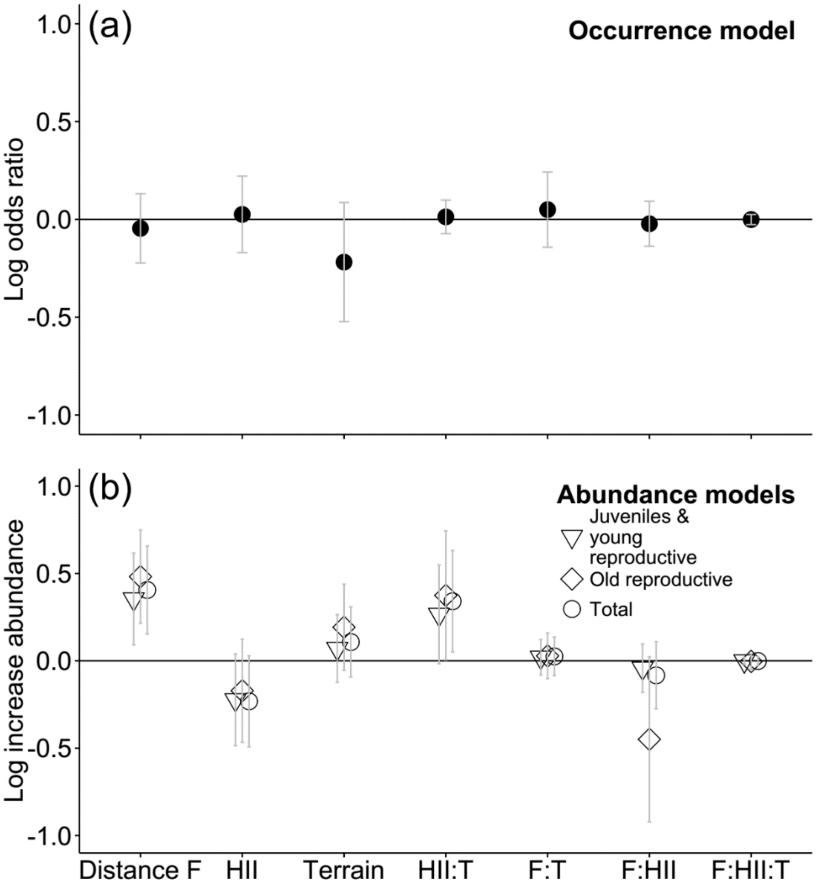


Figure 3 Additive and interaction effects of model parameters for each explanatory variable used to predict occurrence (a) and abundance (b) of *Drosophyllum* populations at its entire range. Magnitude of the effect is defined as the change in the log odds ratio of species presence or increase in log abundance given a one unit increase in predictor value, calculated using model-averaged (± SE) coefficient estimates weighted by AIC_c weights of models. Parameters can change the response positively or negatively, therefore increasing or decreasing the probability of presence/abundance, respectively. Black lines at y = 0 indicate no change in response variables.

6.4.2 Occurrence and abundance at the local scale

At the local scale, variables approximating anthropogenic disturbances (distance to roads/firebreaks and/or percent bare soil) had high AIC_c weights and thus high predictor ranks on the occurrence and abundance of *Drosophyllum* (Fig. 4). Percent bare soil had the highest rank among occurrence and abundance models. Distance to roads and firebreaks was not a significant factor in occurrence models but significantly affected abundance measures (Fig. 4). Terrain roughness did not contribute significantly to model fit when estimating total abundance and abundance of juveniles and young reproductive (Fig. 4).

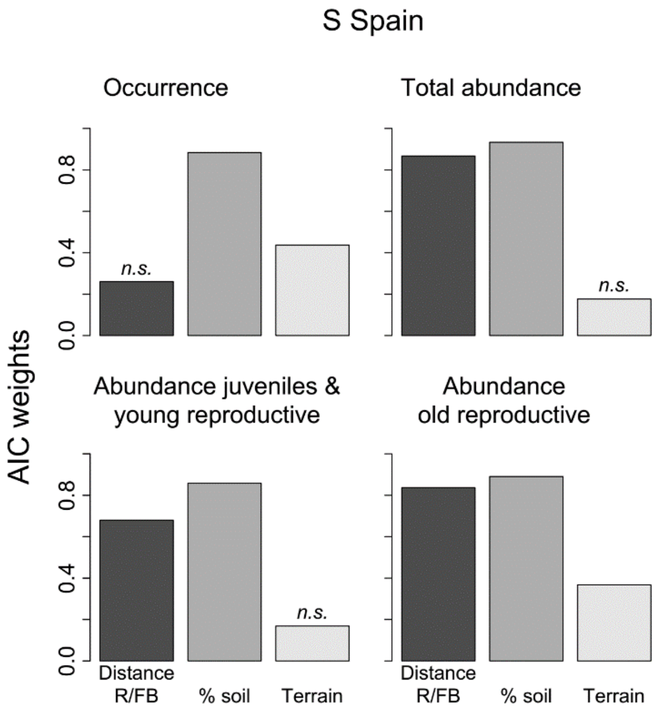


Figure 4 Akaike weights for each explanatory variable used to quantify the occurrence, total abundance, and abundance of different life-cycle classes of *Drosophyllum* in S. Spain (including additive and interaction effects). Predictors depict distance to roads and firebreaks (m), mean percentage of bare soil, and slope (in degrees) variability (terrain roughness). Variables marked as *n.s.* did not provide a significant fit, i.e. absolute AIC_c weights < 0.

The magnitude and direction of the parameter effects overall showed a strong positive effect of predictors approximating disturbances. Probability of occurrence of *Drosophyllum* increased with an increase in percent bare soil, while the parameter effects of distance to roads/firebreaks and terrain roughness were not significant (Fig. 5a). In all abundance models, distance to roads and firebreaks showed the strongest, negative parameter effect (Fig 5 b). Abundances changed positively with an increase in percent bare soil (Fig 5b).

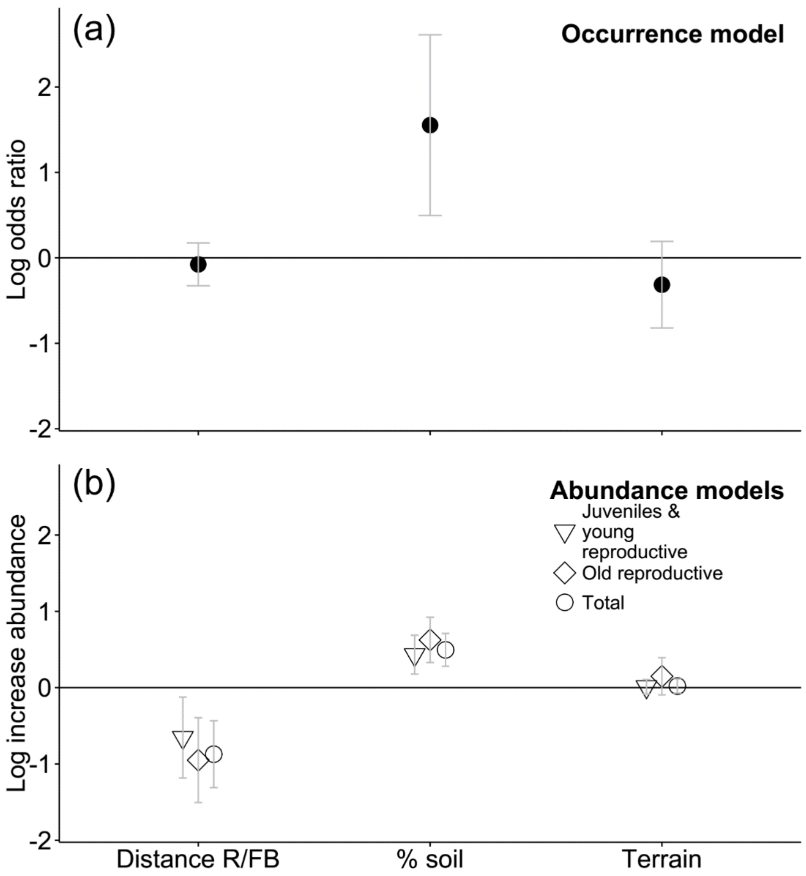


Figure 5 Additive effects of model parameters for each explanatory variable used to quantify the occurrence (a) and abundance (b) of *Drosophyllum* populations in S. Spain. Magnitude of the effect is defined as the change in the log odds ratio of species presence or increase in log abundance given a one unit increase in predictor value and was calculated using model-averaged (± SE) coefficient estimates weighted by AIC_c weights of models. Parameters can change the response positively or negatively, therefore increasing or decreasing the probability of presence/abundance, respectively. Black lines at y = 0 indicate no change in response variables.

6.5 Discussion

Mediterranean heathlands are biodiversity hotspots in which floristic diversity is closely tied to recurrent fires (Keeley *et al.* 2012). In the western Mediterranean Basin however, heathlands are managed under strict fire-suppression policies, and other types of anthropogenic disturbances dominate these systems (Bartolomé *et al.* 2005). These shifts in disturbances may have potentially severe consequences for the distribution, population structure, and future management of endemic heathland species. We show a strong, scale-dependent effect of anthropogenic non-fire disturbances on the occurrence and life-cycle class abundance of *Drosophyllum lusitanicum*, an endemic, fire-dependent, carnivorous subshrub in Mediterranean heathlands. Due to the different quality of disturbances at different spatial scales, local-scale anthropogenic disturbances had a stronger effect on species occurrence than regional-scale disturbances. Such scale-dependent effects of disturbances on populations have been described previously in theoretical (Opdam and Wascher 2004) and empirical (Wayne *et al.* 2006; Yasué 2006; Brunbjerg *et al.* 2014) studies. As we predicted, regional-scale anthropogenic disturbances had a negative effect on life-cycle class abundances of *Drosophyllum*. Contrary to our predictions, however, regional-scale disturbances did not account for the range-wide occurrence of this species. At the local scale though, anthropogenic disturbances had the strongest effects (highest AIC_c,absolute weight, and parameter effect) on both occurrence and life-cycle class abundances of *Drosophyllum*. This local-scale effect was positive, suggesting that, depending on the intensity and quality of the disturbances replacing fires, local populations of endemic post-fire specialists in Mediterranean heathlands can be maintained even under strict fire-suppression policies.

6.5.1 Anthropogenic disturbances, spatial scale, and persistence of a post-fire specialist

Habitat fragmentation and alteration have been identified as major sources of range decline for heathland species (Bartolomé *et al.* 2005; Fagúndez 2013), in particular rare post-fire specialists (Bond and Keeley 2005). We found that regional disturbances are associated with a decrease in population size of *Drosophyllum*. In particular, the strong positive effect of increasing distance to pine plantations highlights the threat of afforestation campaigns on the biodiversity of Mediterranean heathlands, of which *Drosophyllum* is one of the epitomes (Andrés and Ojeda 2002). However, it is surprising that neither of the disturbance sources predicted occurrence of the species at the regional scale, whereas local-scale anthropogenic disturbances were significant drivers of occurrence (Fig. 2; Table 2). This result may be an artifact of our *a-priori* focus on locations where *Drosophyllum* was expected to occur or of the different types and resolution of disturbance variables used at each scale. On the other hand, regional-scale anthropogenic disturbances were significant predictors of population abundance, and our surveying showed that *Drosophyllum* could occur inside heavily disturbed pine plantations while being locally extinct in little-disturbed heathlands (Appendix S1; Table 2). This indicates that the overall occurrence of *Drosophyllum* and the abundance of its life-cycle classes are strongly determined by human disturbances at fine (local) scales.

Table 2 Summary of mean coefficient effect size for predictors describing occurrence and abundance of *Drosophyllum lusitanicum* (L.) Link. populations at two spatial scales. The column headings depict occurrence (OC), total abundance (AB TOT), and abundance of juveniles and young reproductive (AB JYR) and old reproductive individuals (AB OR). Effect sizes are denotes as: ***effect size > 0.5; **effect size 0.3-0.5; *effect size < 0.3; †Highest AIC$_c$ weight/absolute weight; *n.s.* absolute weight < 0 or effect size < 0.05; (+) positive effect; (-) negative effect

Spatial scale	**Predictor category**	**Predictor**	**OC**	**AB TOT**	**AB JYR**	**AB OR**
regional	Human disturbance	(a) distance to needle-leaved forests (m);	*n.s.*	** (+)	** (+)	*** (+)
regional		(b) Human Influence Index;	*n.s.*	* (-)†	** (-)†	* (-)†
regional	Natural disturbance	(c) terrain roughness;	* (-)†	* (+)	* (+)	* (+)
regional	Interactions	(a):(b)	*n.s.*	* (-)	*n.s.*	** (-)
regional		(a):(c)	*n.s.*	*n.s.*	*n.s.*	*n.s.*
regional		(b):(c)	*n.s.*	** (+)	* (+)	** (+)
regional		(a):(b):(c)	*n.s.*	*n.s.*	*n.s.*	*n.s.*
local	Human disturbance	(a) distance to roads and firebreaks (m);	* (-)	*** (-)	*** (-)	*** (-)
local		(b) bare soil (%)	*** (+)†	*** (+)†	** (+)†	*** (+)†
local	Natural disturbance	(c) terrain roughness;	** (-)	*n.s.*	*n.s.*	* (+)

We show that pyrophytic species such as *Drosophyllum*, which respond to indirect fire-related germination cues (e.g., increase in light levels; Correia and Freitas 2002), may significantly benefit from local-scale disturbances providing such cues. The positive effects of non-fire, anthropogenic disturbances have been largely overlooked in studies examining the distribution of pyrophytes, although such effects are well known in other species (Farris *et al.* 2009; Amat *et al.* 2013). However, in a recent study in which the roles of extensive grazing and prescribed burning on heathland biodiversity were examined, Velle *et al.*

(2014) highlighted the positive effect of such disturbances on rates of endemism in heathland communities.

In addition to a positive effect on population persistence, local-scale disturbances can strongly influence the population structure of a post-fire specialist. We found that an increase in local-scale disturbances favored high abundances of all life-cycle classes and therefore the presence of mixed-aged *Drosophyllum* populations in disturbed habitats. Such disturbances do not kill adults of *Drosophyllum* (unlike wildfires) and/or, if maintained, allow for successive recruitment episodes. However, Garrido and collaborators (2003) argued that mixed-aged *Drosophyllum* populations in frequently disturbed habitats experience strong regeneration pressures and may be prone to local extinction. According to these authors, *Drosophyllum* is characterized by a regional assemblage pattern in which massive, post-fire germination from a soil seed bank is vital for population persistence.

Seed banks are known to buffer populations from genetic drift (Dolan *et al.* 2008; Honnay *et al.* 2008). Fires, in turn, provide cues for mass germination from the seed bank, unparalleled by the continuous, small-scale germination in permanently disturbed habitats. This may mean that without fires, most of the seed bank would thin out, with potentially serious consequences for genetic variability in populations. Moreover, a rare fire event over such a depleted seed bank might jeopardize the viability of that mixed-aged, longstanding population (Quintana-Ascencio *et al.* 2003). Nevertheless, *Drosophyllum* is disappearing from undisturbed heathlands under significant fire suppression (M. Paniw, pers. obs.) as it has been documented for other post-fire dwelling carnivorous plants (Jennings and Rohr 2011). Stable populations in disturbed habitats (some known for at least 50 years; Adlassnig *et al.* 2006) are therefore important to prevent the extinction of the species.

6.5.2 Synthesis and conservation perspectives

Anthropogenic disturbances have drastically affected population structures and species distributions in many species of Mediterranean-type ecosystems (Bazzaz 1983; Yates and Ladd 2005, 2010). However, in a changing world characterized by fire suppression and habitat destruction, there are winners and losers associated with human-made disturbances. Post-fire specialists seem to lose under human pressures, such as fire suppression, at the entire range of distribution. However, these species may profit from local disturbances. This is the case for the post-fire specialist used in our analyses, *Drosophyllum*, which has experienced high distribution declines in the past century (Correia and Freitas 2002; Garrido *et al.* 2003). We found detrimental effects of human disturbances on *Drosophyllum* at the regional scale. Nevertheless, the picture is rather different when focusing on the local scale; the occurrence of populations is overwhelmingly associated with open vegetation cover on slopes and heathlands and within open plantations (Juniper *et al.* 1989; Adlassnig *et al.* 2006) - all habitats not characteristic of fire-regulated heathland communities (Garrido *et al.* 2003). Our results at the local scale indeed suggest that larger populations may be found in habitats disturbed by humans and regardless of fire regimes. Consequently, while the species is considered threatened across its range, local-scale anthropogenic disturbances may be considered a haven in fire-less habitats as long as they are edaphically suitable for the species.

6.6 Acknowledgements

We thank M. Gil-López, M. Collado, and the Botany Lab at the University of Coimbra (in particular D. Montesinos) for assisting us with fieldwork. We also thank J. López Tirado, A. Gil, M. Porto, A. Júlia Pereira, S. Gonçalves, and J. L. Rendón for providing information on the locations of Portuguese *Drosophyllum* populations. Financial support was provided by project BREATHAL (CGL2011-28759/BOS; Spanish *Ministerio de Ciencia e Innovación*).

6.7 References

Adamec L. 2009. Ecophysiological investigation on *Drosophyllum lusitanicum*: Why doesn't the plant dry out? *Carnivorous Plant Newsletter* **38**:71–74.

Adlassnig W, Peroutka M, Eder G, Pois W, Lichtscheidl IK. 2006. Ecophysiological observations on *Drosophyllum lusitanicum*. *Ecological Research* **21**:255–262.

Amat ME, Vargas P, Gómez JM. 2013. Effects of human activity on the distribution and abundance of an endangered Mediterranean high-mountain plant (*Erysimum penyalarense*). *Journal for Nature Conservation* **21**:262–271.

Andrés C, Ojeda F. 2002. Effects of afforestation with pines on woody plant diversity of Mediterranean heathlands in southern Spain. *Biodiversity Conservation* **11**:1511–1520.

ANTHOS - Sistema de información sobre las plantas de España. 2014. *Ministerio de Agricultura, Alimentacíon, y Medio Ambiente*. <http://www.anthos.es/>

Arino O, Casadio S, Serpe D. 2012. Global night-time fire season timing and fire count trends using ATSR instrument series. *Remote Sensing of Evironment* **116**:1-13.

Bartolomé J, Plaixats J, Fanlo R, Boada M. 2005. Conservation of isolated Atlantic heathlands in the Mediterranean region: effects of land-use changes in the Montseny biosphere reserve (Spain). *Biological Conservation* **122**:81–88.

Barton K. 2014. MuMIn: Multi-model inference. *R package version 1.10.5*. <http://CRAN.R-project.org/package=MuMIn>

Bazzaz FA. 1983. Characteristics of populations in relation to disturbance in natural and man-modified ecosystems. In: Mooney H, Gondron, M. (eds.) *Disturbance and Ecosystems. Components of Response. Ecological Studies*. Springer, 259-275.

BOJA no. 107. 1994. Decreto 104/1994, de 10 de Mayo, por el que se establece el Catálogo Andaluz de la Flora Silvestre Amenazada. Junta de Andalucía, Sevilla

Bond WJ, Keeley JE. 2005. Fire as global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology and Evolution* **7**:387-384.

Brunbjerg AK, Svenning JC, Ejrnæs R. 2014. Experimental evidence for disturbance as key to the conservation of dune grassland. *Biological Conservation* **174**:101-110.

Burnham KP, Anderson DR, Huyvaert KP. 2011. AIC model selection and multimodel inference in behavioral ecology : some background , observations , and comparisons. *Behavioral Ecology and Sociobiology* **65**:23–35.

Caswell H. 2001. *Matrix Population Models: Construction, Analysis and Interpretation* (2nd ed.). Sinauer Associates.

Correia E, Freitas H. 2002. *Drosophyllum lusitanicum*, an endangered West Mediterranean endemic carnivorous plant: threats and its ability to control available resources. *Botanical Journal of the Linnean Society* **140**:383–390.

Cowling R, Ojeda F, Lamont BB, Rundel PW, Lechmere-Oertel R. 2005. Rainfall reliability, a neglected factor in explaining convergence and divergence of plant traits in fire-prone mediterranean-climate ecosystems. *Global Ecology and Biogeorgraphy* **14**:509-519.

Dolan RW, Quintana-Ascencio PF, Menges ES. 2008. Genetic change following fire in populations of a seed-banking perennial plant. *Oecologia* **158**:355–360.

Easterling MR, Ellner SP, Dixon PM. 2000. Size-specific sensitivity: Applying a new structured population model. *Ecology* **81**:694-708.

European Topic Centre on Spatial Information and Analysis. 2013. CORINE 2006. - European Environmental Agency. <a47ee0d3248146908f72a8fde9939d9d>.

Fagúndez J. 2013. Heathlands confronting global change: drivers of biodiversity loss from past to future scenarios. *Annals of Botany* **111**:151–172.

Farris E, Pisanu S, Ceccherelli G, Filigheddu R. 2009. Effects of the management regime on the performance of the endangered Mediterranean *Centaurea horrida* Badarò (Asteraceae). *Journal for Nature Conservation* **17**:15–24.

Fernandes PM, Davies GM, Ascoli D, Fernández C, Moreira F, Rigolot E, Stoof CR, Vega JA, Molina, D. 2013. Prescribed burning in southern Europe: developing fire management in a dynamic landscape. *Frontiers in Ecology and Environment* **11**:e4–e14.

FLORA ON. 2014. Sociedade Portuguesa de Botanica. <http://www.flora-on.pt/>

Garrido B, Hampe A, Marañón T, Arroyo J. 2003. Regional differences in land use affect population performance of the threatened insectivorous plant *Drosophyllum lusitanicum* (Droseraceae). *Diversity and Distributions* **9**:335–350.

Güthlin D, Storch I, Küchenhoff H. 2013. Landscape variables associated with relative abundance of generalist mesopredators. *Landscape Ecology* **28**:1687–1696.

Heubl G, Bringmann G, Meimberg H. 2006. Molecular phylogeny and character evolution of carnivorous plant families in Caryophyllales - revisited. *Plant Biology* **8**:821-830.

Hobbs RJ, Richardson DM, Davis GW. 1995. Mediterranean-type ecosystems: Opportunities and constraints for studying the function of biodiveristy. In Richardson DM, Davis GW. (eds.) *Mediterranean-type Ecosytems: The Function of Biodiveristy*. Springer, 1-42.

Honnay O, Bossuyt B, Jacquemyn H, Shimono A, Uchiyama K. 2008. Can a seed bank maintain the genetic variation in the above ground plant population? *Oikos* **117**:1–5.

Jennings DE, Rohr JR. 2011. A review of the conservation threats to carnivorous plants. *Biological Conservation* **144**:1356–1363.

Juniper BE, Robins RJ, Joel DM. 1989. *The Carnivorous Plants*. AP.

Junta de Andalucía, Consejería de Medio Ambiente. 2007. Mapa de usos y coberturas vegetales del suelo de Andalucía. <http://www.juntadeandalucia.es/medioambiente/site/web/rediam>.

Junta de Andalucía, Consejería de Medio Ambiente. 2013. Plan de Prevención y Extinción de Incendios forestales de Andalucía (INFOCA)

Keeley JE. 1987. Role of fire in seed germination of woody taxa in California chaparral. *Ecology* **68**:434-443.

Keeley JE. Bond WJ. 1997. Convergent seed germination in South African fynbos and Californian chaparral. *Plant Ecology* **133**:153-167.

Keeley JE, Bond WJ, Bradstock RA, Pausas JG, Rundel PW. 2012. *Fire in Mediterranean Ecosystem: Ecology, Evolution and Management*. Cambridge University Press.

Keeley JE, Pausas JG, Rundel PW, Bond WJ, Bradstock RA. 2011. Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Sciences* **16**:406–411.

Lawson DM, Regan HM, Zedler PH, Franklin J. 2010. Cumulative effects of land use, altered fire regime and climate change on persistence of *Ceanothus verrucosus*, a rare, fire-dependent plant species. *Global Change Biology* **16**:2518–2529.

McAlpine CA, Rhodes JR, Bowen ME, Lunney D, Callaghan JG, Mitchell DL, Possingham HP. 2008. Can multiscale models of species' distribution be generalized from region to region? A case study of the koala. *Journal of Applied Ecology* **45**:558-567.

Médail F, Quézel P. 2001. Biodiversity hotspots in the Mediterranean Basin: setting global conservation priorities. *Conservation Biology* **13**:1510-1513.

Metcalf CJE, McMahon SM, Salguero-Gómez R, Jongejans E. 2013. *IPMpack*: an R package for integral projection models. *Methods in Ecology and Evolution* **4**:195-200.

Moreira B, Tormo J, Estrelles E, Pausas JG. 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany* **105**:627–635.

NASA Land Processes Distributed Active Archive Center (LP DAAC). 2001. ASTER L1B. USGS/Earth Resources Observation and Science (EROS) Center, South Dakota.

Ojeda F. 2001. El fuego como factor clave en la evolucion de plantas mediterraneas. In: Zamora R, Pugnaire FI. (eds.). *Ecosistemas Mediterraneos. Analisis Funcional*. CSIC-AEET, 319–349.

Ojeda F, Marañón T, Arroyo J. 1996. Patterns of ecological, chorological and taxonomic diversity on both sides of the Strait of Gibraltar. *Journal of Vegetation Science* **7**:63-72.

Ojeda F, Marañón T, Arroyo J. 2000. Plant diversity in the Aljibe Mountains (S. Spain): a comprehensive account. *Biodiversity and Conservation* **9**:1323–1343.

Ojeda F, Pausas JG, Verdú M. 2010. Soil shapes community structure through fire. *Oecologia* **163**:729-735.

Onset Computer Corporation. 2013. HOBO Data Loggers. Madrid.

Ooi MKJ, Denham AJ, Santana VM, Auld TD. 2014. Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. *Ecology and Evolution* **4**:656-671.

Ortega-Olivencia A, Carrasco Claver JP, Devesa Alcaraz JA. 1995. Floral and reproductive biology of *Drosophyllum lusitanicum* (L.) Link (Droseraceae). *Botanical Journal of the Linnean Society* **118**:331–351.

Pavlovic NB. 1994. Disturbance-dependent persistence of rare plants: anthropogenic impacts and restoration implications. In Bowles ML, Whelan CJ. (eds.) *Restoration of Endangered Species: Conceptual Issues, Planning and Implementation*. Cambridge Univeristy Press, 159-193.

Preston CA, Baldwin IT. 1999. Positive and negative signals regulate germination in post-fire annual, *Nicotiana attenuata*. *Ecology* **80**: 481–494.

Opdam P, Wascher D. 2004. Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. *Biological Conservation* **117**:285-297.

Quintana-Ascencio PF, Dolan RW, Menges ES. 1998. *Hypericum cumulicola* demography in unoccupied and occupied Florida scrub patches with different time-since-fire. *Journal of Ecology* **86**:640–651.

Quintana-Ascencio PF, Menges ES, Weekley CW. 2003. A fire-explicit population viability analysis of *Hypericum cumulicola* in Florida rosemary scrub. *Conservation Biology* **17**:433–449.

Quintana-Ascencio PF, Weekley CW, Menges ES. 2007. Comparative demography of a rare species in Florida scrub and road habitats. *Biological Conservation* **137**:263-270.

Thuiller W, Slingsby JA, Privett SD, Cowling RM. 2007. Stochastic species turnover and stable coexistence in a species-rich, fire-prone plant community. *PloS One* **2**:e938.

VanDerWal J, Beaumont L, Zimmerman N, Lorch P. 2012. climates: Methods for working with weather and climate. R package version 0.1-1.3

Velle LG, Nilsen LS, Norderhaug A, Vandvik V. 2014. Does prescribed burning result in the homogenization of coastal heathlands? *Global Change Biology* **20**:1429-1440.

Ver Hoef JM, Boveng PL. 2007. Quasi-Poisson vs. negative binomial regression: how should we model overdispersed count data? *Ecology* **88**:2766–2772.

Wayne AF, Cowling A, Lindenmayer DB, Ward CG, Vellios CV, Donnelly CF, Calver MC. 2006. The abundance of a threatened arboreal marsupial in relation to anthropogenic disturbances at local and landscape scales in Mediterranean-type forests in south western Australia. *Biological Conservation* **127***:*463-476.

Wenger SJ, Freeman MC. 2008. Estimating species occurrence, abundance, and detection probability using zero-inflated distributions. *Ecology* **89**:2953–2959.

Wildlife Conservation Society (WCS) and Center for International Earth Science Information Network (CIESIN), Columbia University. 2005. Last of the Wild Project, Version 2: Last of the Wild Dataset (Geographic). NASA Socioeconomic Data and Applications Center (SEDAC).

Wilson JP, Gallant JC. 2000. *Terrain Analysis: Principles and Applications*. Wiley.

Yasué M. 2006. Environmental factors and spatial scale influence shorebirds' responses to human disturbance. *Biological Conservation* **128**:47-54.

Yates CJ, Ladd PG. 2005. Relative importance of reproductive biology and establishment ecology for persistence of a rare shrub in a fragmented landscape. *Conservation Biology* **19:**239-249.

Yates CJ, Ladd PG. 2010. Using population viability analysis to predict the effect of fire on the extinction risk of an endangered shrub *Verticordia fimbrilepis* subsp. *fimbrilepis* in a fragmented landscape. *Plant Ecology* **211**:305-319.

Zipkin EF, Leirness JB, Kinland BP, O'Connell AF, Silverman ED. 2014. Fitting statistical distributions to sea duck count data: Implications for survey design and abundance estimation. *Statistical Methodology* **17**:67–81.

6.8 Appendix S1 - Description of sampling locations

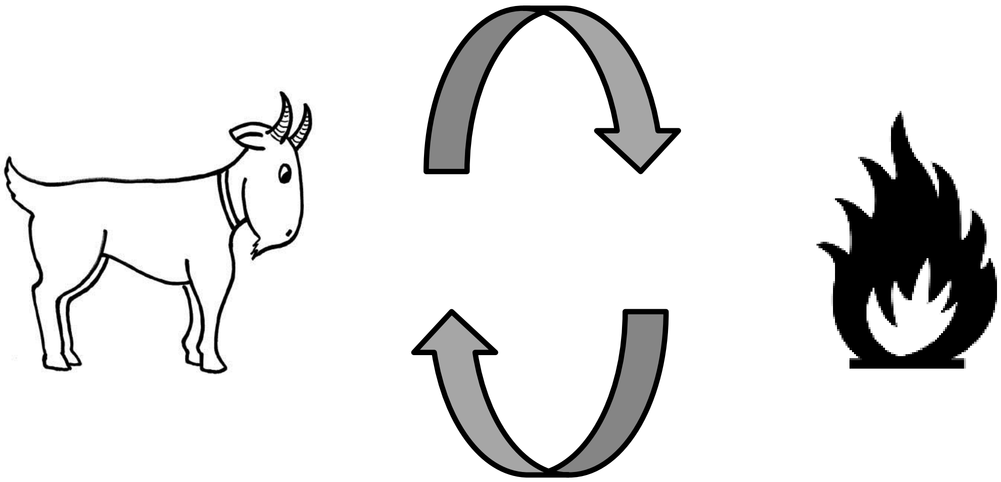
To model the occurrence and life-cycle class abundance of *Drosophyllum lusitanicum* L., Link (Drosophyllaceae), 74 sites across the range of the species were sampled. The table below provides information on the sampling sites. In the locations where populations were present (presence = "yes"), the number of juveniles and young and old reproductive plants was recorded in sixty 1 × 1 m quadrats. Habitat type was recorded for all locations.

Chapter 6 – Appendix S1 – continued

ID	**Country**	**Presence**	**Area (m2)**	**Habitat type**	**Juveniles**	**Young reproductive**	**Old reproductive**	**Month of sampling**
1	Spain	no	0	dense heathland	0	0	0	Oct 2011
2	Spain	no	0	dense heathland	0	0	0	Oct 2011
3	Spain	no	0	dense heathland	0	0	0	Oct 2011
4	Spain	no	0	dense heathland	0	0	0	Oct 2011
5	Spain	yes	240	dense heathland	27	32	82	May 2013
6	Spain	yes	300	fire break	20	25	25	May 2013
7	Spain	no	0	dense heathland	0	0	0	Mar 2014
8	Spain	yes	60	dense heathland	35	0	0	Mar 2014
9	Spain	no	0	dense heathland	0	0	0	Oct 2011
10	Spain	yes	100	dense heathland	0	3	5	Mar 2014
11	Spain	no	0	dense heathland	0	0	0	Oct 2011
12	Morocco	yes	300	disturbed heathland	57	8	2	spring13
13	Morocco	no	0	dense heathland	0	0	0	Oct 2011
14	Morocco	no	0	disturbed heathland	0	0	0	Oct 2011
15	Morocco	no	0	pine plantation	0	0	0	Oct 2011
16	Morocco	yes	400	road-side disturbed patch	50	9	19	June 2013
17	Morocco	yes	120	pine plantation	126	4	25	June 2013
18	Morocco	no	0	disturbed heathland	0	0	0	Sept 2012
19	Morocco	yes	240	road-side, dense pine stand	34	9	20	June 2013
20	Morocco	no	0	small heathland patch surrounded by settlement	0	0	0	Sept 2012
21	Morocco	yes	400	disturbed heathland	47	17	40	June 2013
22	Morocco	yes	60	road-side, dense pine stand	3	0	3	June 2013
23	Spain	yes	500	heathland burned 2009	23	2	15	Apr 2014
24	Spain	yes	100	dense heathland	1	2	2	Apr 2014
25	Spain	yes	300	heathland burned 2008	3	3	2	Apr 2014
26	Spain	yes	100	dense heathland	0	4	15	Apr 2014
	Spain	no	0	sparse eucalipt plantation	0	0	0	Apr 2013
27	Spain	yes	540	disturbed heathland	16	8	8	Apr 2014
28	Spain	yes	350	heathland burned 2010	6	20	2	Apr 2014
29	Spain	yes	640	heathland burned	52	21	35	Apr 2014

				2009				
30	Morocco	yes	850	fire break	93	76	132	June 2013
31	Morocco	yes	400	sparse pine plantation	35	20	40	June 2013
32	Morocco	yes	325	disturbed heathland	7	15	28	June 2013
33	Morocco	yes	680	fire break, sparse pine plantation	21	2	33	June 2013
34	Spain	yes	650	disturbed heathland	221	113	87	June 2013
35	Spain	yes	600	disturbed heathland	19	18	17	June 2013
36	Spain	yes	400	fire break	65	103	66	June 2013
37	Portugal	yes	200	road-side pine plantation	13	26	43	Mar 2013
38	Portugal	yes	180	eucalipt plantation	10	51	19	Mar 2013
39	Portugal	no	0	cultivated land	0	0	0	Mar 2013
40	Portugal	yes	950	road-side eucalipt plantation	52	24	46	Mar 2013
41	Portugal	yes	60	road-side distubed patch	8	10	28	Mar 2013
42	Portugal	yes	60	road-side distubed patch	31	28	23	Mar 2013
43	Portugal	no	0	road-side oak plantation	0	0	0	Mar 2013
44	Portugal	no	0	dune	0	0	0	Mar 2013
45	Spain	yes	60	sparse pine plantation	1	7	0	Apr 2013
46	Spain	yes	300	disturbed patch (quarry)	196	99	83	Mar 2014
47	Spain	yes	60	pine plantation	15	0	0	Mar 2014
48	Spain	yes	120	road-side distubed patch	76	16	21	Mar 2014
49	Spain	yes	100	dense heathland	126	48	129	Mar 2014
50	Spain	yes	60	road-side distubed patch	6	5	5	Mar 2014
51	Spain	yes	60	road-side distubed patch	30	17	42	Mar 2014
52	Spain	yes	100	pine plantation	94	11	13	Mar 2014
53	Spain	yes	60	dense heathland	8	5	1	Mar 2014
54	Spain	yes	80	pine plantation	62	5	10	Mar 2014
55	Spain	yes	1100	fire break	104	17	51	Mar 2014
56	Spain	yes	360	fire break	45	13	60	Mar 2014
57	Spain	yes	500	heathland/ strongly	32	1	39	Mar 2014

				disturbed pine plantation				
58	Spain	yes	240	sparse cork oak & pine plantation	68	80	90	Mar 2014
59	Spain	yes	900	recently plowed cork oak plantation	150	58	156	Mar 2014
60	Spain	yes	60	road-side, managed pine stand	12	2	18	Mar 2014
61	Spain	no	0	strongly disturbed pine plantation	0	0	0	Mar 2014
62	Portugal	no	0	cultivated land	0	0	0	Mar 2014
63	Portugal	yes	400	sparse cork oak plantation	194	0	6	Mar 2014
64	Portugal	no	0	dense heathland	0	0	0	Mar 2014
65	Portugal	yes	60	small heathland patch surrounded by settlement	7	1	3	Mar 2014
66	Portugal	yes	60	recently plowed cork oak plantation	56	8	4	Mar 2014
67	Portugal	no	0	sparse cork oak plantation	0	0	0	Mar 2014
	Portugal	yes	60	sparse eucalipt plantation	102	11	31	Mar 2014
68	Portugal	yes	0	densely vegetated cork oak forest	0	0	0	Mar 2014
69	Portugal	yes	90	disturbed cork oak stand	29	17	31	Mar 2014
70	Portugal	yes	150	disturbed pine stand/ heathland	39	19	38	Mar 2014
71	Portugal	no	0	recently plowed cork oak plantation	0	0	0	Mar 2014
72	Portugal	no	0	recently plowed cork oak plantation	0	0	0	Mar 2014
73	Portugal	no	0	sparse cork oak/ pine stand	0	0	0	Mar 2014
74	Portugal	no	0	recently plowed cork oak plantation	0	0	0	Mar 2014



CHAPTER 7

Interacting livestock and fire may both threaten and increase viability of a fire-adapted Mediterranean carnivorous subshrub

CHAPTER 7

Interacting livestock and fire may both threaten and increase viability of a fire-adapted Mediterranean carnivorous subshrub

Maria Paniw, Pedro F. Quintana-Ascencio, Fernando Ojeda, and Roberto Salguero-Gómez

This chapter is under review in *Journal of Applied Ecology*.

7.1 Abstract

Fires are widespread natural disturbances and determine population dynamics of many species. However, fire regimes are being increasingly modified by and interact with human activities, while the effect of such interactions on population dynamics of fire-adapted species remain poorly understood. We used demographic data of the carnivorous, post-fire recruiting plant *Drosophyllum lusitanicum*, endemic to heathlands in the southwestern Mediterranean Basin, to investigate whether increasing human disturbances in fire-prone ecosystems may pose a threat or an opportunity to improve population viability. We fit stochastic integral projection models and simulated population dynamics under different combinations of two key disturbance types affecting populations: fire and livestock browsing/trampling. We used perturbation analyses to determine potential long-term consequences of maintaining fundamentally different disturbance types. Despite most populations inhabiting browsed habitats, simulations showed a generally higher extinction risk in populations under high livestock pressure compared with ones under low or moderate pressure. Extinction risk decreased when fire return intervals shortened in populations under low/moderate livestock pressure; however, the opposite pattern emerged in heavily browsed populations, where short intervals between fires increased extinction. Elasticity analyses showed that decreases in viability under frequent disturbance interactions (heavy browsing and frequent fire) may be explained by selection against seed dormancy in populations with frequent browsing/trampling, potentially causing population collapse when a fire kills above-ground plants without populations being able to recover from a seed bank. Biodiversity patterns in the Mediterranean Basin have been shaped by humans for millennia, and integrating moderate human activities into fire management may increase the success of species conservation. However, replacing fires by human disturbances, a currently widespread strategy in the southwestern Mediterranean Basin and elsewhere, may have severe consequences for fire-adapted species, fundamentally altering population dynamics and selection pressures and decreasing viability.

Keywords: *Drosophyllum lusitanicum*, elasticities, fire, integral projection models (IPM), livestock disturbance, quasi-extinction, stochastic population growth rate (log λ_s)

7.2 Introduction

Natural disturbances are key drivers of population dynamics (Tuljapurkar 1990; Boyce *et al.* 2006; Turner 2010). Among the various disturbance regimes to which natural populations are exposed, fires are the most widespread, regularly affecting 50 % of the Earth’s surface (Bond and Keeley 2005; Chuvieco *et al*. 2008). In turn, many plant species in fire-prone habitats evolved strategies that link critical life-cycle transitions to fire, e.g. post-fire recruitment from persistent seed banks (Bond and Keeley 2005). However, human activities increasingly interact with natural fire regimes (Lawson *et al*. 2010; Keeley *et al*. 2012). Beyond merely changing fire regimes through fire suppression policies or changes to land management (Valdecantos *et al*. 2008; Steel *et al*. 2015), humans have introduced small-scale disturbances, typically involving vegetation removal, harvesting of plant parts, or intense livestock browsing, that co-occur with fires and affect an increasing number of fire-adapted species (e.g., Lawson *et al*. 2010; Mandle *et al*. 2015; Tye *et al*. 2016). Yet, the demographic consequences of multiple, potentially interacting disturbance regimes remain poorly understood in many fire-prone ecosystems (Ehrlén *et al*. 2016; Tye *et al*. 2016).

Population dynamics of fire-adapted species may not necessarily be negatively affected by human disturbances. For example, when post-fire regeneration is triggered by indirect cues such as increased light levels or removal of allelopathic compounds (Ooi *et al.* 2014; Renne *et al.* 2014), disturbances that mimic the effect of fire of removing vegetation may allow for continuing recruitment even as fire return intervals decrease (Bond and Kelley 2005; Quintana-Ascencio *et al*. 2007). In addition, human disturbances may decrease competition between plants and therefore increase survival and growth (Sánchez-Velásquez *et al*. 2002; Tye *et al*. 2016).

However, small-scale, chronic human disturbances are fundamentally different from rare fire events and can lead to lasting changes in population structure and dynamics of fire-adapted species (Quintana-Ascencio *et al*. 2007; Lawson *et al*. 2010; Chapter 6). For example, by significantly increasing plant longevity or fecundity, disturbances such as repeated vegetation removal exert novel selection pressures on organisms (Palkovacs *et al*. 2012), and their interaction with periodic high-intensity fires may severely destabilize populations and result in extinction (Mandle *et al.* 2015; Darabant *et al*. 2016). For conservation management, quantifying both the potential long-term changes in population dynamics under human disturbances and the demographic effects of disturbance interactions in fire-prone ecosystems will be critical to assess the viability of fire-adapted species under increasing human pressures (Ashley *et al*. 2003; González-Varo *et al*. 2015).

Here, we use *Drosophyllum lusitanicum* (L.), Link (Drosophyllaceae) (*Drosophyllum* hereafter) to investigate the effects of interacting disturbances on population dynamics of a fire-adapted plant species. This rare carnivorous subshrub is associated with fire-prone, highly biodiverse heathlands in the SW Iberian Peninsula and N Morocco (Garrido *et al.* 2003). In natural heathlands, *Drosophyllum* has a life cycle typical of a short-lived seeder species (e.g. Menges and Quintana-Ascencio 2004; Fig 1a), germinating from a persistent seed bank after fire (Müller and Deil 2001). Seed germination is triggered by both heat and removal of surrounding vegetation (Correia and Freitas 2002; Chapter 5). However, with prevailing fire suppression policies across the species' range (Turco *et al*. 2016), most populations persist in habitats where chronic human disturbances - shrub slashing or browsing and trampling by livestock and game - mimic the removal of shrub canopy by fire (Chapter 6). Populations exposed to human disturbances show a markedly different population structure (longer above-ground survival of adult plants and annual recruitment; Chapter 6) than fire-disturbed heathland populations but experience fire occasionally (Plan INFOCA 2012; Fig. 1). With increasing local extinctions across the species' range (Correia and Freitas 2002), assessing population viability under different combinations of disturbances is not only important for conservation of heathland biodiversity but may also shed light on the potential fate of fire-adapted seeder species in Mediterranean ecosystems.

We quantified the dynamics of *Drosophyllum* populations exposed to variable time since last fire and two levels of browsing/trampling pressure by domestic ungulates (Fig 1b). We asked (i) under what conditions disturbance interactions may cause population extinction *vs*. persistence. As population dynamics in human-disturbed habitats may differ from ones in natural heathlands (Chapters 5, 6), we also asked (ii) how chronic disturbances may change selection pressures and what consequences such changes may have for management of this species. To answer the questions, we parameterized integral projection models (IPMs) and evaluated the effect of interactions between the two disturbance types on the stochastic population growth rate (log λ_s) and probability of quasi-extinction ($P_q(t)$) using stochastic simulations while assessing parameter uncertainty under a Bayesian framework. Finally, we used prospective perturbations of vital rates to investigate selection pressures imposed by various regimes of interacting disturbances. We provide fully commented *R* scripts of our analyses, which can be adapted to quantify population-level effects of multiple disturbances in a wide range of systems.

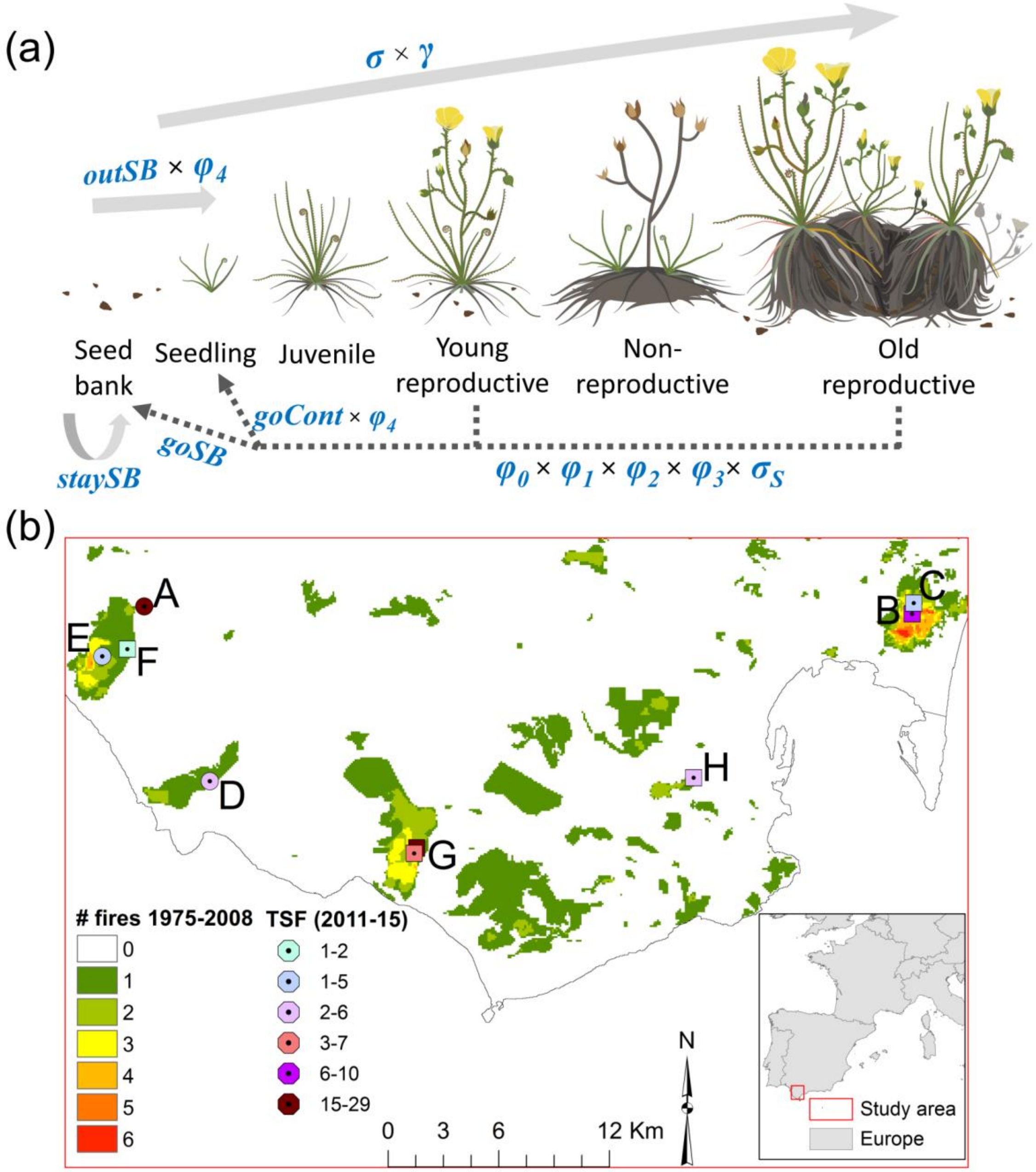


Figure 1 The carnivorous subshrub *Drosophyllum lusitanicum* shows life-cycle adaptations to recurrent fires and occurs in a fire-prone Mediterranean biodiversity hotspot. (a) In the integral projection models, life-cycle transitions (here simplified to stages) were represented by 12 vital rates (blue); Solid and dashed arrows represent transitions of survival [above-ground (σ) or seed bank (*staySB*, *outSB*)]/growth [above-ground (γ) or seedling size (φ_4)] and fecundity [flowering probability (φ_0), # stalks (φ_1), # flowers/stalk (φ_2), # seeds/flower (φ_3), immediate germination (*goCont*), and seed-bank ingression (*goSB*) or egression (*outSB*)], respectively; σ_S – above-ground seed survival. (b) The study sites (different letters) in which demographic data were collected all had burned at least once since 1975 and differed in time-since fire (TSF) and livestock pressure, populations exposed to either high (squares) or low (circles) browsing and trampling of vegetation by domestic ungulates.

7.3 Materials and methods

7.3.1 Study system and demographic data

To quantify vital-rate transitions in *Drosophyllum*, we parameterized integral projection models (IPMs; Easterling *et al*. 2000) with census and experimental field and laboratory data. We estimated vital rates of individuals with above-ground biomass from five annual censuses (every April 2011-2015) of a total of 2,378 individuals in eight populations (Fig. 1b). The populations differed with respect to (i) livestock pressure (*LS*) from browsing/trampling, experiencing either high (*HLS*; human-disturbed populations) or low (*LLS*; natural fire-disturbed heathlands) pressure; and (ii) time-since-fire (*TSF* hereafter), between 1 and >26 years (Fig. 1b; Table S1.1 in Appendix S1). As Mediterranean heathland habitats do not change significantly in species composition and structure >3 years after fire (Ojeda *et al*. 1996), we transformed TSF into a categorical variable consisting of 1, 2, 3, or >3 years since fire.

The above-ground vital rates obtained from the census data included survival (σ), growth (γ), probability of flowering (φ_0), number of flowering stalks (φ_1), number of flowers per stalk (φ_2), number of seeds per flower (φ_3), and seedling size distribution the next year (φ_4) (Fig. 1a). We used plant *size* = log(# of leaves × length of longest leaf (cm)), after model selection for σ, γ, φ_0 and φ_1, as the continuous state variable in all IPMs (see below). We also quantified above-ground seed survival from the demographic census data as $\sigma_S = 1 -$ flower damage (Appendix S1). We then used this parameter to modify vital rates describing seed production (φ_0, φ_1, φ_2, φ_3, and φ_4).

We performed two field seed-burial experiments and a greenhouse germination trial to quantify seed fates and thereby the discrete, size-independent component of IPMs (Table 1). Overall, > 5,100 seeds were used in the experiments (Fig. 1a; details in Appendix S1 and chapter 5). Both field experiments were designed to ensure that seed-bank dynamics were measured at the same time scale as the rest of the species' life cycle modeled. We buried seeds in open (recently burned) and vegetated (long unburned) heathlands and estimated seed survival in the soil, *i.e.*, seed-bank stasis (*staySB*), and seedling establishment, *i.e.*, the probability of establishment in the spring following seed dispersal (*goCont*) and the probability of egression from the seed bank at least two springs after dispersal (*outSB*) (Fig. 1a). We defined the proportion of seeds entering the seed bank (*goSB*) as 1- *goCont* - ω_S, where ω_S = seedling mortality prior to the census (Appendix S1). In greenhouse trials, we exposed seeds to heat and smoke treatments and quantified germination, which we used as a proxy for seed-bank egression after fires (*outSB* in TSF_0). For TSF_0 and TSF_1, *staySB* was estimated from an examination of *Drosophyllum* seeds in soil samples from recently burned patches (Appendix S1).

7.3.2 Model parameterization

We fit all vital-rate models in a Bayesian framework (as in Chapter 5), using normal (μ = 0; $1/\theta^2 = 1\times10^{-06}$) or uniform uninformative priors (Appendix S1). We used MCMC sampling to estimate the distributions of 206 model parameters quantifying vital rates. The MCMC sampling was run for 2,100,000 steps using three chains, and the parameter distributions were obtained from the last 100,000 MCMC samples, subsampling every 500th value (see `vitalRateModels.R` in Appendix S2). Convergence after the burn-in of 2,000,000 steps was assessed visually and with posterior predictive checks.

We modeled the above-ground vital rates as functions of the continuous predictor *size* using generalized linear mixed models (GLMMs). We accounted for environmental variability in all the models by including TSF and LS as fixed effects, and used *site* as a random effect (Table 1). Using DIC criteria, we chose the most plausible model for each vital rate (Table 1; see Appendix S1 for all candidate models), testing interactions between size, TSF, and LS. We also described the variance (τ) of the predicted distributions of mean growth (γ) and seedling size (φ_4) as functions of TSF and LS, which provided a better model fit than assuming homoscedasticity of variance (Table 1). The number of seeds per flower (φ_3) did not differ significantly between populations, and we treated it as a constant = 9.8 in all models.

Vital rates describing immediate germination (*goCont*) or seed-bank dynamics (*staySB*, *outSB*) were defined as binomial functions, *i.e.*, proportions of total seed number in open and vegetated experimental patches using *block* as random effect (Table 1). We then associated predictions obtained for the seed-bank models with TSF categories in each

LS state, assuming that estimates from open and vegetated patches represented seed-bank dynamics under HLS and LLS, respectively (Table S1.2).

Table 1 Parameterization of the models used to describe vital rates of *Drosophyllum lusitanicum*. The models shown described the data best among several candidate models. Parameter superscripts indicate parameter names in the R scripts (Appendix S2). *TSF* – time since last fire. *LS* – lifestock pressure. *PFS* – post-fire habitat state. The parameters modeled were: α_0 - intercept; α_j, α_k, α_p - mean response at each TSF level *j*, LS level *k*, or PFS level *p* compared with α_0; α_{jk} - TSF × LS interaction; β_c - slope for *size*; β_{jc} , β_{kc} - TSF × *size* and LS × *size* interaction, respectively; α_s, α_b - random effect on α_0 for each site *s* or block *b*. Distributions B, ℵ, and NB - Bernoulli, normal, and negative binomial distribution, respectively. ΔDIC indicate the difference in values between the chosen model and the second-best model with fewer parameters, indicated by superscript letters a-f (Appendix S1).

Vital rate	Parameters	Link function	Likelihood distribution	ΔDIC
Survival (σ)	$\mu^{surv} = \alpha_0 + \alpha_j[TSF] + \alpha_k[LS] + \alpha_{jk}[TSF \times LS]$ $+(\beta_c + \beta_{jc}[TSF] + \beta_{kc}[LS]) \times size + \alpha_s[site]$	logit(σ)	$\sigma \sim B(\mu^{surv})$	-82.0[f]
Growth (γ)	$\mu^{gr} = \alpha_0 + \alpha_j[TSF] + \alpha_k[LS] + \alpha_{jk}[TSF \times LS]$ $+(\beta_c + \beta_{jc}[TSF] + \beta_{kc}[LS]) \times size + \alpha_s[site]$ $\log(\tau^{gr}) = \alpha_0 + \alpha_j[TSF] + \alpha_k[LS] + \alpha_{jk}[TSF \times LS]$	identity	$\gamma \sim \aleph(\mu^{gr}, \tau^{gr})$	-25.0[f] -112.0[ii]
Probability of flowering (φ_0)	$\mu^{fl} = \alpha_0 + \alpha_j[TSF] + \alpha_k[LS] + \alpha_{jk}[TSF \times LS]$ $+(\beta_c + \beta_{jc}[TSF] + \beta_{kc}[LS]) \times size + \alpha_s[site]$	logit(φ_0)	$\varphi_0 \sim B(\mu^{fl})$	-90.0[f]
Number of flowering stalks (φ_1)	$\mu^{fs} = \alpha_0 + \alpha_j[TSF] + \alpha_k[LS] + \alpha_{jk}[TSF \times LS]$ $+(\beta_c + \beta_{jc}[TSF] + \beta_{kc}[LS]) \times size + \alpha_s[site]$	log(φ_1)	$\varphi_1 \sim NB(\rho^{fs}, \mu^{fs})$	-5.0[f]
Number of flowers per stalk (φ_2)	$\mu^{fps} = \alpha_0 + \alpha_j[TSF] + \alpha_k[LS] + \alpha_{jk}[TSF \times LS]$ $+ \beta_c \times size + \alpha_s[site]$	log(φ_2)	$\varphi_2 \sim NB(\rho^{fps}, \mu^{fps})$	-7.0[c]
Seedling size (φ_4)	$\mu^{sds} = \alpha_0 + \alpha_j[TSF] + \alpha_k[LS] + \alpha_{jk}[TSF \times LS]$ $+ \alpha_s[site]$ $\log(\tau^{sds}) = \alpha_0 + \alpha_j[TSF]$	identity	$\varphi_4 \sim \aleph(\mu^{sds}, \tau^{sds})$	-5.0[d] -48.0[h]
Immediate germination (*goCont*)	$\mu^{goCont} = \alpha_0 + \alpha_p[PFS] + \alpha_b[block]$	logit(*goCont*)	*goCont* ~ $B(\mu^{goCont})$	-38.2[a]
Stasis is seed bank (*staySB*)	$\mu^{staySB} = \alpha_0 + \alpha_p[PFS] + \alpha_b[block]$	logit(*staySB*)	*staySB* ~ $B(\mu^{staySB})$	-6.8[a]
Egression from seed bank (*outSB*)	$\mu^{outSB} = \alpha_0 + \alpha_p[PFS] + \alpha_b[block]$	logit(*outSB*)	*outSB* ~ $B(\mu^{outSB})$	-206.0[a]

7.3.3 IPM construction

In order to associate environmental states with vital rates in stochastic simulations (see below), we built IPMs from the 600 posterior estimates of vital rates for each combination of the five TSF, two LS, and eight site-effect estimates (see makeIPM.R in Appendix S2). The IPMs consisted of two coupled equations integrated over $L = 0$ and $U = 9.6$ sizes x at t to give a vector of sizes y at $t+1$. The lower and upper integration limits corresponded to 0.9 × minimum observed size and 1.1 × maximum observed size, respectively. The first of the two equations describes seed-bank dynamics (S) at $t + 1$ through the contribution of seeds produced by above-ground individuals (*goSB*) and seeds remaining in the seedbank (*staySB*) at t:

$$\mathrm{S(t+1) = S(t)staySB} + \int_{\mathrm{L}}^{\mathrm{U}} \varphi_0(\mathrm{x})\,\varphi_1(\mathrm{x})\varphi_2(\mathrm{x})\varphi_3\sigma_{\mathrm{S}}\mathrm{goSBn(x,t)dx} \qquad (1)$$

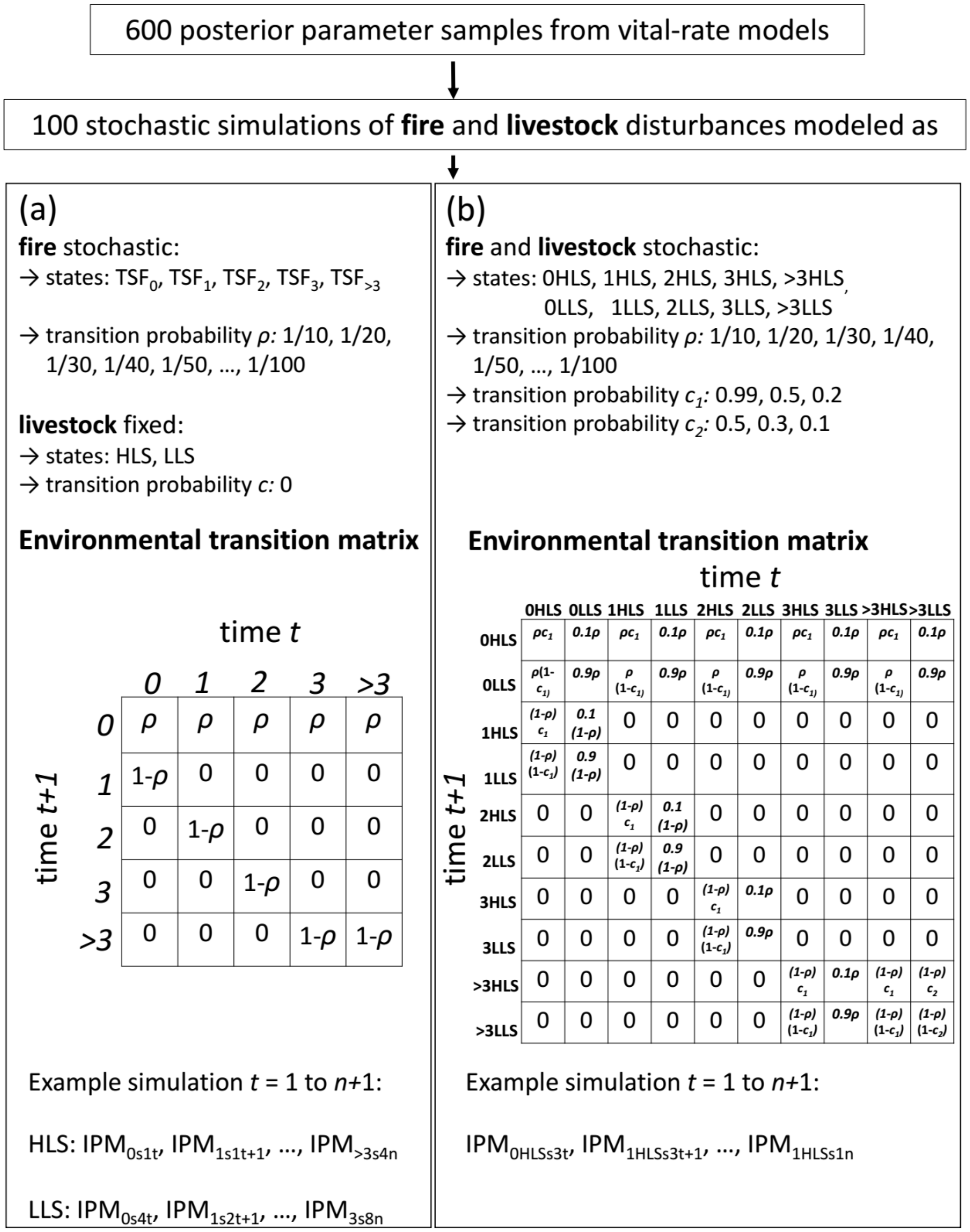
The second equation describes the dynamics of above-ground individuals through establishment of seedlings from the seed bank, survival of established individuals, and contributions of seedlings by reproductive individuals at t:

$$\mathrm{n(y,t+1) = S(t)outSB}\varphi_3(\mathrm{y}) + \int_{\mathrm{L}}^{\mathrm{U}} [\sigma(\mathrm{x})\gamma(\mathrm{y,x}) + \varphi_0(\mathrm{x})\varphi_1(\mathrm{x})\varphi_2(\mathrm{x})\varphi_3\sigma_{\mathrm{S}}\mathrm{goCont}\,\varphi_4(\mathrm{y})]\mathrm{n(x,t)dx} \qquad (2)$$

IPMs for TSF_0 (burning) consisted of stasis in and germination from the seed bank, with 0 transition probabilities elsewhere, reflecting the death of above-ground individuals by fire.

7.3.4 Stochastic simulations

We investigated the effects of disturbance interactions on viability and selection pressures of *Drosophyllum* by simulating population dynamics from the IPMs. Environmental variability in the simulations consisted of stochastic transitions between TSF and LS states. We simulated stochastic TSF transitions as a Markov-chain process with states corresponding to TSF categories: 0, 1, 2, 3, and > 3 years after fire, and transitions corresponding to probability of fire (ρ) = 1/fire return interval (Fig. 2). We used 10 fire-return intervals, from 10 to 100 years at 10-year increments (Fig. 2), which characterize the fire-frequency range in the study region (Ojeda 2009).



600 posterior parameter samples from vital-rate models

100 stochastic simulations of **fire** and **livestock** disturbances modeled as

(a)

fire stochastic:

→ states: TSF_0, TSF_1, TSF_2, TSF_3, $TSF_{>3}$

→ transition probability ρ: 1/10, 1/20, 1/30, 1/40, 1/50, …, 1/100

livestock fixed:

→ states: HLS, LLS

→ transition probability c: 0

Environmental transition matrix

time t

time $t+1$	0	1	2	3	>3
0	ρ	ρ	ρ	ρ	ρ
1	$1-\rho$	0	0	0	0
2	0	$1-\rho$	0	0	0
3	0	0	$1-\rho$	0	0
>3	0	0	0	$1-\rho$	$1-\rho$

Example simulation t = 1 to n+1:

HLS: IPM_{0s1t}, IPM_{1s1t+1}, …, $IPM_{>3s4n}$

LLS: IPM_{0s4t}, IPM_{1s2t+1}, …, IPM_{3s8n}

(b)

fire and **livestock** stochastic:

→ states: 0HLS, 1HLS, 2HLS, 3HLS, >3HLS, 0LLS, 1LLS, 2LLS, 3LLS, >3LLS

→ transition probability ρ: 1/10, 1/20, 1/30, 1/40, 1/50, …, 1/100

→ transition probability c_1: 0.99, 0.5, 0.2

→ transition probability c_2: 0.5, 0.3, 0.1

Environmental transition matrix

time t

time $t+1$	0HLS	0LLS	1HLS	1LLS	2HLS	2LLS	3HLS	3LLS	>3HLS	>3LLS
0HLS	ρc_1	0.1ρ	ρc_1	0.1ρ	ρc_1	0.1ρ	ρc_1	0.1ρ	ρc_1	0.1ρ
0LLS	$\rho(1-c_1)$	0.9ρ	$\rho(1-c_1)$	0.9ρ	$\rho(1-c_1)$	0.9ρ	$\rho(1-c_1)$	0.9ρ	$\rho(1-c_1)$	0.9ρ
1HLS	$(1-\rho)c_1$	$0.1(1-\rho)$	0	0	0	0	0	0	0	0
1LLS	$(1-\rho)(1-c_1)$	$0.9(1-\rho)$	0	0	0	0	0	0	0	0
2HLS	0	0	$(1-\rho)c_1$	$0.1(1-\rho)$	0	0	0	0	0	0
2LLS	0	0	$(1-\rho)(1-c_1)$	$0.9(1-\rho)$	0	0	0	0	0	0
3HLS	0	0	0	0	$(1-\rho)c_1$	0.1ρ	0	0	0	0
3LLS	0	0	0	0	$(1-\rho)(1-c_1)$	0.9ρ	0	0	0	0
>3HLS	0	0	0	0	0	0	$(1-\rho)c_1$	0.1ρ	$(1-\rho)c_1$	$(1-\rho)c_2$
>3LLS	0	0	0	0	0	0	$(1-\rho)(1-c_1)$	0.9ρ	$(1-\rho)(1-c_1)$	$(1-\rho)(1-c_2)$

Example simulation t = 1 to n+1:

$IPM_{0HLSs3t}$, $IPM_{1HLSs3t+1}$, …, $IPM_{1HLSs1n}$

Figure 2 Description of stochastic simulations used to model the effects of fire and livestock browsing/trampling on the population dynamics of *Drosophyllum lusitanicum*. We performed 100 simulations for each of 600 posterior parameter samples, by considering (a) only stochastic transitions, with probability ρ, in fire states but assuming fixed livestock-pressure states, and (b) stochastic transitions in fire and livestock-pressure states (with transition probabilities defined by c_1 and c_2). The resulting environmental transition matrices produced different sequences of IPMs representing each state (examples). TSF: time since fire (0, 1, 2, 3, or >3 years); HLS: high livestock pressure; LLS: low livestock pressure; *s*: random sites (1-8).

Stochastic transitions between HLS and LLS were based on different scenarios of heathland conservation. First, we explored the differences in dynamics of populations where high livestock pressure (HLS) is maintained (no conservation) compared with dynamics in natural fire-disturbed populations under low livestock pressure (LLS). We therefore modeled populations fixed in their respective LS state. Here, fires could occur in each LS state independently, and stochastic simulations varied for TSF transitions only (Fig. 2a; `sLambdafixedLS.R` in Appendix S2).

Second, we explored the effects on population dynamics when livestock pressure, in addition to fire, varied in a given population, representing variable conservation efforts (Paniw *et al.*, 2015). We therefore considered transitions between LS states and integrated TSF and LS into one Markov chain with states determined by each combination of TSF and LS (Fig. 2; `sLambdaStochLS.R` in Appendix S2). LS transitions were defined by three probabilities (c_1) of a population experiencing HLS at t and remaining in HLS at t+1, depicting low to high conservation of natural heathlands: 0.99 (high Pr(HLS)), 0.5 (moderate Pr(HLS)), or 0.2 (low Pr(HLS)) (Fig. 2b). Accordingly, populations transitioned from HLS to LLS with probabilities = 1 – Pr(HLS). In turn, populations experiencing LLS at t could face high livestock pressure at t+1 with probabilities c_2: 0.5, 0.3, or 0.1 in $\mathrm{TSF}_{>3}$. The latter probabilities were derived from estimates of rates of human disturbance (% of habitat loss in the last 20 years) in mature heathlands across the range of *Drosophyllum*: in Portugal where habitat degradation is largest (0.5), in Morocco where degradation is moderate (0.3), and in Southern Spain where the proportion of natural heathland habitats is largest (0.1) (Chapter 6). In TSF_3, populations under LLS transitioned to HLS with probabilities arbitrarily fixed at 0.1 to mimic low herbivory pressure in natural habitats.

For each scenario of heathland conservation, we ran 100 simulations, each for 4,500 years (Fig. 2). We included parameter uncertainty by running the 100 simulations for each of the 600 MCMC samples of parameters (Table 1; Chapter 5). At each of the 4,500 iterations during a simulation, an environmental state (combination of TSF and LS) was picked based on the state transition probabilities given by the Markov chain. That state was represented by one of eight IPMs, corresponding to each of the eight estimates of the random site effect at a given TSF×LS combination (Fig. 2). For each simulation, we calculated the stochastic population growth rate, log λ_s, as in Trotter *et al.* (2013) discarding the first 500 years. We also obtained the probability of quasi-extinction at t=150 years, P_q(150 years) where populations were considered extinct when the total number of individuals (including seeds in the seed bank) N<100.

To infer differences in selection pressures on life-history strategies of populations exposed to different combinations of fire and livestock disturbances, we perturbed each vital rate used to compose the IPMs by its mean, μ, and standard deviation, σ, across all environmental states (Haridas and Tuljapurkar 2005; see Appendix S3 for details and *R* scripts in Appendix S2 for examples). We then used the chain rule to calculate (i) how these perturbations affected the IPM kernels, and (ii) how the latter in turn affected log λ_s. These calculations provided us with elasticities, $\mathrm{E}_{\mathrm{a}}^{\mu}$ and $\mathrm{E}_{\mathrm{a}}^{\sigma}$, of a = log λ_s to changes in the mean and variance of vital rates, respectively. These two measures quantify the strength of selection pressures on life-history traits in stochastic environments (Haridas and Tuljapurkar 2005). Here, we used a subset of four fire return-intervals and excluded parameter uncertainty for simplicity and computational efficiency. We calculated average (± 95 % non-parametric CI) $\mathrm{E}_{\mathrm{a}}^{\mu}$ and $\mathrm{E}_{\mathrm{a}}^{\sigma}$ across the 100 stochastic simulations.

7.4 Results

7.4.1 Disturbance interactions and population viability

Our simulations of the stochastic population growth rate, log λ_s, showed significant interactive effects between fire regimes and livestock pressure on *Drosophyllum* populations. In populations maintained under low livestock pressure (LLS) but experiencing varying fire regimes, mean log λ_s (across simulations and parameters) decreased and mean probability of quasi-extinction P_q(150 years) increased monotonically as the fire return interval *shortened* (Fig. 3 a, b). However, populations maintained under high browsing (HLS) showed the opposite trend with mean log λ_s increasing and mean P_q(150 years) decreasing monotonically with *extended* fire return interval (Fig. 3a, b). In populations maintained under HLS, lowest P_q(150 years) ≈ 0.5, remaining high even at a fire return interval of 100 years. When livestock pressure, in addition to fire, was assumed to vary through time in a given population, log λ_s and P_q(150 years) patterns in populations under high and low/moderate Pr(HLS) were very similar to ones in populations maintained under HLS and LLS, respectively (Fig. 3c, d). However, populations under moderate browsing, or moderate Pr(HLS), had highest log λ_s across all fire return intervals, ranging from 0.15 (10 year return interval) to 0.05 (100 year interval) - much higher compared with populations maintained under LLS, ranging from 0.13 (10 year interval) to -0.05 (100 year interval) (Fig 3 a, c). Consequently, P_q(150 years) were lowest (< 0.10) under moderate browsing at a fire return interval of 10 years.

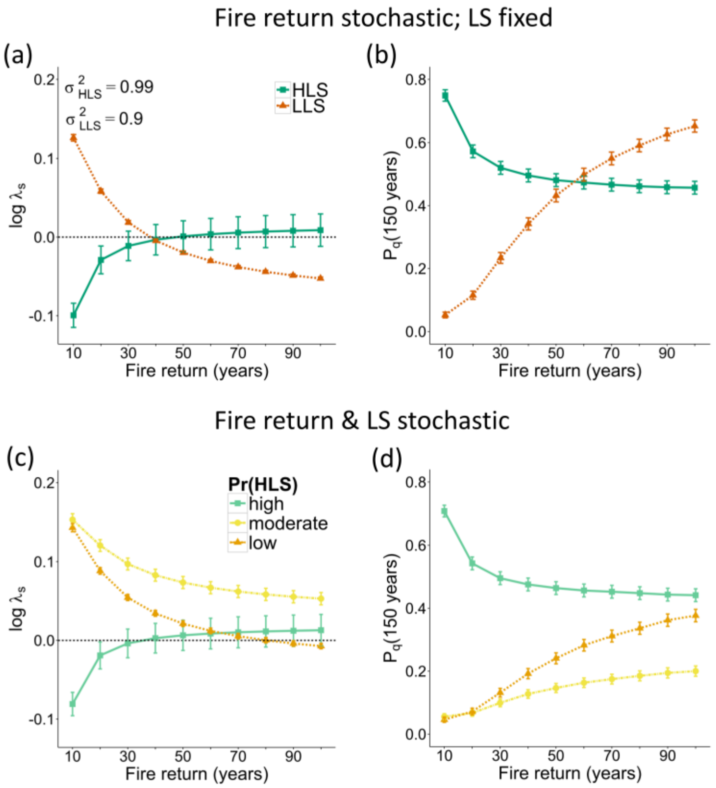


Figure 3 Stochastic population growth rate, log λ_s (a, c) and probability of quasi-extinction after 150 years, P_q(150 years) (b, d), across stochastic simulations of interactions of 10 fire return intervals and livestock (LS) pressure, high (HLS) or low (LLS). For each fire return interval, LS states were either fixed (a, b) or fluctuated stochastically (c, d). In the latter, probabilities to change LS states were determined by high, intermediate, and low probability of HLS in time *t*. Values at each fire return × LS combination depict mean of 60,000 log λ_s from 600 samples of the posterior parameter distributions and 100 stochastic simulations of log λ_s obtained for each parameter value. Error bars depict variance attributed to parameter uncertainty. σ^2 = proportion of log λ_s variance explained by parameter uncertainty for simulations using HLS and LLS. Bars of log λ_s crossing horizontal dotted line correspond to constant population sizes through time.

A great deal of uncertainty, largely due to vital-rate parameter estimation (Fig. 1), was associated with the mean estimates of log λ_s across simulations (Fig 3a, c; Fig. S3.1a, c in Appendix S3). High parameter uncertainty resulted in the 95 % CI of log λ_s estimates always including 0 values, implying little certainty in effect of both disturbances on long-term stochastic dynamics (Fig. S3.1a, c in Appendix S3). However, parameter uncertainty did not change the general trend in the response of log λ_s and P_q(150 years) to varying fire and livestock disturbances.

7.4.2 Selection of life-history strategies under different disturbance regimes

Selection pressures, quantified by elasticities $\mathrm{E}_{\mathrm{a}}^{\mu}$ and $\mathrm{E}_{\mathrm{a}}^{\sigma}$, differed starkly in populations maintained under HLS compared to ones exposed to LLS and largely disturbed by fire. Whereas under LLS, mean increases in seed-bank stasis (*staySB*) and egression (*outSB*) were critical in increasing log λ_s, vital rates describing survival (σ), growth (γ), flowering (φ_0), and immediate germination (*goCont*) were under much stronger selection relative to other vital rates under HLS (Fig. 4). The importance of σ, γ, φ_0 as well as *goCont* and *outSB* increased with extending fire return interval in populations maintained under HLS (Fig. 4a). The same occurred for *staySB* and *outSB* in populations under LLS.

Our simulations showed that vital-rate variation was largely selected for and against in populations under HLS and LLS, respectively. In HLS populations, increases in the standard deviation of above-ground vital rates were favored across fire return intervals, although $\mathrm{E}_{\mathrm{a}}^{\sigma}$ were small compared to LLS populations (Fig. 4b). In LLS populations, variability in above-ground vital rates (Table 1) as well as *staySB* and *outSB* would potentially threaten populations, while variability in the remaining vital rates had negligible effects log λ_s (Fig. 4b; Appendix S3).

$\mathrm{E}_{\mathrm{a}}^{\mu}$ and $\mathrm{E}_{\mathrm{a}}^{\sigma}$ for vital rates in populations exposed to varying livestock pressures under high probability of HLS were similar to the ones maintained under HLS while those with low probability of HLS were similar to ones maintained under LLS. When probabilities of changing from LS states were intermediate, increases in above-ground vital rates and seed-bank stasis resulted in high $\mathrm{E}_{\mathrm{a}}^{\mu}$ and $\mathrm{E}_{\mathrm{a}}^{\sigma}$with increasing fire return interval (Fig. S3.3).

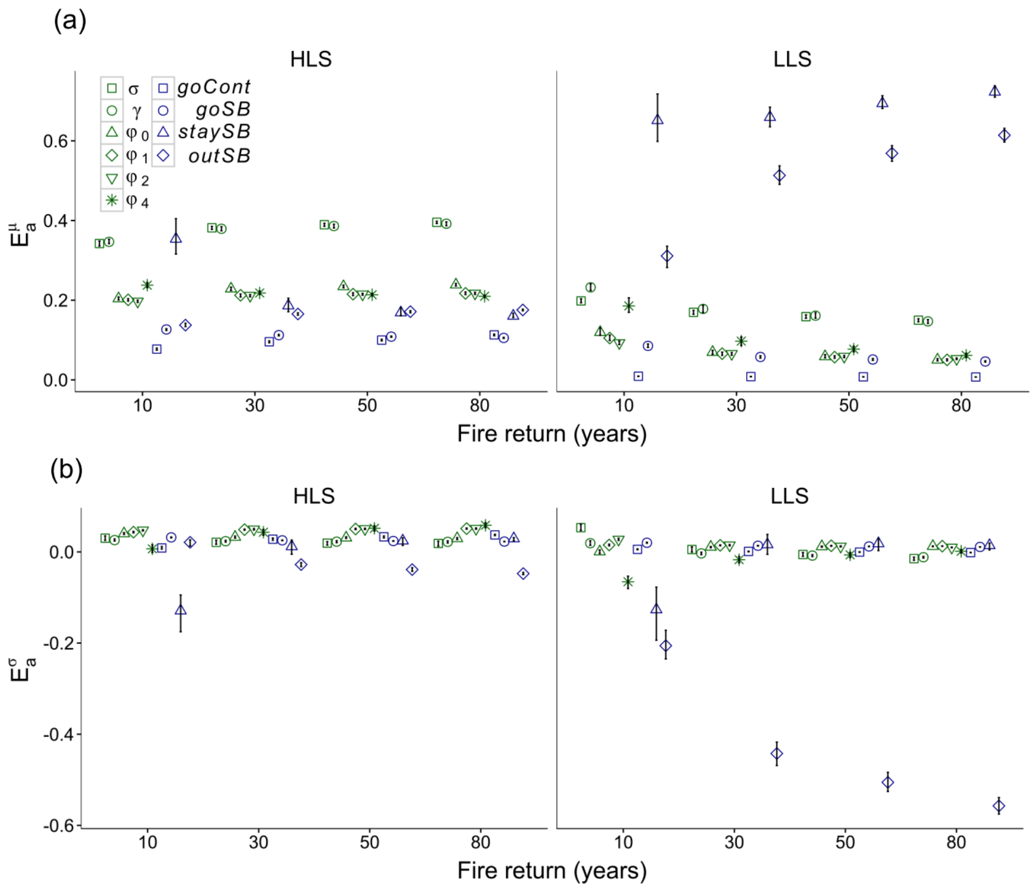


Figure 4 Elasticities of log λ_s to changes in (a) the average (E_a^{μ}) and (b) standard deviation (E_a^{σ}) of 10 vital rates obtained from stochastic simulations of four fire return intervals using vital rates from populations exposed to high (HLS) and low (LLS) livestock pressure. See Appendix S3 for details on calculations. Vital rates denoted by green and orange colors represent above-ground processes [survival (σ), growth (γ, φ_4), and reproduction (φ_0, φ_1, φ_2)] and seed fates [immediate germination (*goCont*) and seed-bank ingression (*goSB*), stasis (*staySB*) and egression (*outSB*)], respectively. Error bars show a 95 % non-parametric CI calculated from 100 simulations at each fire return.

7.5 Discussion

In Mediterranean and other fire-prone ecosystems, human disturbances have been increasingly replacing or interacting with natural fire disturbances in the last century (Turner 2010; Duwyn and MacDougall 2015; Tye *et al*. 2016). Our study suggests that combining different disturbance regimes, rather than substituting human disturbances for fire, may enhance the conservation of fire-adapted seeder species (Fuhlendorf *et al*. 2009; Fernandes *et al*. 2013). On the other hand, fire management aimed at chronic removal of potential fuel to decrease the probability of burning (Valdecantos *et al*. 2008) may compromise the viability of species and may therefore decrease local biodiversity. This is in part because chronic small-scale disturbances may select against life-history adaptations to severe disturbance events and compromise populations when such disturbances inevitably occur. By assessing differences in selection pressures exerted by fundamentally different disturbances, this study highlights emerging issues for the management of fire-adapted species increasingly found in human-disturbed habitats (Bonebrake *et al*. 2014).

Due to the high parameter uncertainty associated with vital-rate estimates in our analyses, which has been demonstrated in stochastic population models for other fire-adapted species (Evans *et al.* 2010), we cannot reliably predict the exact long-term fates of our studied populations. However, our results allow us to compare potential selection pressures and population dynamics under different combinations of disturbance regimes and infer management strategies (e.g., Mandle *et al.* 2015).

7.5.1 Human-fire interactions are opportunity and threat to conservation

Many species with flexible strategies to buffer environmental variability may have the potential to adapt to human disturbances (McKinney and Lockwood 1999; Hendry *et al.* 2008). Some species even show higher growth rates in human-disturbed habitats compared to naturally-disturbed ones (e.g., Vieira-Neto *et al*. 2016; Tye *et al*. 2016). Our simulations showed that growth rates were consistently higher and risk of extinction lower in *Drosophyllum* populations under varying livestock pressures compared with ones maintained under low pressures. The positive effects of browsing may be explained by the fact that for a large number of species, including *Drosophyllum*, moderate levels of livestock browsing and trampling may be typical of prehistoric conditions where ungulates were abundant and created open space for periodic recruitment in between large-scale disturbance events (Pykälä 2000; Velle *et al.* 2014). Such periodic recruitment and subsequent seed input into the seed bank has been shown to ensure population viability under a wide range of natural disturbance regimes for threatened (Pardini *et al*. 2015) and invasive (Renne and Tracy 2007) species alike. In the southwestern Mediterranean Basin, where fire suppression remains a management goal (Andrés and Ojeda 2002; Valdecantos *et al*. 2008), our study shows that recurrent fires, in combination with browsing, are instead needed to conserve heathland biodiversity (Baeza *et al*. 2007; Fernandes *et al.* 2013).

Despite human activities potentially playing a positive role in the conservation of fire-adapted species, our simulations suggest that substituting fires by human disturbances may compromise population viability. For *Drosophyllum*, populations maintained under high browsing pressure showed consistently high P_q(150 years) ≈ 0.5, even when fire return intervals extended (Fig. 3). When also burned frequently, as is likely to occur despite fire suppression (Syphard *et al*. 2009), such populations are not viable (Fig. 3). Frequent disturbance interactions have been shown to reduce population viability of a number of species (*e.g*., Lawson et al. 2010; Mandle *et al*. 2015; Darabant *et al*. 2016). In this sense, the apparent persistence of *Drosophyllum* populations in human-disturbed habitats (Chapter 6) may constitute an extinction debt by which true extinction will inevitably follows a time lag inversely proportional to rates of population turnover – in our case slowed by the seed bank (Tilman *et al*. 1994; González-Varo *et al*. 2015).

7.5.2 Understanding selection pressures under different disturbances to improve management

In this study, we aimed to understand the demographic processes that may lead to population collapse under frequent human and fire disturbances. Elasticity analyses showed that human disturbance favored survival and growth of established *Drosophyllum* individuals and yearly germination over seed-bank stasis, which was selected for in fire-disturbed populations. As immediate germination *vs.* ingression into the seed bank constitutes a direct trade-off (Cohen 1966), a depleted soil seed bank may be expected in human-disturbed populations. In such populations, higher fitness (and viability) may be achieved through increases in survival of reproductive individuals, which may confer more tolerance to year-to-year environmental variation and high seedling mortality caused by the nature of human disturbances (Morris *et al.* 2008). The fact that elasticities of log λ_s to increases in vital-rate variability are almost negligible for critical above-ground vital rates (Fig. 4b) may indicate that increased longevity in human-disturbed populations is buffering against variability in vital rates (Morris *et al*. 2008). At the same time, high levels of browsing/trampling may increase damage to reproductive structures and mortality of plants, either directly or indirectly via exposure to adverse microhabitat conditions (e.g., solar radiation or wind; Peñuelas *et al.* 2007; M. Paniw, unpbl. data). Therefore, when human disturbances occur frequently or co-occur with fires, the increased environmental variability leads to higher mortality of aboveground individuals, which cannot be compensated by germination from a diminished seed bank. This phenomenon may explain the frequent population collapse in human-disturbed habitats of *Drosophyllum* (Garrido *et al.* 2003) as well as other fire-dependent species (Quintana-Ascencio *et al.* 2007).

It is alarming then that most natural populations of *Drosophyllum* are found in human-disturbed habitats, some persisting even >50 years after fire (chapter 6). Conservation of *Drosophyllum* populations under high livestock pressure must differ to conservation in fire-disturbed, natural heathlands. Whereas in the latter, introduction of moderate browsing disturbances while preserving the seed bank is important, the former must protect large above-ground individuals and attempt to reestablish a seed bank before fire management can be introduced. Considering selection pressures exerted by different disturbance types is therefore crucial for a proper management of populations. At the same time, a full analysis of life-history evolution and its application to management - beyond the scope of this study - would need to consider vital-rate trade-offs. In human-disturbed populations of *Drosophyllum*, where several above-ground vital rates are under strong selection (Fig. 4), such tradeoffs and plant physiology will likely constrain potential adaptations and population dynamics in the absence of fires (Benton *et al*. 2006).

Overall, our analyses emphasize that detailed analyses of various environmental drivers are needed in a world where human activities increasingly affect natural ecosystems (Turner 2010). We encourage ecologists to explore on interactions of environmental drivers while assessing uncertainty in analyses, which allows for more robust interpretations of patterns (Evans *et al*. 2010; Elderd and Miller 2016).

7.6 Acknowledgements

M. Gil-López, M. Scott, and M. Collado-Aliaño helped in data collection, and A. Jiménez' CITI team (UCA) granted access to parallel processing. This study was financed by BREATHAL (CGL2011-28759/BOS; Spanish *Ministerio de Economía y Competitividad*).

M.P. was supported by a FPI scholarship and by a travel bursary granted by the Spanish *Ministerio de Economía y Competitividad*, R.S.-G. by the Australian Research Council (DE140100505), and P.F.Q.-A. by NSF-DEB 1347247 & NSF-DEB 0812753.

7.7 References

Andrés C, Ojeda F. 2002. Effects of afforestation with pines on woody plant diversity of Mediterranean heathlands in southern Spain. *Biodiversity and Conservation* **11**:1511–1520.

Ashley MV, Willson MF, Pergams OR, O'Dowd DJ, Gende SM, Brown JS. 2003. Evolutionarily enlightened management. *Biological Conservation* **111**:115-123.

Baeza MJ, Valdecantos A, Alloza JA, Vallejo VR. 2007. Human disturbance and environmental factors as drivers of long-term post-fire regeneration patterns in Mediterranean forests. *Journal of Vegetation Science **18**:243-252.*

Benton TG, Plaistow SJ, Coulson TN. 2006. Complex population dynamics and complex causation: devils, details and demography. *Proceedings of the Royal Society of London B: Biological Sciences* **273**:1173-1181.

Bonebrake TC, Syphard AD, Franklin J, Anderson KE, Akçakaya HR, Mizerek T, Winchell C, Regan HM. 2014. Fire management, managed relocation, and land conservation options for long-lived obligate seeding plants under global changes in climate, urbanization, and fire regime. *Conservation Biology* **28**: 1057-1067.

Bond WJ, Keeley JE. 2005. Fire as a global ‘herbivore’: the ecology and evolution of flammable ecosystems. *Trends in Ecology and Evolution* **20**:387-394.

Boyce MS, Haridas CV, Lee CT, NCEAS Stochastic Demography Working Group. 2006. Demography in an increasingly variable world. *Trends in Ecology and Evolution* **21**:141-148.

Chuvieco E, Giglio L, Justice C. 2008. Global characterization of fire activity: toward defining fire regimes from Earth observation data. *Global Change Biology* **14**:1488-1502.

Cohen D. 1966. Optimizing reproduction in a randomly varying environment. *Journal of Theoretical Biology* **12**:119-129.

Correia E, Freitas H. 2002. *Drosophyllum lusitanicum*, an endangered West Mediterranean endemic carnivorous plant: threats and its ability to control available resources. *Botanical Journal of the Linnaean Society* **140**:383–390.

Darabant A, Staudhammer CL, Rai PB, Gratzer G. 2016. Burning for enhanced non-timber forest product yield may jeopardize the resource base through interactive effects. *Journal of Applied Ecology*, in press.

Duwyn A, MacDougall AS. 2015. When anthropogenic-related disturbances overwhelm demographic persistence mechanisms. *Journal of Ecology* **103**:761–768.

Easterling MR, Ellner SP, Dixon PM. 2000. Size-specific sensitivity: applying a new structured population model. *Ecology* **81**:694–708.

Ehrlén J, Morris WF, von Euler T, Dahlgren JP. 2016. Advancing environmentally explicit structured population models of plants. *Journal of Ecology* **104**:292-305.

Elderd BD, Miller TE. 2016. Quantifying demographic uncertainty: Bayesian methods for Integral Projection Models (IPMs). *Ecological Monographs* **86**:125-144.

Evans ME, Holsinger KE, Menges ES. 2010. Fire, vital rates, and population viability: a hierarchical Bayesian analysis of the endangered Florida scrub mint. *Ecological Monographs* **80**:627-649.

Fuhlendorf SD, Engle DM, Kerby JA, Hamilton R. 2009. Pyric herbivory: rewilding landscapes through the recoupling of fire and grazing. *Conservation Biology* **23**:588-98.

Fernandes PM, Davies GM, Ascoli D, Fernández C, Moreira F, Rigolot E, Stoof CR, Vega JA, Molina D. 2013. Prescribed burning in southern Europe: developing fire management in a dynamic landscape. *Frontiers in Ecology and Environment* **11**:e4–e14.

Garrido B, Hampe A, Maranon T, Arroyo J. 2003. Regional differences in land use affect population performance of the threatened insectivorous plant *Drosophyllum lusitanicum* (Droseraceae). *Diversity and Distributions* **9**:335–350.

González-Varo JP, Albaladejo RG, Aizen MA, Arroyo J, Aparicio A. 2015. Extinction debt of a common shrub in a fragmented landscape. *Journal of Applied Ecology* 52:580-589.

Haridas CV, Tuljapurkar S. 2005. Elasticities in variable environments: properties and implications. *The American Naturalist* **166**:481–495.

Hendry AP, Farrugia TJ, Kinnison MT. 2008. Human influences on rates of phenotypic change in wild animal populations. *Molecular Ecology* **17**:20-29.

Keeley JE, Bond WJ, Bradstock RA, Pausas JG, Rundel PW. 2012. *Fire in Mediterranean Ecosystem: Ecology, Evolution and Management*. Cambridge University Press.

Lawson DM, Regan HM, Zedler PH, Franklin J. 2010. Cumulative effects of land use, altered fire regime and climate change on persistence of *Ceanothus verrucosus*, a rare, fire-dependent plant species. *Global Change Biology* **16**:2518–2529.

Mandle L, Ticktin T, Zuidema PA. 2015. Resilience of palm populations to disturbance is determined by interactive effects of fire, herbivory and harvest. *Journal of Ecology* **103**:1032-1043.

McKinney ML, Lockwood JL. 1999. Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends in Ecology and Evolution* **14**:450-453.

Menges ES, Quintana-Ascencio PF. 2004. Population viability with fire in *Eryngium cuneifolium*: deciphering a decade of demographic data. *Ecological Monographs* **74**:79-99.

Morris WF, Pfister CA, Tuliapurkar S, Haridas CV, Boggs CL, Boyce MS, *et al.* 2008. Longevity can buffer plant and animal populations against changing climatic variability. *Ecology* **89**:19–25.

Müller J, Deil U. 2001. Ecology and structure of *Drosophyllum lusitanicum* (L.) Link populations in the south-western of the Iberian Peninsula. *Acta Botánica Malacitana* **26**:47-68.

Ojeda F. 2009. 4030 Brezales secos europeos. In: *VV. AA.* Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España. Ministerio de Medio Ambiente, y Medio Rural y Marino, Madrid, Spain.

Ojeda F, Marañón T, Arroyo J. 1996. Postfire regeneration of a Mediterranean heathland in southern Spain. *International Journal of Wildland Fire* **6**:191-198.

Palkovacs EP, Kinnison MT, Correa C, Dalton CM, Hendry AP. 2012. Fates beyond traits: ecological consequences of human-induced trait change. *Evolutionary Applications* **5**:183-191.

Pardini EA, Vickstrom KE, Knight TM. 2015. Early successional microhabitats allow the persistence of endangered plants in coastal sand dunes. *PloS One* **10**:e0119567.

Peñuelas J, Prieto P, Beier C, Cesaraccio C, De Angelis P, de Dato G, Emmett BA, Estiarte M, Garadnai J, Gorissen A, Láng EK. 2007. Response of plant species richness and primary productivity in shrublands along a north–south gradient in Europe to seven years of experimental warming and drought: reductions in primary productivity in the heat and drought year of 2003. *Global Change Biology* **13**:2563-2581.

Plan INFOCA – Incedios forestales. 2012. *Junta de Andalucia: Consejería de Medioambiente y Ordenación del Territorio*. <http://www.juntadeandalucia.es/medioambiente>

Pykälä J. 2000. Mitigating human effects on European biodiversity through traditional animal husbandry. *Conservation Biology* **14**:705-712.

Quintana-Ascencio PF, Weekley CW, Menges ES. 2007. Comparative demography of a rare species in Florida scrub and road habitats. *Biological Conservation* **137**:263–270.

Renne IJ, Tracy BF. 2007. Disturbance persistence in managed grasslands: shifts in aboveground community structure and the weed seed bank. *Plant Ecology* **190**:71-80.

Sánchez-Velásquez LR, Ezcurra E, Martínez-Ramos M, Álvarez-Buylla E, Lorente R. 2002. Population dynamics of *Zea diploperennis*, an endangered perennial herb: effect of slash and burn practice. *Journal of Ecology* **90**:684-692.

Syphard AD, Radeloff VC, Hawbaker TJ, Stewart SI. 2009. Conservation threats due to human-caused increases in fire frequency in Mediterranean-climate ecosystems. *Conservation Biology* **23**:758-69.

Steel ZL, Safford HD, Viers JH. 2015. The fire frequency-severity relationship and the legacy of fire suppression in California forests. *Ecosphere* **6**:1-23.

Tilman D, May RM, Lehman CL, Nowak MA. 1994. Habitat destruction and extinction debt. *Nature* **371**:65–66.

Trotter MV, Krishna-Kumar S, Tuljapurkar S. 2013. Beyond the mean: sensitivities of the variance of population growth. *Methods in Ecology and Evolution* **4**:290-298.

Tuljapurkar S. 1990. *Population Dynamics in Variable Environments*. Springer.

Turco M, Bedia J, Di Liberto F, Fiorucci P, von Hardenberg J, Koutsias N, Llasat MC, Xystrakis F, Provenzale A. 2016. Decreasing fires in Mediterranean Europe. *PLoS One* **16**:e0150663.

Turner MG. 2010. Disturbance and landscape dynamics in a changing world. *Ecology* **91**:2833-2849.

Tye MR, Menges ES, Weekley C, Quintana-Ascencio PF, Salguero-Gómez R. 2016. A demographic ménage à trois: interactions between disturbances both amplify and dampen population dynamics of an endemic plant. *Journal of Ecology*, in press.

Valdecantos A, Baeza MJ, Vallejo VR. 2008. Vegetation management for promoting ecosystem resilience in fire-prone Mediterranean shrublands. *Restoration Ecology* **17**:414-421.

Velle LG, Nilsen LS, Norderhaug A, Vandvik V. 2014. Does prescribed burning result in biotic homogenization of coastal heathlands? *Global Change Biology* **20**:1429–1440.

Vieira-Neto EH, Vasconcelos HL, Bruna EM. 2016. Roads increase population growth rates of a native leaf-cutter ant in Neotropical savannahs. *Journal of Applied Ecology* **53**:983-992.

Vilà M, Terradas J. 1995. Effects of competition and disturbance on the resprouting performance of the Mediterranean shrub *Erica multiflora* L. (Ericaceae). *American Journal of Botany* **82**:1241-1248

7.8 Appendix S1 - Details on study design and parameterization of vital-rate models

Here, we provide details on (A) the biology of the study species *Drosophyllum lusitanicum* (L.) Link. (Drosophyllaceae; *Drosophyllum* hereafter) and experiments performed to obtained seed related vital rates; (B) the parameterization of the Bayesian model to quantify vital rates and construct IPMs; and (C) diagnostic checks for convergence of the MCMC chains.

7.8.1 Study sites and censuses/experiments

The eight sites with *Drosophyllum* populations censused in this study differed in two disturbance types: fires (from 1 to > 20 years since last fire - TSF) and browsing by large mammalian herbivores (high or low livestock pressure, LS). Figure S1.1 shows examples of sited affected by different disturbances while Table S1.1 shows all combinations of TSF × LS by site and year.

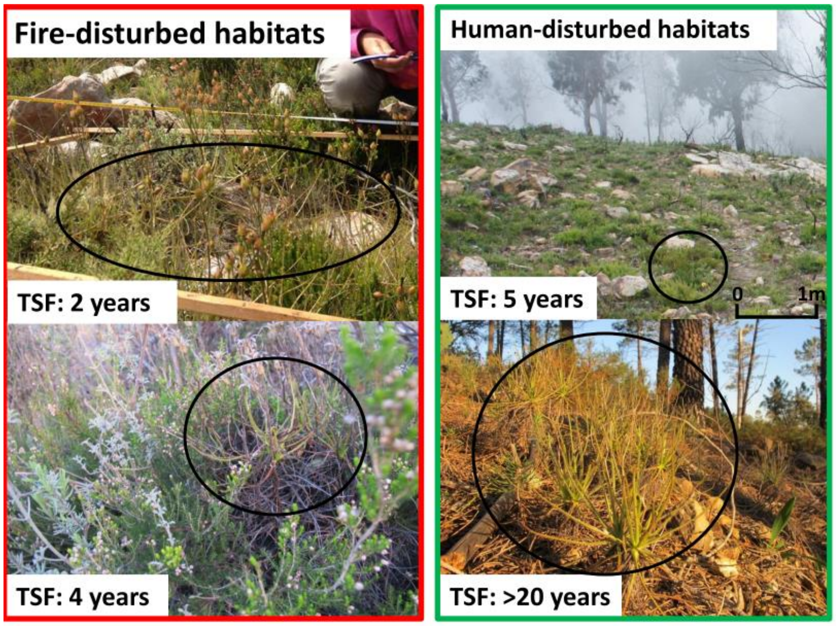


Figure S1.1 Fire-disturbed (red) and human-disturbed (green) habitats in different time-since-fire (TSF) states. In fire-disturbed habitats, *Drosophyllum* plants (circled) germinate, stimulated by heat, and reproduce, replenishing the seed bank, in the first years after fires. Germination events are large. The upper-left photo shows M. Paniw censusing > 200 individuals in 1 m^2. As soon as four years after fires, above-ground *Drosophyllum* individual are outcompeted by shrubs. In human disturbed habitats, open space for recruitment is maintained, and plants germinate into these open microhabitats annually, regardless of TSF.

Table S1.1 Time-since-fire (TSF, different colors) and livestock pressure [LS; either high (white background) or low (grey background) pressure] of the 8 populations monitored at each site-year combination. Populations that burned in a given year (September), were censused the following April at TSF$_1$

	Site A	Site B	Site C	Site D	Site E	Site F	Site G	Site H
2011	**TSF$_{>3}$**	**TSF$_{>3}$**	**burned**	**TSF$_2$**	**TSF$_1$**	**NA**	**TSF$_3$/ TSF$_{>3}$**	**TSF$_2$**
2012	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_1$**	**TSF$_3$**	**TSF$_2$**	**NA**	**TSF$_{>3}$**	**TSF$_3$**
2013	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_2$**	**TSF$_{>3}$**	**TSF$_3$**	**burned**	**TSF$_{>3}$**	**TSF$_{>3}$**
2014	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_3$**	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_1$**	**TSF$_{>3}$**	**TSF$_{>3}$**
2015	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_{>3}$**	**TSF$_2$**	**TSF$_{>3}$**	**TSF$_{>3}$**

We positioned HOBO data loggers (Onset Computer Corporation, 2013) 1 m above ground within each population to record temperature (°C) and relative humidity (%) in hourly intervals from January 2013-December 2015. The data loggers confirmed lack of microclimate differences among sites and years with the exception of summer relative humidity, which was higher at two sites in 2013 (Fig. S1.2). However, no climatic differences between sites occurred in the critical spring growing season (March-May).

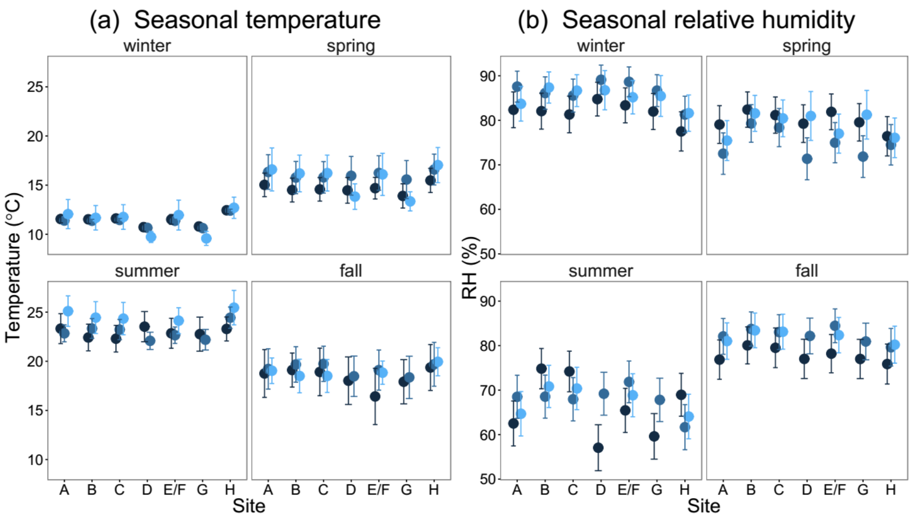


Figure S1.2 Mean (± S.E.) of seasonal temperature (a) and relative humidity (b) measured with HOBO data loggers at the eight study sites (site G is pooled across two populations) in 2013(dark blue), 2014 (medium blue) and 2015 (light blue). The two populations pooled in G were measured with the same HOBO. Sites E and F were also measured with the same HOBO.

7.8.2 Demographic censuses

We collected individual-level data in annual censuses between April 2011 and April 2015. Details of the design of the censuses can be found in Chapter 5 (Appendix S2). We quantified the size of all tagged individuals by measuring the sum of rosettes, number of leaves per rosette, and the length of the longest leaf. Individuals range in size between 1-17 rosettes, and each rosette contains ~ 14 leaves (11.8 ± 5.6 cm in leaf length) but can contain up to 45 leaves. Rosettes are produced each growing season, at the onset of the rainy season (Adlassnig *et al*. 2006).

We estimate the production of viable seeds per flower from randomly collected fruits of reproductive *Drosophyllum* individuals across all populations in which plants flowered in August 2012 and July 2014. We also quantified the number of damaged flower heads (mainly from herbivory or wind) in each population and year and averaged flower damage across time-since-fire (TSF) and livestock pressure (LS) categories (Fig. S1.3).

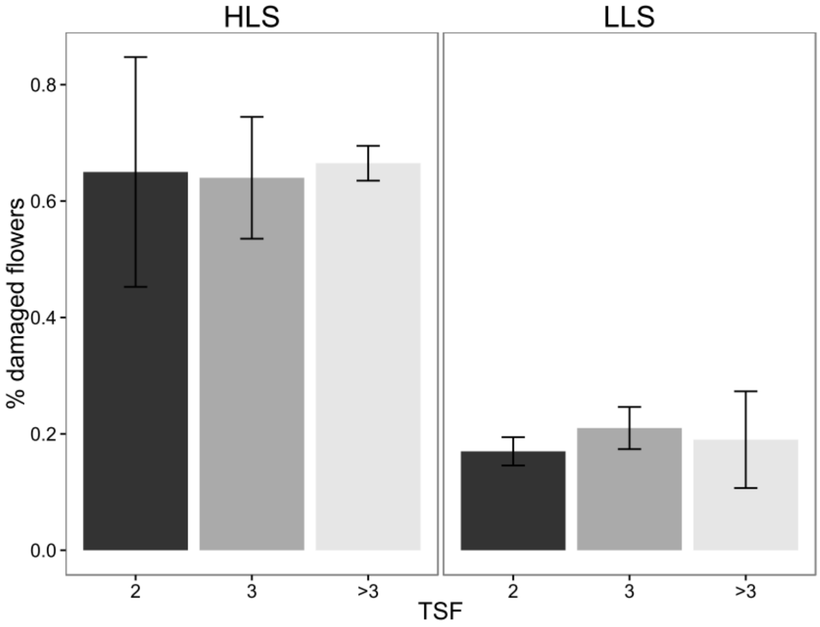


Figure S1.3 Mean (± S.E.) percentage of flowers damaged on *Drosophyllum* individuals. HLS: high livestock pressure; LLS: low livestock pressure; TSF: time since last fire (years).

7.8.3 Seed-addition experiment – immediate and seed-bank germination

We quantified immediate germination/seedling recruitment (*goCont* in the main text) and recruitment from the seed bank (*outSB* in the main text) from an *in-situ* experiment initially conducted at a natural-heathland site (close to site C; 36° 12' 16" N, 5° 21' 39" W) in August 2012 and repeated in August 2013. The experimental design is described in detail in Appendix S2 of Chapter 5. Briefly, we established seven randomized paired blocks with two treatments in each: burned/open (last fire in the summer of 2011; 15-30 % vegetation cover) and unburned/covered (last fire in the summer of 2005; > 75 % vegetation cover) patches. We sowed seeds in each patch type and recorded the number of seedlings in the spring (April) following sowing. Seeds that did not germinate in the following growing season, eight months after sowing, were considered the input into the seed bank. Seeds that germinated in the second growing season, 20 months after sowing, were considered germination out of the seed bank. In 2014 and 2015, we recorded seedling emergence in winter (February), in order to estimate the mortality of newly emerged seedlings from emergence to establishment in April (Fig. S1.4).

7.8.4 Estimating correction parameters for seed and seedling survival between two censuses

We used the information on flower damage (Fig. S1.3) to estimate the parameter σ_S = (1 – flower damage) depicting proportion of seeds surviving above ground. The σ_S parameters in HLS habitats were estimated to be σ_S = 0.35, 0.35, and 0.37 in TSF 2, 3, and >3, respectively. The equivalent estimates in LLS habitats were σ_S = 0.18, 0.20, and 0.18 (Table S.1.3 below). We used our estimates of seedling mortality before establishment to define seed-bank ingression as 1- *goCont* - ω_S, where ω_S = mortality in the seedling stage (Fig. S1.4). We averaged ω_S across all treatments so that this rate corresponded to 0.03 in all models.

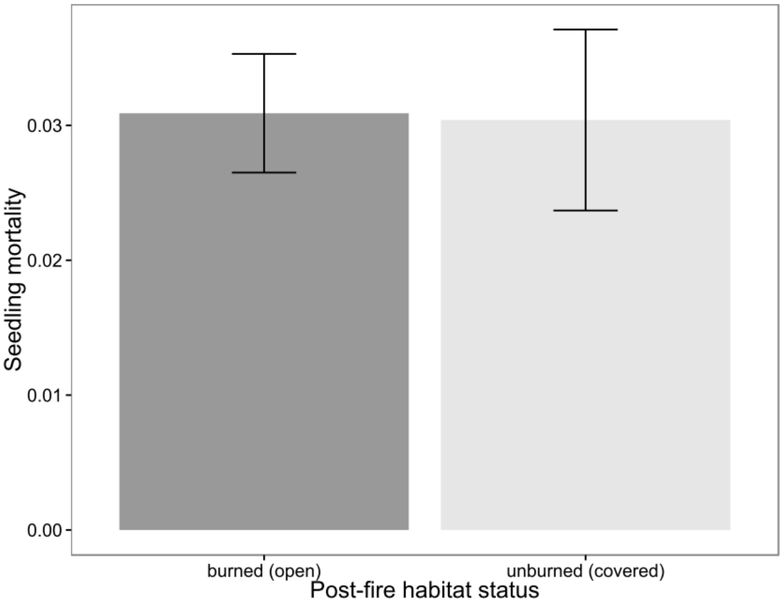


Figure S1.4 Mean (± S.E.) mortality of 50 sowed seeds as seedlings (before establishment as recruits) as function of burned and unburned habitat patches and two years in which mortality was measured.

During our censuses, we likely overestimated seedling germination in long-unburned populations under low livestock pressure due to a biased effort to find census locations of *Drosophyllum* populations. That is, we censused long-unburned heathlands where we spotted *Drosophyllum* plants, even if few and in declining populations. These plants likely persisted due to favorable microhabitat conditions that allowed for germination in the absence of fire; but these conditions are not presentative of the majority of long-unburned habitats, where populations persist only in the seed bank (M. Paniw, pers. obs.). As a consequence, we multiplied *goCont* and *outSB* by 0.2 in $TSF_{>3}$ to approximate the lowest germination rates we observed in the seed burial experiments, *i.e.*, mean(0.02, 0.00)=0.1

7.8.5 Growth-chamber experiment – seedling germination in response to fire cues

We estimated germination from the seed bank after fire in a growth-chamber experiments as we did not have permission to perform *in-situ* burning experiments. We applied three *fire* treatments to *Drosophyllum* seeds: (a) heating seeds at 100 ºC for 5 minutes; (b) incubating seeds in a smoke solution (see Jaeger *et al*. 1996; Moreira *et al*. 2010) for 24 h;

and (c) combining treatments (a) and (b). Treated seeds along with dry (unmanipulated) and wet (incubation in distilled water) controls were placed in 10 petri dishes on moist filter paper (25 seeds per dish) and incubated for 3 months at 20 (± 2) ºC in darkness. Germination was checked every 2-3 days the first 1.5 months and twice a week thereafter. We quantified germination from the seed bank after fire (TSF_0, see main text) as 0.81 which corresponded to germination results from the *fire × heat* treatment, multiplied by the probability of seedling establishment, which we quantified as 0.84 and 0.45 corresponding to the proportion of emerged seedlings in February that survived to April in covered and open habitat patches, respectively, from the experiment detailed above (data not shown).

7.8.6 Seed-burial experiment – survival in the seed bank

Lastly, we estimated long-term seed-bank survival or stasis (*staySB* in main text) from an *in-situ* experiment identical to the germination experiment in design and location (see Appendix S2 in Chapter 5). Seed-bank survival was measured from bags buried for 1.5 years in the soil, *i.e.*, bags buried in September 2012 and 2013 and retrieved in April 2013 and 2015, respectively. We pooled the data across the two years since we did not have enough data to model year as a random effect.

7.8.7 Soil samples – density of seeds in soil in distinct post-fire habitats

Additional seed-bank analyses conducted in the spring of 2012, in which we collected 20 random soil samples from the eight study populations and counted viable *Drosophyllum* seeds in the samples, indicated depleted seed banks in early post-fire habitats (Fig. S1.5). We therefore assumed that only a small proportion of seeds (0.05-0.1) survives in the seed bank after fire (Table S1.3)

7.8.8 Vital rates, model parameterization, and IPM construction

As the seed-addition and seed-burial experiments were performed in burned and unburned patches and not in continuous time-since fire (TSF) habitats as was the case for above-ground vital-rate transitions, we linked the results of the experiments to TSF by assigning seed dynamics from burned patches (B) to TSF_1/TSF_2 in LLS and $TSF_{>3}$ in HLS habitats, since the browsing disturbance in populations experiencing HLS mimics the effect of fire of opening space. Similarly, results from unburned patches (U) were generally assigned to

TSF_3/$TSF_{>3}$ in populations experiencing LLS (Table S1.3). The approach of linking experimental data of seed dynamics to different post-fire states of populations is common (e.g., Quintana-Ascencio et al. 2003) but it downscales parameter estimates. However, as we only defined five TSF levels, an interpolation of experimental results was rather straightforward. In addition, actual germination rates in HLS and LLS populations across TSF levels agreed with the experimental data. For example, in populations experiencing HLS, actual germination = (# recruits at t+1)/(estimated # seeds at t) calculated from demographic data was 0.02 and 0.07 in TSF_3 and $TSF_{>3}$, respectively. Similarly, in populations experiencing LLS, actual germination was 0.06 and 0.004 in TSF_3 and $TSF_{>3}$, respectively. This does not deviate too much from experimental values for *goCont* + *outSB*. Using our estimates of seed dynamics (Table S1.3), we also simulated the number of seedlings produced at each TSF category assuming different initial numbers of seeds in the seed bank. Our simulations agreed with the range of seedling numbers observed during demographic censuses (Fig. S1.6).

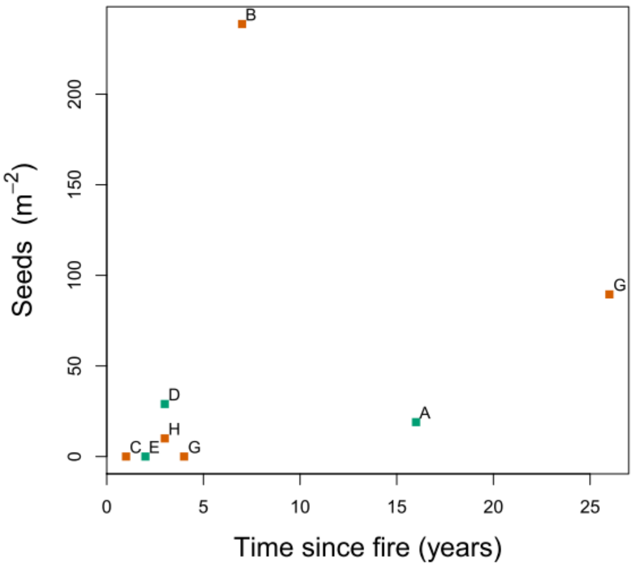


Figure S1.5 Number of seeds per m^2 (extrapolated from 20 samples in 4 × 4 cm cores) in *Drosophyllum* populations (letters) characterized by different time since fire and livestock pressure (green: high pressure; orange: low pressure). Note that parts of site G burned in 2010, and this site therefore consists of two time-since-fire states.

Table S1.2 Summary statistics of demographic data based on five years of censuses of the carnivorous plant *Drosophyllum lusitanicum* at eight sites. The mean (± S.E.) vital rates of continuous stages (above-ground biomass) are provided for habitats experiencing high (HLS; white background) and low (LLS; grey background) livestock pressure *N* is the total number of individuals available to estimate a given vital rate.

		TSF$_0$	TSF$_1$	TSF$_2$	TSF$_3$	TSF$_{>3}$
Survival (σ)	HLS	0	0.73 (0.04) N = 103	0.42 (0.03) N = 357	0.28 (0.03) N = 170	0.38 (0.02) N = 576
	LLS	0	0.63 (0.02) N = 852	0.79 (0.02) N = 558	0.20 (0.06) N = 514	0.38 (0.02) N = 349
Growth (γ)	HLS	0	0.50 (0.06) N = 75	-0.33 (0.11) N = 150	0.49 (0.14) N = 47	0.50 (0.05) N = 225
	LLS	0	2.0 (0.03) N = 541	-0.01 (0.03) N = 442	0.2 (0.06) N = 104	0.2 (0.05) N = 147
Flowering probability (φ_0)	HLS	0	0	0.24 (0.01) N = 357	0.37 (0.02) N = 170	0.12 (0.02) N= 648
	LLS	0	0	0.12 (0.01) N = 602	0.49 (0.02) N = 514	0.44 (0.02) N= 491
# flowering stalks (φ_1)	HLS	0	0	1.0 (0.01) N = 87	1.2 (0.06) N = 63	1.2 (0.06) N = 94
	LLS	0	0	1.5 (0.23) N = 71	1.8 (0.14) N = 254	1.8 (0.11) N = 205
# flowers/stalk (φ_2)	HLS	0	0	4.3 (0.29) N = 85	2.9 (0.32) N = 63	4.0 (0.20) N = 94
	LLS	0	0	6.1 (0.36) N = 71	3.4 (0.1) N = 254	2.9 (0.1) N = 205
# seeds/flower (φ_3)	HLS	0	0	9.8	9.8	9.8
	LLS	0	0	9.8	9.8	9.8
Seedling size (φ_4)	HLS	4.5 (0.02) N = 103	4.5 (0.02) N = 103	3.7 (0.16) N = 20	3.4 (0.08) N = 18	3.6 (0.03) N = 432
	LLS	3.04 (0.02) N = 849	3.04 (0.02) N = 849	4.03 (0.16) N = 13	3.76 (0.08) N = 37	3.72 (0.03) N = 203

Table S1.3 Extrapolation of seed-related vital rates calculated from field experiments to time since fire (*TSF*) categories and high (HLS) or low (LLS) livestock browsing pressure used to build integral projection models (IPMs) for *Drosophyllum lusitanicum*. The four vital rates estimated in *burned/open* (B) and *unburned/covered* (U) heathland patches (see methods) were modeled as binomial functions (Table 1); Constant values (†) of vital rates were obtained from soil seed bank cencuses (*staySB* in $\mathrm{TSF}_{0,1}$), a greenhouse germination trial (*outSB* in TSF_{0}), measurements of seedling mortality (*goSB* in $\mathrm{TSF}_{2,3,>3}$), or censuses of actual field germination (c; see Appendix S1 for details); σ_S is seed survival in $\mathrm{TSF}_{2,3,>3}$ and high (H) and low (L) livestock pressure (see main text).

		TSF_0	TSF_1	TSF_2	TSF_3	$\mathrm{TSF}_{>3}$
Immediate germination (*goCont*)	HLS	0	0	σ_{S2H} × *goCont*_U	σ_{S3H} × *goCont*_B	$\sigma_{S>3H}$ × *goCont*_B
	LLS	0	0	σ_{S2L} × *goCont*_U	σ_{S3L} × *goCont*_U	$\sigma_{S>3L}$ × *goCont*_U × c†
				R=14	R=14	R=14
Ingression into seed bank (*goSB*)	HLS	0	0	σ_{S2H} × (1-*goCont*_U-0.03†)	σ_{S3H} × (1-*goCont*_B-0.03†)	$\sigma_{S>3H}$ × (1-*goCont*_B-0.03†)
	LLS	0	0	σ_{S2L} × (1-*goCont*_U-0.03†)	σ_{S3L} × (1-*goCont*_U-0.03†)	$\sigma_{S>3L}$ × (1-*goCont*_U-0.03†)
Stasis in seed bank (*staySB*)	HLS	0.1†	0.05†	*staySB*_B	*staySB*_B	*staySB*_B
	LLS	0.1†	0.05†	*staySB*_B	*staySB*_U	*staySB*_U
				R=49	R=105	R=105
Egression from seed bank (*outSB*)	HLS	0.36†	*outSB*_B× 0.45	*outSB*_U	*outSB*_B	*outSB*_B
	LLS	0.68†	*outSB*_B× 0.84	*outSB*_U	*outSB*_U	*outSB*_U × c†
			R=21	R=21	R=21	R=21

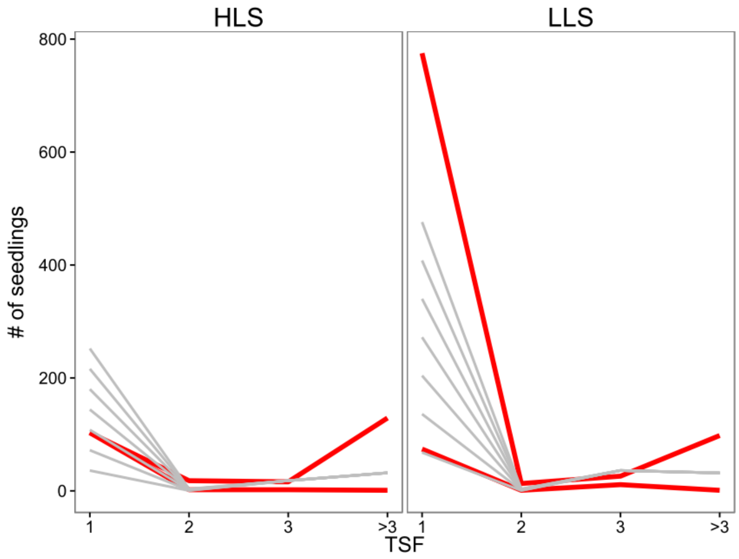


Figure S1.6 Observed (red) and simulated (grey) range of number of seedlings as a function of time-since-fire (TSF) and livestock pressure (LS; two panels, high pressure, HLS, and little pressure, LLS) categories. Simulations began with a vector of seeds in the seed bank (100-700, in intervals of 100) at TSF_0. The seeds progressed through TSF states. In each state, seed fate was determined by multiplying the seeds numbers by the rates of *goCont*, *goSB*, *staySB*, and *outSB* corresponding to each TSF (see Table S1.1).

We fitted all vital-rate models in a Bayesian framework, comparing model fit using DIC, which is a Bayesian modeling equivalent of the AIC measure used in frequentist statistics (Spiegelhalter *et al*. 2002). Differences of ≥ -5 in DIC suggest substantially better fit of the best model compared to the second best model with fewer parameters. With the exception of number of seeds per flower (φ_3), which was treated as a constant as it was not predicted by size of reproductive individuals (Likelihood ratio test, D = 1.4, *d.f.* = 1), we defined several candidate models for the remaining above-ground vital rates (σ, γ, φ_0, φ_1, φ_2, and φ_4; Table S1.2; Fig. 1 in main text):

(a) intercept-only model,

(b) including only *size* as predictor,

(c) including *size* + *TSF* as predictors,

(d) including *size* + *TSF* + *LS* as predictors,

(e) including *size* + *TSF* + *LS* + *TSF* × *LS* as predictors,

(f) including *size* + *TSF* + *LS* + *TSF* × *LS* + *size* × *TSF* as predictors,

(g) including *size* + *TSF* + *LS* + *TSF* × *LS* + *size* × *TSF* + *size* × *LS* as predictors.

No data were available to link seedling size (φ_4) in time *t*+1 to parent size in *t*, and we therefore excluded the size variable from all candidate models for this vital rate. Growth and seedling size were assumed to be normally distributed (Table S1.2). We tested the homoscedasticity (h) assumption of the variance component (τ) of these normal distributions by fitting log(τ) as functions of (i) *TSF*, (ii) *TSF* + *LS*, and (iii) *TSF* × *LS*.

For below-ground, seed-related vital rates, candidate models were an intercept-only model and a model including the post-fire status of experimental patches, *PFS*, as predictor. The random *site* and *block* effects were included in all candidate models describing above- and below-ground vital rates, respectively. Ideally, both random temporal and spatial variation should have been included, but our data did not offer enough degrees of freedom, as year × site interactions already depicted variation in TSF.

The parameterization of the vital-rate models within the Bayesian framework using OpenBUGS (*i.e.*, `vitalRateModels.R`) followed a standard form. For example, the likelihood function of survival was described as:

```
for ( j in 1:NtotalSURV ) {

surv[j] ~ dbern( mu.surv[j] )

mu.surv[j] <- 1/(1+exp(-( a0.surv + a1.surv[TSF.surv[j]] +
a2.surv[LS.surv[j]] + a1a2.surv[TSF.surv[j],LS.surv[j]] +
(bc.surv + bcTSF.surv[TSF.surv[j]] + bcLS.surv[LS.surv[j]]) *
size.surv[j] + aS.surv[site.surv[j]])))

}
```

This means that for each data record, *j*, where survival (either 0 or 1) is known for `NtotalSURV` individuals, probability of survival, `surv[j]`, is estimated as a Bernoulli distribution, `dbern`. The parameter describing the shape of the distribution, `mu.surv[j]`, is a function, with associated parameters, of the categorical variables time since fire, `a1.surv[TSF.surv[j]]`, livestock pressure , `a2.surv[LS.surv[j]]`, their interaction, `a1a2.surv[TSF.surv[j],LS.surv[j]]`, and site, `aS.surv[site.surv[j]]`. Each individual *j* is associated with one level of TSF (`TSF.surv[j]`), LS (`LS.surv[j]`), and site (`site.surv[j]`). In addition, survival is described by a slope, `bc.surv`, associated with the continuous variable size, `size.surv[j]`, the interaction of TSF and size, `bcTSF.surv[TSF.surv[j]] * size.surv[j]`, and the interaction of LS and size, `bcLS.surv[LS.surv[j]] * size.surv[j]`. The parameter `a0.surv` describes the overall mean of the survival data.

To make the models identifiable, we used the sum-to-zero constraint (Kaufman and Sain 2010) on all categorical variables. This constrains the difference between the model mean, α_0, and the parameters at each level of a categorical variable, e.g., $\alpha_{1[\mathrm{TSF}]}$, to sum to zero. In addition, we *z*-transformed the continuous state variable size ($\mu = 0$; $\theta = 1$) in the likelihood functions describing survival (σ) and probability of flowering (φ_0) to facilitate convergence of the three chains (Kruschke 2010).

We used normal uninformative priors ($\mu = 0$; $1/\theta^2 = 1\times10^{-6}$) for most fixed factors and for the Gamma-distributed rate parameters, ρ, of the Poisson-Gamma mixture models for the number of flowering stalks (φ_1) and number of flowers per stalk (φ_2). The τ parameters describing the standard deviation in the growth (γ) and seedling-size (φ_3) likelihood functions were modeled as functions of TSF× LS and TSF only, respectively. We used hyperpriors for the random site and block effects. The hyperpriors were defined as a normal distribution $\mathrm{N}(0, \tau)$ in which the precision, τ to be estimated using the prior $\sigma \sim \mathrm{unif}(0, 100)$ for the linear and $\sigma \sim \mathrm{unif}(0, 10)$ for the logistic regressions, respectively.

7.8.9 Bayesian diagnostics

The best-fit models showed a good fit to the data (Figs. S1.7 & S1.8).

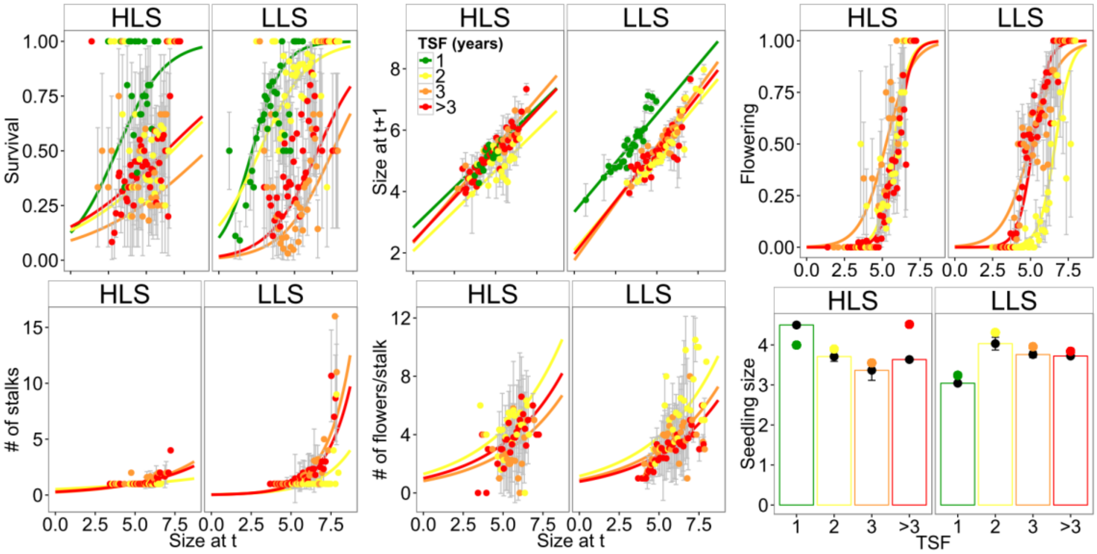


Figure S1.7 Predictions of vital rates (lines**;** points for seedling size) describing population dynamics of the continuous state (above-ground individuals) in the life cycle of the carnivorous plant *Drosophyllum* as a function of time-since-fire (TSF; different colors of lines/points) and livestock pressure (LS; two panels, high pressure, HLS, and little pressure, LLS) categories. Predictions were obtained with a Bayesian model. The points (bars for seedling size) represent mean observed values (± S.E.) of the response variables for each TSF category within certain size ranges (0.1–9.0 at interval of 0.1) at *t* for display purposes.

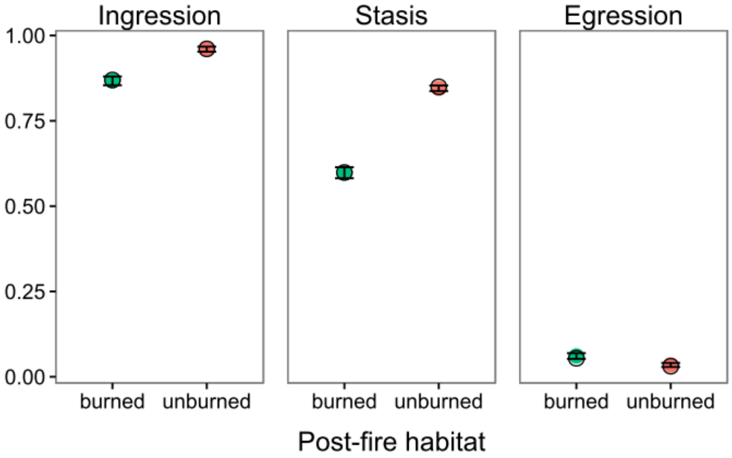


Figure S1.8 Predictions of vital rates (black hollow points) describing seed-bank dynamics in burned (open) and unburned (covered) patches where seed-addition and seed-burial experiments were performed. Predictions were obtained with a Bayesian model. The filled covered points represent observed values (± S.E.). The parameters depict ingression into (*goSB* = 1 - *goCont*), stasis in (*staySB*), and egression from the seed bank (*outSB*).

We assessed convergence of the chains within the Bayesian framework in several ways: using trace plots and the Gelman-Rubin-Brooks diagnostic of convergence (Brooks and Gelman 1998), plotting priors *vs*. posteriors, and performing posterior predictive checks as described in Kéry (2010, p. 247). Fig. S1.9 shows an example plot of a predictive check for survival (σ). Bayesian *p*-values ≈ 0.5 indicate good model performance (Kéry 2010).

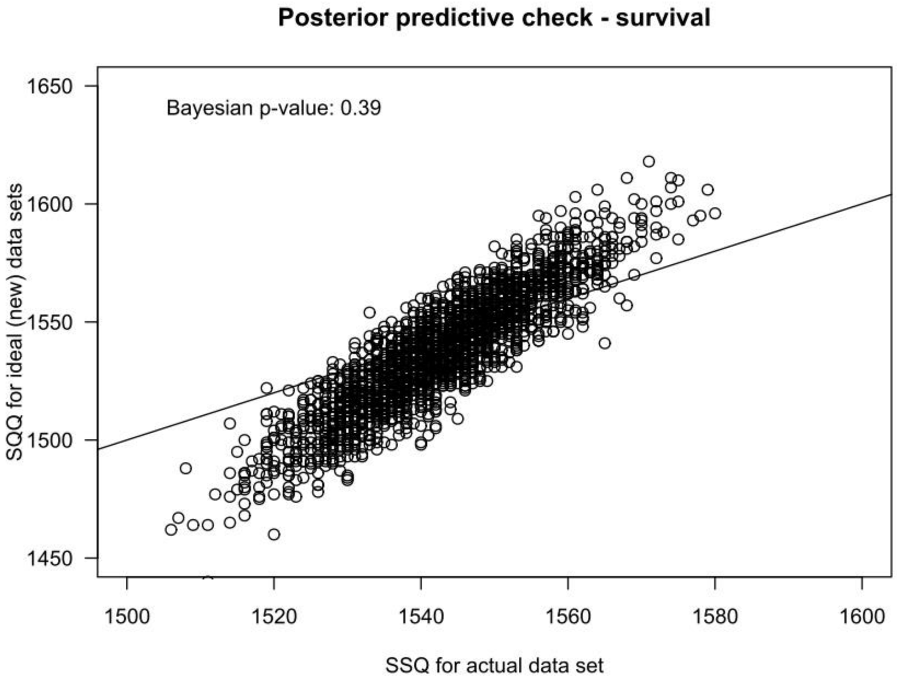


Figure S1.9 Graphical posterior predictive check of the model adequacy for the vital rate survival (σ). The Bayesian *p*-value is equal to the proportion of symbols above the 1:1 line.

7.8.10 References

Adlassnig W, Peroutka M, Eder G, Pois W, Lichtscheidl IK. 2006. Ecophysiological observations on *Drosophyllum lusitanicum*. *Ecological Research* **21**:255–262.

Brooks SP, Gelman, A. 1998. General methods for monitoring convergence of iterative simulations. *Journal of Computational and Graphical Statistics* **7**:434–455.

Jäger AK, Light ME, Van Staden J. 1996 Effects of source of plant material and temperature on the production of smoke extracts that promote germination of light-sensitive lettuce seeds. *Environmental and Experimental Botany* **36**:421-429.

Kéry M. 2010. *Introduction to WinBUGS for Ecologists: A Bayesian Approach to Regression, ANOVA, Mixed Models and Related Analyses*. Academic Press.

Moreira B, Tormo J, Estrelles E, Pausas JG. 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany* **105***:*627-635.

Onset Computer Corporation. 2013. HOBO Data Loggers. Madrid.

Spiegelhalter DJ, Best NG, Carlin BP, Van Der Linde A. 2002. Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* **64**:583-639.

7.9 Appendix S2 - Overview of the R code provided in the manuscript

Here, we provide an overview of the R scripts and data files to accompany the main text. The .R files should be opened with an R editor (e.g., R Studio). The R code is fully commented and intended to replicate the analyses used in the manuscript. To understand variable names, the reader is referred to the main text itself. Please note that the R code focuses largely on the analyses of stochastic elasticity and therefore uses the average values of the posterior samples of vital-rate parameters. For code to simulate population dynamics for each parameter sample separately and calculate contribution of parameter uncertainty to the variance in the stochastic population growth rate, the reader may consult the appendices in Chapter 5. All files can be found on a CD attached to the back cover and at https://github.com/MariaPaniw/Drosophyllum-Population-Models.

Data files:

dataDroso.csv: Demographic transitions of *Drosophyllum lusitanicum* populations recorded in five annual censuses (from 2011to 2015) in eight populations differing in time-since-fire (TSF) and livestock browsing (LS) in their habitats. These data are used to quantify vital rates of above-ground individuals.

dataDrosoSB.csv: Seed fates (in a binary format) inferred from two experiments. These data are used to quantify the transitions related to the seedbank.

In case the reader wishes to forego the step of fitting the Bayesian models, which can be very time consuming (> 24 h on 3.40 GHz processor), we provided a *mcmcOUT.csv* file with 600 posterior parameter values for each of the parameters estimated with Bayesian models using uninformative priors.

R code:

`vitalRateModels.R`: Executes and saves the results of a Bayesian model quantifying all vital rates using uniformed priors; illustrates basic diagnostics that can be run on the results of an MCMC run (i.e., the posterior parameter distribution) to check for model convergence and autocorrelation of the posterior samples.

`makeIPM.R`: Demonstrates how to construct IPMs including continuous (above-ground) and discrete (seedbank) transitions for parameter means of the Bayesian models; saves IPMs for each combination of TSF (n =5) × LS (n = 2) × site (n = 8) (Part A). The code also saves IPMs for each TSF × LS averaged over sites (Part B). Site-specific and average kernel transitions for the vital rate growth (γ in main text) are also saved. The IPMs in Part A are used to calculate the stochastic population growth rate log λ_s, and extinction probability, $P_q(t)$ by t = 300 years. The IPMs in parts A and B are used to calculate elasticities of log λ_s to changes in mean transitions ($\mathrm{E}_{\mathrm{a}}^{\mathrm{S\mu}}$) across environmental states (TSF and LS) and in the standard deviation of transitions ($\mathrm{E}_{\mathrm{a}}^{\mathrm{S\sigma}}$). The script is based on the supporting material in Ellner and Rees (2006), *Am. Nat.*, 167, 410-428.

`sLambdaFixedLS.R`: Runs and plots results of simulations of log λ_s and elasticities using IPMs constructed for mean parameter values. The simulations consider fire as a stochastic process in which transitions between TSF states are based on fire return interval. Transitions between LS are not considered.

`sLambdaStochLS.R`: Runs and plots results of simulations of log λ_s and elasticities using IPMs constructed for mean parameter values. Here, the growth kernel transitions are also included alongside the full IPMs. The simulations consider both fire and livestock browsing as an integrated stochastic process in which transitions between combinations of TSF × LS states are based on fire return interval and browsing management efforts.

The simulations in `sLambdaFixedLS.R` and `sLambdaStochLS.R` are based on Tuljapurkar et al. (2003), *Amer. Naturalist*, 162, 489-502.

`elastVR.R`: Runs and plots results of simulations of log λ_s and elasticities using IPMs constructed for lower vital rate survival (σ). The simulations consider fire as a stochastic process in which transitions between TSF states are based on fire return interval. Transitions between LS are not considered.

7.10 Appendix S3 - Additional modeling results

Here, we present box plots showing the distributions of log λ_s when stochastic simulations of time-since-fire (TSF) and livestock pressure (LS) habitat states included parameter uncertainty or when simulations were performed using mean parameter estimates (that is, variance of log λ_s was only due to process variability in the stochastic simulations). By fitting vital-rate regressions in a Bayesian framework, we obtained parameter uncertainty from MCMC posterior parameter samples. Contribution of parameter uncertainty to overall variance of log λ_s was calculated as described in Evans *et al*. (2010) and Chapter 5. That is, we were able to partition the variation in log λ_s estimates according to parameter uncertainty and process variability by fitting a GLMM to the estimates of log λ_s treating the posterior parameters as a random effect. To test whether log λ_s estimates differed significantly from 0, confidence intervals around all simulated distributions were obtained from the 2.5 and 97.5 quantiles of the raw distributions.

We also explain how elasticities of log λ_s to changes in vital rates were calculated. We then show plots of summed elasticities of log λ_s to changes in the mean (E_a^{μ}) and standard deviation (E_a^{σ}) of lower-level vital rates when stochastic simulations assumed transition probabilities between LS states.

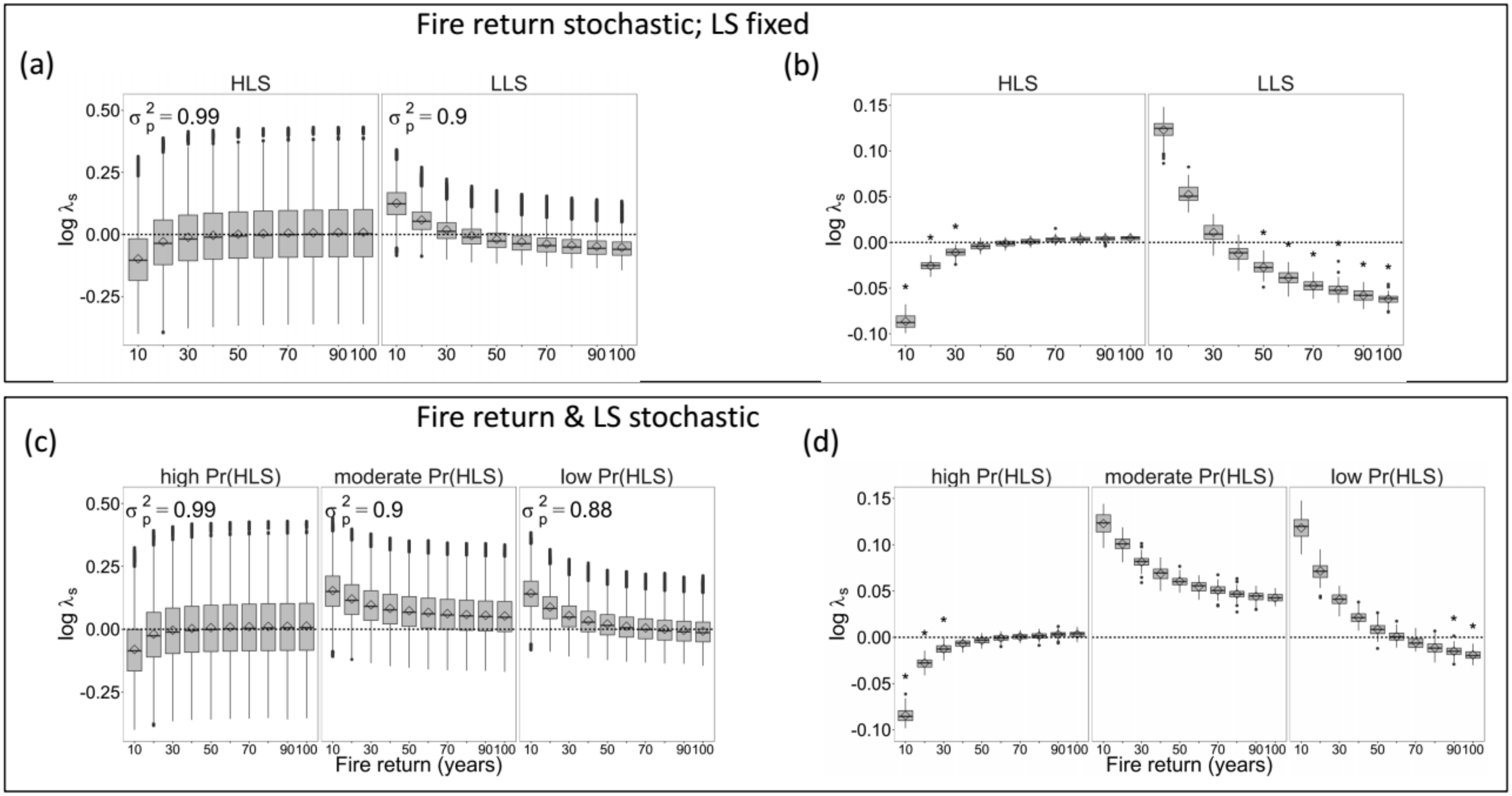


Figure S3.1 Variation in simulated stochastic population growth rate estimates (log λ_s). Box-and-whisker plots show the distribution of log λ_s as function of fire return interval (*x*-axis) and different assumptions of LS transitions: fixed high (HLS) and low (LLS) livestock pressures in habitats (1st box) or decreasing probability (Pr) of HLS (2nd box). At each fire return interval, log λ_s consist either of 60,000 estimates based on 100 stochastic simulations run using IPMs constructed from each of 600 posterior parameter samples (a, c); or 100 estimates based on stochastic simulations using mean parameter values (b, d). Diamonds indicate the mean estimate of log λ_s. The black horizontal dashed lines indicate stable population sizes. “*” indicate the 95 % non-parametric CI around the mean of log $\lambda_s < 0$.

7.10.1 Elasticities calculations for lower-level vital rates

Elasticities of a = log λ_s to IPM kernel transitions *ij* can be calculated using the formula derived by Tuljapurkar (1990) and Tuljapurkar *et al*. (2003):

$$\mathrm{E_{aij}} = \lim_{\mathrm{T}\to\infty} \left(\frac{1}{\mathrm{T}}\right) \sum_{\mathrm{t=1}}^{\mathrm{T\text{-}1}} \frac{\mathrm{V_i(t)'C_{ij}(t)U_j(t\text{-}1)}}{\lambda(\mathrm{t})\langle \mathrm{V(t)'U(t)} \rangle} \qquad \text{(F.1)}$$

where **V** and **U** are the left and right eigenvectors associated with λ at each iteration *t* and *t*-1, respectively, and $\mathrm{C_{ij}(t)}$ denotes the IPM of proportional changes in entries *ij*. Elasticities to changes in mean transitions can be calculated by defining $\mathrm{C_{ij}(t)} = \mu_{\mathrm{ij}}$, where μ_{ij} is the average transition *ij* across a sequence of environmental states during simulations. Similarly, elasticities to changes in the standard deviation of transitions can be calculated by defining $\mathrm{C_{ij}(t)} = \mathrm{K_{ij}(t)}\text{-}\mu_{\mathrm{ij}}$, where $\mathrm{K_{ij}}$ (t) is the IPM kernel values for transition *ij* at time *t*.

The perturbation kernel $\mathrm{C(t)}$ can be derived from lower-level vital rates using the chain rule (Haridas and Tuljapurkar 2005). At each iteration *t*, elasticities of log λ_s to changes in the mean of vital rates were calculated by perturbing the predicted values of each vital-rate model VR_j(t) to VR_j(t) + $\overline{\mathrm{VR_J}}$, where $\overline{\mathrm{VR_J}}$ are the predicted values of the average model describing VR_j across environmental states. The resulting changes in the kernel $\mathrm{C(t)}$ were substituted into equation F1. For elasticities of log λ_s to changes in the standard deviation of a vital rate, the vital rate VR_j(t) would be perturbed from VR_j(t) to VR_j(t) + VR_j(t) - $\overline{\mathrm{VR_J(t)}}$. The script `elastVR.R` in Appendix S2 shows how to perform the elasticity calculations for survival (σ). Figure S3.2 shows the elasticity results for simulations including transitions in LS states (see main text). Note that in the main text (Figure 4) and the figure below, $\mathrm{E_a^{\mu}}$ and $\mathrm{E_a^{\sigma}}$ were summed across all IPM kernel entries containing a given vital rate.

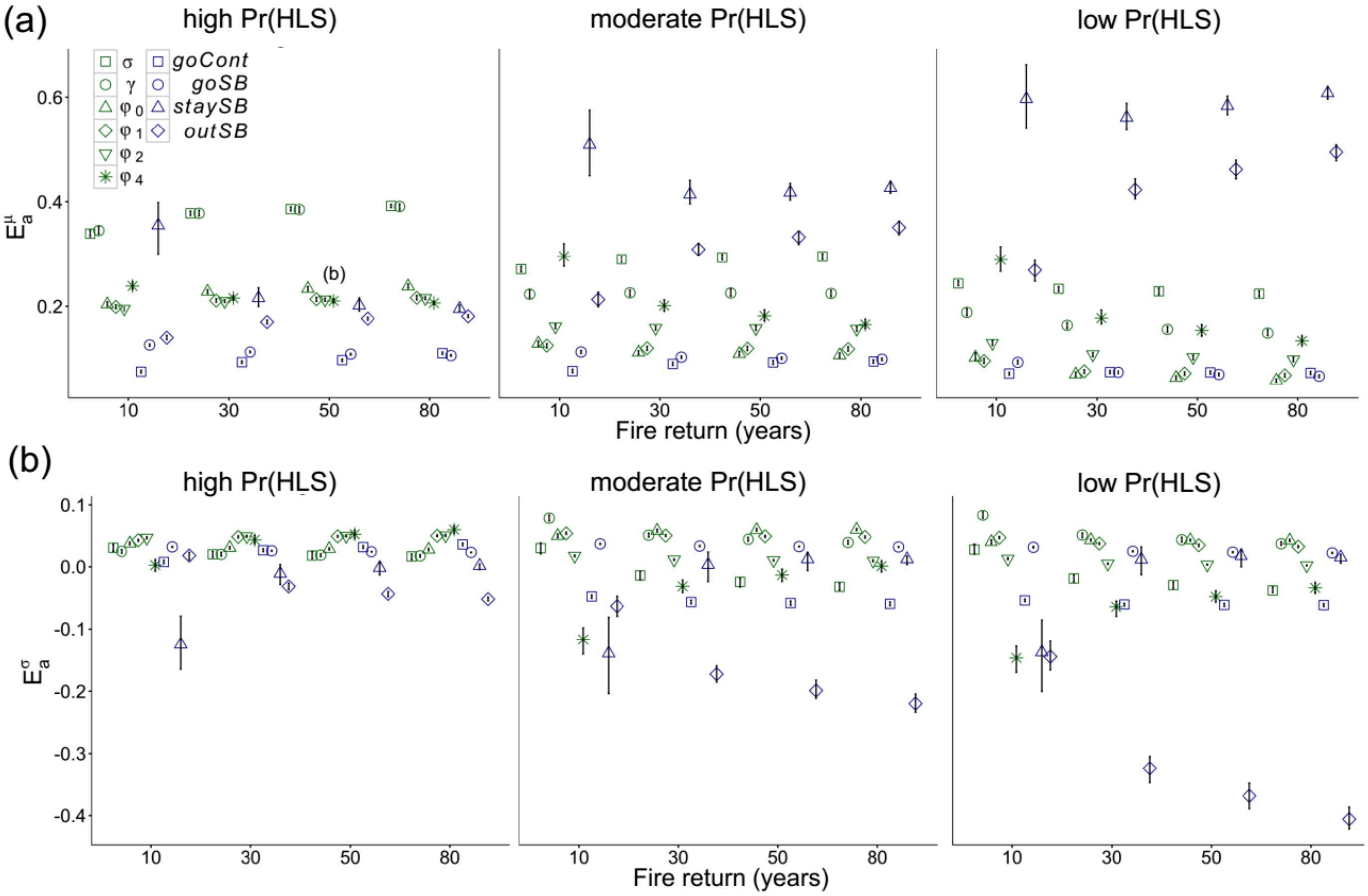


Figure S3.2 Elasticities of log λ_s to changes in (a) the average (E_a^μ) and (b) standard deviation (E_a^σ) of 10 vital rates obtained from stochastic simulations that assumed stochastic transitions of time-since-fire and livestock pressure. Stochastic transitions were based on four fire return intervals and different probabilities of high livestock pressure (HLS). Vital rates denoted by green and blue colors represent above-ground processes [survival (σ), growth (γ, φ_4), and reproduction (φ_0, φ_1, φ_2)] and seed fates [immediate germination (*goCont*) and seed-bank ingression (*goSB*), stasis (*staySB*) and egression (*outSB*)], respectively. Error bars show a 95 % non-parametric CI calculated from 100 simulations at each fire return.

CHAPTER 8

Transient facilitation of resprouting shrubs in fire-prone habitats

CHAPTER 8

Transient facilitation of resprouting shrubs in fire-prone habitats

Maria Paniw, Roberto Salguero-Gómez, and Fernando Ojeda

This chapter is under review in *Journal of Plant Ecology*.

8.1 Abstract

Fires play a crucial role mediating species interactions in the Mediterranean Basin, with one prominent example being the nursing effect of post-fire resprouting shrubs on tree recruits, which then outcompete their benefactors throughout succession. Yet, the community structuring role of resprouting shrubs as potential facilitators of post-fire recruiting subshrub species, which are commonly outcompeted in late post-fire stages, has been overlooked. The aims of this work were to investigate (i) whether proximity to resprouting shrubs increased the demographic performance of a fire-adapted carnivorous subshrub and (ii) whether mature shrubs negatively affected the performance of established plants through interference with prey capture. To evaluate the facilitative effects of resprouting shrubs, we sowed seeds of *Drosophyllum lusitanicum*, a carnivorous, seeder pyrophyte, into two microhabitats in recently burned heathland patches defined by proximity to resprouting shrubs. We monitored key demographic rates of emerged seedlings for two years. To test for competitive effects of mature shrubs on plant performance, we placed greenhouse-reared, potted plants into distinct microhabitats in neighboring burned and unburned heathland patches, the latter dominated by mature shrubs, and monitored prey capture. Both experiments were performed in the Aljibe Mountains at the Northern Strait of Gibraltar and were replicated in two years. Resprouting shrubs significantly improved survival, juvenile size, and flowering probability compared with open microhabitats, and had no significantly negative effects on the growth of recruits. Prey capture was significantly lower in unburned heathland patches compared with burned ones. However, microhabitat did not affect prey capture. Our findings suggest that not only periodic fires, removing biomass in mature stands, but also resprouting neighbors, increasing establishment success after fires, may be important for the viability of early successional pyrophytes.

Keywords: competition, *Drosophyllum lusitanicum*, early successional species, habitat succession, Mediterranean heathlands, pyrophyte

8.2 Introduction

Species interactions such as facilitation and competition drive biodiversity and community composition (Bertness and Callaway 1994; Holmgren *et al.* 1997; Wright *et al.* 2014). The prevalence and importance of facilitation over competition in natural communities, meanwhile, have been linked to increases in abiotic stress such as drought or frost (Bertness and Callaway1994; but see Holmgren and Scheffer 2010), where facilitation has been shown to increase biodiversity (He *et al.* 2013; Soliveres *et al.* 2015). Mediterranean shrublands are diverse ecosystems where seasonal drought typically constitutes a severe, periodic stress (Lloret *et al.* 2004; Moreno *et al.* 2011). In the Mediterranean Basin, shrub species resilient to droughts (Zeppel *et al.* 2015) may increase the survival of tree and shrub seedlings recruiting under or nearby them (Raffaele and Veblen 1998; Gómez-Aparicio *et al.* 2004). Facilitation in this setting happens via the increased soil moisture and/or nutrient content, low evapotranspiration, protection from ultraviolet radiation or herbivory, and/or buffering against high temperatures and winds provided by the nursing plant (Callaway 1995; Baraza *et al.* 2006; He *et al.* 2013).

Fires play a key role in mediating facilitative plant interactions in Mediterranean heathlands (Keeley *et al.* 2012). In early post-fire stages, where the effects of drought stress are most pronounced (Peñuelas *et al.* 2007), plant diversity is also highest (Ojeda *et al.* 1996; Keeley *et al*., 2012). This diversity is largely driven by a high abundance of post-fire recruiting plant species, usually short-lived herbs and subshrubs, which disappear as shrub cover increases with time since fire (Ojeda *et al.* 1996; Calvo *et al.* 2002; Yates and Ladd 2010). It has been assumed that, after fire, resprouting, drought-resilient shrubs may act as nurse plants for seedlings of short-lived, post-fire dwelling subshrub species (Verdú *et al*. 2009), but this assumption has rarely been investigated experimentally (He *et al*. 2013).

The question of whether growing under or in close proximity to resprouting shrubs improves the performance of a short-lived, post-fire dweller is complicated by the fact that such facilitative interactions change with habitat succession. With shrub maturing typically within 3-4 years after fires in Mediterranean heathlands (Calvo *et al.* 2002; Céspedes *et al.* 2014), facilitation by resprouting shrubs may eventually turn into competition for light and resources against smaller post-fire recruiting subshrubs (Vilà and Sardans 1999). They may thus be preferentially found in open patches, risking higher mortality due to adverse environmental conditions (e.g., solar radiation, wind, or drought) in early post-fire habitats but avoiding competition for longer during habitat succession. The presence of post-fire recruiting species close to resprouting shrubs may then simply be the result of propagule concentration by shrubs (Callaway 1995). To investigate the net effects of facilitation *vs.* competition, a study must span a reasonable time interval to capture habitat succession (He *et al*. 2013).

In addition, it has been repeatedly demonstrated that the choice of demographic performance estimator (e.g., survival, growth, and reproduction) by which the effects of facilitation or competition are measured may strongly affect results (Maestre *et al*. 2005; He *et al*. 2013). For example, several studies investigating facilitation under high abiotic stress in arid ecosystems have found no effect of neighbors on plants survival and growth but a strong facilitative effect on fecundity of target plants (Donovan and Richards 2000; Maestre *et al*. 2005). Therefore, studies must consider several performance estimators to gain a full picture of the effects of neighbors on the performance of a target species.

Disentangling the roles of facilitation and competition along ecological succession will shed light on the dependence of early successional species on the presence of community structuring species (Dickie *et al.* 2005). For example, selective removal of resprouting shrubs for lignotuber harvesting (Ojeda *et al.* 1996) or heathland afforestation with pines (Andrés and Ojeda 2002) may subsequently affect the success of post-fire dwelling species in fire-prone Mediterranean heathlands if resprouting shrubs act as nursing plants in early post-fire regeneration stages. To test the hypothesis that resprouting shrubs provide key facilitative services to short-lived, post-fire subshrub species, we quantified the effects of shrub neighbors on the demographic performance of the Mediterranean heathland endemic *Drosophyllum lusitanicum* (Drosophyllaceae), a short-lived, carnivorous pyrophyte (chapter 6). In order to base our results on several performance measures, we estimated seed germination as well as survival, growth, and reproduction in two microhabitats, close to shrubs and open, from a recently burned heathland patch. To account for habitat succession, we monitored the performance of emerged seedlings for two consecutive years. We also recorded trapped insects on the sticky leaves of greenhouse reared, potted young individuals placed in recently burned and neighboring unburned sites in order to test for negative effects of fully developed shrubs in mature communities via interference with nutrient acquisition (*i.e.* insect capture). In addition, we noted leaf damage on the potted plants in order to test whether shrubs protect individuals from solar radiation and/or wind.

8.3 Materials and methods

8.3.1 Study species and sites

Drosophyllum lusitanicum (L.) Link (Drosophyllaceae; *Drosophyllum* hereafter) is a short-lived, carnivorous perennial subshrub endemic to the Western Mediterranean Basin (Garrido *et al.* 2003) and tightly associated with fire-prone Mediterranean heathlands (Müller and Deil 2001). These heathland habitats are characterized by a Mediterranean climate regime and occur on highly acidic, infertile, siliceous soils (Ojeda *et al.* 2010). Their dominant vegetation consists of shrubs in the Ericaceae (*Calluna vulgaris*, *Erica australis, E. umbellata*, and *E. scoparia*) and Fabaceae families (e.g. *Stauracanthus boivini*, *Pterospartum tridentatum*, *Genista tridens*). *Drosophyllum* populations are threatened by habitat degradation (Correia and Freitas 2002; Garrido *et al.* 2003). In natural heathlands, population dynamics of this species are linked to recurrent fires, since its seed germination is stimulated by both direct (heat and smoke; chapter 5) and indirect (opening of vegetation; chapter 6) fire-related cues. Consequently, *Drosophyllum*'s highest population densities are attained during early post-fire stages, typically 1-3 years after fires (chapter 6). During such stages, emerging seedlings are typically exposed to high levels of seasonal (summer) drought stress (Adlassnig *et al.* 2006), and resprouting shrubs may thus act as nursing plants. However, germination is increasingly inhibited by mature shrubs and accumulation of ground litter in heathlands ≥ 4 years after fires; chapter 5). In addition, shrubs may interfere with insect capture as has been shown for other carnivorous plant species (Schulze *et al.* 2001), but this has not been investigated for *Drosophyllum*.

To quantify the extent of facilitation and/or competition by shrubs during post-fire habitat succession, we carried out two experiments replicated at two Mediterranean heathland sites within the Aljibe Mountains, at the northern side of the Strait of Gibraltar (Fig. 1). Parts of the two study sites burned by wildfires (see below). Natural *Drosophyllum* populations occur at both sites, but were located > 200 m away from the experimental settings.

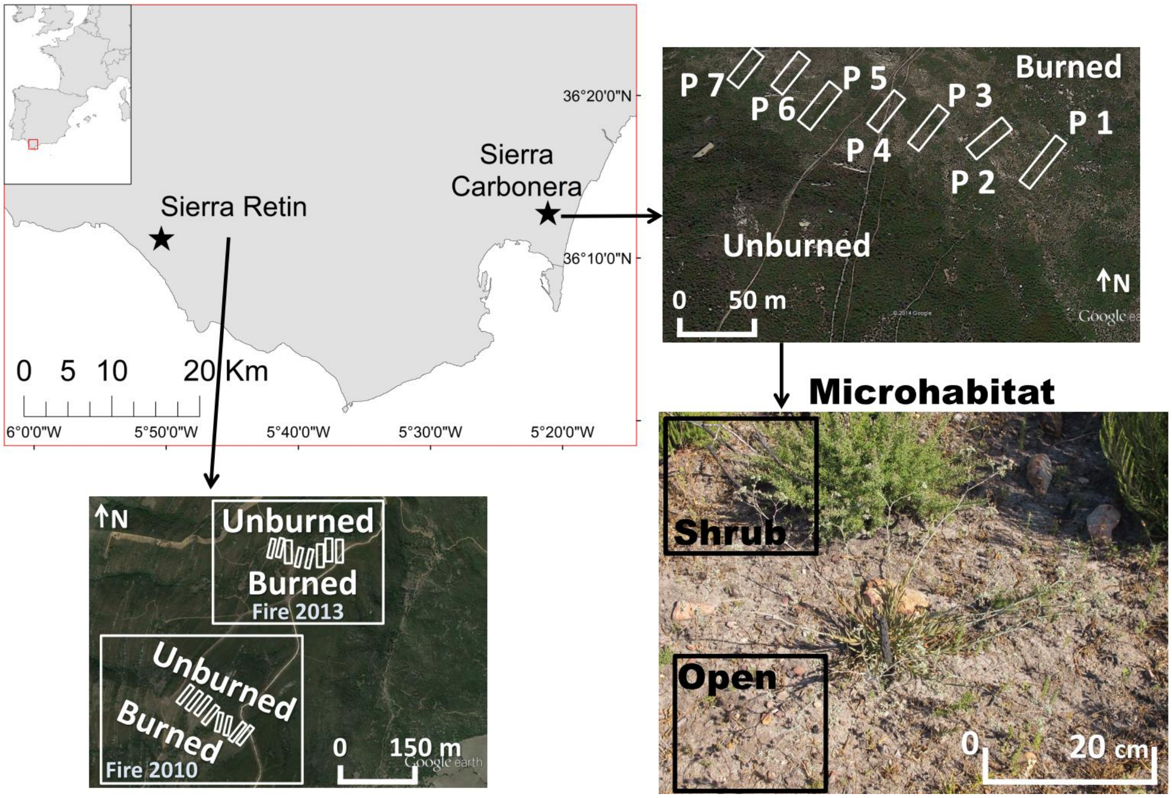


Figure 1 Location of the two sites within the Aljibe Mountains at the Northern Straits of Gibraltar (red box) used in this study and experimental design at each study site. At Sierra Carbonera, the seed-sowing experiment was designed as random plots (P) in a burned heathland patch adjacent to an unburned one. Within each plot, two microhabitats (open and close to shrub) were used. At Retin, the prey-capture experiment was designed at two sites as paired plots in adjacent burned and unburned heathland patches.

8.3.2 Seed-sowing experiment

To test the interspecific effects of resprouting shrubs on the vital rates germination, survival, growth, and reproduction of *Drosophyllum* individuals, we conducted a seed-sowing experiment at a burned heathland site (Sierra Carbonera; fire in August 2011). We established seven paired plots perpendicular to the main elevation gradient (Fig. 1) and sowed two cohorts of seeds, in August 2012 and in 2013, to track the aforementioned vital rates. The experiment did not assess germination in response to direct fire cues but rather relative germination in response to indirect cues (chapter 6) in distinct microhabitats during early stages of habitat succession. We therefore did not pretreat seeds, e.g., exposing them to heat.

In each of the seven plots, we distinguished two types of microhabitats: *open* and *shrub* (Fig. 1). We chose the most abundant shrub species in each plot as shrub microhabitat, which were either *Erica scoparia* or *Stauracanthus boivini*. Both species had similar, rounded/conical crowns and show similar growth rates after fire (M. Paniw, unpubl.), and we assumed that neighbor identity would not significantly affect our results (Correia and Freitas 2002).We chose exemplars of ≥ 20 cm crown diameter as neighbors to ensure that neighbors were large enough to potentially affect the performance of *Drosophyllum* seedlings. In each microhabitat, we sowed 50 seeds, randomly collected from >80 individuals across five *Drosophyllum* populations, in squares (20 × 20 cm^2, 0.5-1 cm deep), using one and two squares per microhabitat treatment for the 2012 and 2013 cohort, respectively. The centers of the squares were randomly positioned > 30 cm and < 20 cm away from the edge of resprouting shrubs for the open and shrub microhabitats, respectively.

In three of the seven plots, we created a control treatment by digging up soil in three 20 × 20 cm^2 squares in *open* and *shrub* microhabitats, respectively, without seed sowing so as to control for potential germination of naturally occurring *Drosophyllum* seeds after mechanical disturbance**.** In this control treatment, the *open* microhabitats were picked randomly, while *shrub* microhabitats consisted of the same shrub that was used as a neighbor for a sowing treatment (see above). We only used three plots because active seed dispersal does not occur in *Drosophyllum*, and we therefore did not expect to find naturally occurring seeds in our experimental plots (Ortega-Olivencia *et al.* 1995). We recorded the proportion of seeds that germinated and their consequent number and length (cm) of leaves in each square in April 2013 and 2014, eight months after sowing when germination rates in natural *Drosophyllum* populations are highest (chapter 6). We tagged all emerged plants and followed their fate in 6-month intervals until April of 2014 and 2015, or twenty months after sowing, for the 2012 and 2013 cohort, respectively.

8.3.3 Prey capture experiments

In order to test for any negative effects of shrub cover on established *Drosophyllum* individuals, we quantified prey-capture rates in nine-month-old potted plants placed in burned and unburned habitat patches. *Drosophyllum* individuals in this experiment were grown in clay pots under greenhouse conditions (20 ºC, 85 % humidity, and daily 50mL watering with decalcified water) at the University of Cadiz from seeds collected randomly in five natural populations from southern Spain (Fig. 1). We initially performed this experiment in early May 2013, within the growing season of natural *Drosophyllum* populations, at a heathland site in Sierra Retin (Fig. 1). The last fire occurred in August 2010 at this site. We established seven paired plots perpendicular to the main elevation gradient, each plot consisting of burned and unburned (>30 years after last fire) subplots. After another fire occurred at a different site in Sierra Retin in August 2013, we implemented the same experimental design in early May 2014 at the newly burned and an adjacent unburned (>30 years after last fire) patch (Fig. 1). Although the burned patches in 2013 and 2014 were not in the same post-fire successional stage, we grouped them into the "burned" treatment as both were in an early post-fire stage (< 3 years since fire).

Within each burned and unburned subplot for 2013 and 2014, we distinguished two microhabitats, *open* and *shrub*, using the aforementioned criteria for Sierra Carbonera. We placed two potted individuals of *Drosophyllum* free of insect prey at each microhabitat within each subplot across the 14 plots (seven per year/site). Shrub neighbors consisted of *Erica scoparia*, *Calluna vulgaris*, *Quercus lusitanica*, or *Stauracanthus boivini*. The potted *Drosophyllum* individuals were watered daily with 100 mL of decalcified water during seven days to prevent desiccation. We then took the individuals to the laboratory for quantification, size estimation and identification to at least the taxonomic order of every trapped insects per individual. To assess whether shrubs protect *Drosophyllum* individuals from physical damage, we also examined all mature leaves for visual damage such as desiccation or broken-off parts (Online Resource 1).

8.3.4 Statistical analyses

For the seed-sowing experiment, we fitted generalized mixed effect models (GLMMs) to describe each vital rate separately (seedling recruitment, seedling and juvenile survival probability, and flowering probability) as a function of microhabitat (open *vs*. shrub). We fitted GLMMs with a binomial error distribution for germination, survival, and flowering, and used a normal error distribution to model size, measured as leaf number × length of longest leaf (cm) on the logarithmic scale. Models were fitted separately for each year because we did not have enough temporal replicates to include year as a random effect.

For the prey-capture experiment, we fitted GLMMs describing number of insects and number of visibly damaged leaves as functions of post-fire habitat state (burned *vs.* unburned), microhabitat (open *vs*. shrub), and their interaction. Here, analyses were performed separately for each of the two replicates of the experiment because we could not separate the effect of year *vs*. site. We used number of leaves per plant as an offset in the models, thereby treating the two responses as proportions but allowing the models to be fit as count data in a GLMM framework. We fitted the two models using a negative binomial error distribution as simple Poisson models showed overdispersion, *i.e.*, the ratio of squared Pearson residuals and residual degrees of freedom was >1 (χ^2, $p < 0.01$; Ver Hoef and Boveng 2007).

All analyses were performed with the *lme4* package in *R* (Bates *et al.* 2014). In all models, we used plot as a random effect on the model mean. We used likelihood ratio tests to determine significant differences between treatments (Vuong 1989). These tests compare increasingly complex, or nested, models to simpler ones (starting with intercept-only models). When significant effect of microhabitat or post-fire state, we applied a *post-hoc* Tukey’s honestly significant difference (HSD) test to the linear predictors using the R package *multcomp* (Hothorn *et al*. 2008) to detect significant pairwise differences between treatment levels.

8.4 Results

8.4.1 Seed-sowing experiment

The microhabitat (open *vs*. shrub) in burned plots markedly, positively affected several vital rates *Drosophyllum* individuals, although only for seeds sowed in 2012, one year after fire (Figs. 2 and 3). Seedling survival was significantly higher when seeds were sowed close to resprouting shrubs than in the open for the 2012 cohort (χ^2 deviance = 4.6, df = 1; $p < 0.05$; Fig. 2b). On the other hand, none of the three recruitment vital rates (germination, seedling size, and seedling survival) differed significantly between open and shrub microhabitats for the 2013 cohort (Fig. 2). For the 2012 cohort, juvenile size was also significantly higher in shrub microhabitat (χ^2 deviance = 5.1, df = 1; $p < 0.05$; Fig. 3b); and recruiting plants in the shrub microhabitat had a significantly higher probability of flowering after 20 months than recruiting plants in the open (χ^2 deviance = 4.7, df = 1; $p < 0.05$; Fig. 3c). No recruiting plant from the 2013 cohort flowered 20 months after emergence, so that statistical comparison of flowering were not possible (Fig. 3c). No germination occurred in the control treatment, and we therefore excluded it from statistical analyses.

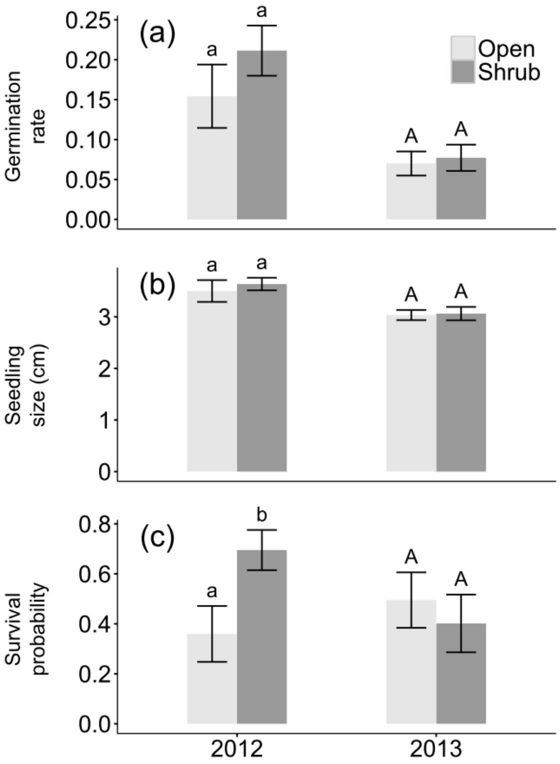


Figure 2 Effects of microhabitat (open and close to shrubs) in burned heathland patches on (a) germination rate, (b) seedling size (cm), and (c) seedling survival probability eight months following sowing of *Drosophyllum lusitanicum* seeds sown in September 2012 and 2013. Error bars indicate ± 1 SE. Different small (large) letters indicate significant differences (Tukey´s HSD, $p < 0.05$) of group means between microhabitat for the treatment in 2012 (2013, respectively).

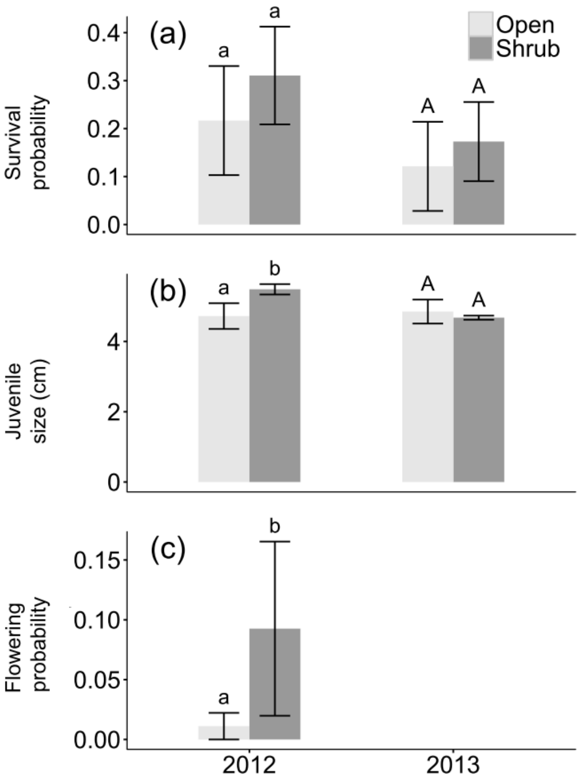


Figure 3 Effects of microhabitat (open and close to shrubs) in burned heathland patches on (a) survival probability, (b) size (cm), and (c) flowering probability of individuals 20 months following sowing of *Drosophyllum lusitanicum* seeds in September 2012 and September 2013. Error bars indicate ± 1 SE. Different small (large) letters indicate significant differences (Tukey´s HSD, $p < 0.05$) of group means between microhabitat for the treatment in 2012 (2013, respectively). Note that none of the individuals in the 2013 cohort flowered.

8.4.2 Prey capture experiments

Overall, insect capture was significantly higher in potted individuals placed in the burned subplots than in neighboring unburned ones in both years/sites in the study (2013: χ^2 deviance = 37.0, df = 1, $p < 0.01$; 2014: χ^2 deviance = 14.9, df = 1, $p < 0.01$), regardless of shrub/open microhabitat (Fig. 4a). The majority of prey consisted of flies (Diptera) of various sizes, and we did not detect a difference in prey diversity between post-fire state or microhabitat (Online Resource 2). Potted individuals showed a significant higher proportion of damaged leaves in burned subplots (2013: χ^2 deviance = 13.1, df = 1, $p < 0.01$; 2014: χ^2 deviance = 50.3, df = 1, $p < 0.01$) and, within burned subplots, in open microhabitats (2013: χ^2 deviance = 10.2, df = 1, $p < 0.01$; 2014: χ^2 deviance = 4.2, df = 1, $p < 0.05$) (Fig. 4).

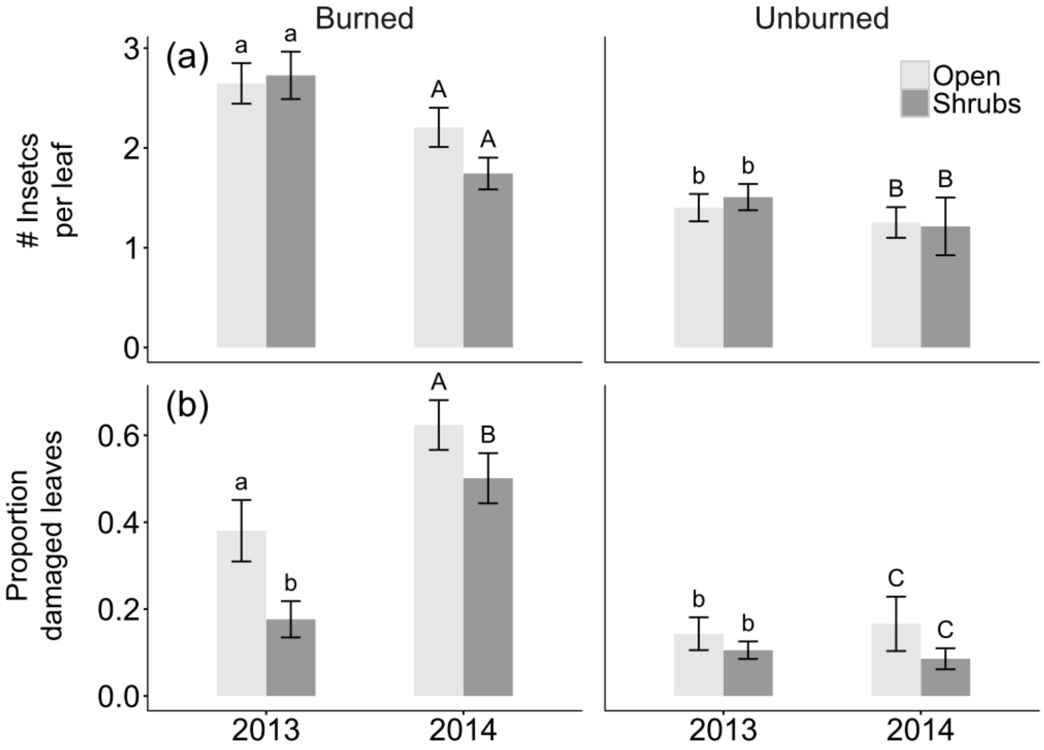


Figure 4 Effects of fire state of the habitat (burned and unburned) and microhabitat (open and close to shrubs) on (a) general insect capture and (b) proportion damaged leaves of *Drosophyllum lusitanicum* (L.) plants. Error bars indicate ± 1 SE. All tests were performed separately for both years/sites. Different small (large) letters indicate significant differences (Tukey´s HSD, $p < 0.05$) of group means between microhabitat for the treatment in 2012 (2013, respectively).

8.5 Discussion

Community structure is a driver of interactions among species that often determines their coexistence (Amarasekare 2003; Dickie *et al.* 2005; Callaway 2007) as well as the overall resistance of the community to climatic extremes (Cavieres *et al.* 2013). Fires in Mediterranean heathlands remove biomass and, while this provides post-fire recruiting species with the opportunity to colonize otherwise rather competitive habitats, it also exposes them to harsh environmental conditions typical of Mediterranean summers. Our results showed a strong yet transient facilitative effect of resprouting shrubs on the demographic performance of a short-lived, post-fire dwelling, subshrub species. We therefore provide novel evidence for the importance of resprouting shrubs on the post-fire recovery of heathland biodiversity (Sedláková and Chytrý 1999; Soliveres *et al.* 2015). Given the role of resprouting shrubs as ecosystem engineers, it is alarming that these shrub communities are being increasingly altered by habitat degradation such as active afforestation campaigns, which permanently change heathland community structure and composition by replacing shrub vegetation with trees (Andrés and Ojeda 2002).

Recruits as well as established individuals of *Drosophyllum*, the epitome of short-lived, post-fire recruiting species in Mediterranean heathlands (Andrés and Ojeda 2002; Paniw *et al.* 2015), benefit from a nursing effect of resprouting shrubs in early post-fire stages (Figs. 2 and 3). In Mediterranean ecosystems, facilitation occurs typically when the abiotic stress, which the benefactor species alleviates, constitutes a non-resource stress for interacting species, such as temperature (Maestre *et al.* 2003). If a resource stress, e.g. water or nutrients, is the main abiotic stress and the niches of interacting species overlap, a shift to facilitation is not likely (Maestre *et al.* 2003, 2009). In our case, resprouting heathland shrubs and *Drosophyllum* plants do not compete for resources since the carnivorous *Drosophyllum* obtains nutrients from prey capture and a substantial amount of water in form of dew and mist absorbed by the mucilage droplets on stalked leaf glands (Adlassning *et al.* 2006; Adamec 2009; chapter 4). In early post-fire habitats therefore, where summer drought may otherwise prove detrimental to seedling establishment and growth to reproduction, resprouting shrubs may create a favorable microhabitat for individuals by decreasing exposure to solar radiation and wind (Goméz-Aparicio *et al.* 2004; Adlassnig *et al.* 2006) and thereby preventing *Drosophyllum* leaf damage and dessication (Fig. 4). As *Drosophyllum* relies on the production of sticky mucilage on leaf trichomes for prey capture and nutrient absorption, favorable microhabitat conditions in close proximity to shrubs that allow for the maintenance of healthy leaf structures are key for the performance of individuals (Adlassnig *et al.* 2006; Bertol *et al*. 2015). These key facilitative effects were however transient.

With increasing time-since fire and vegetation recovery, our study showed that facilitative effects of resprouting shrubs diminish dramatically. A close proximity to resprouting shrub neighbors benefited recruitment only in the 2012 seed cohort, where seeds were sowed in a patch that burned just one year before. By 2014, when the 2013 cohort emerged from the soil, the differences in vital rates between the open and shrub microhabitat vanished. As the experiment was only performed in two years, we cannot discard that facilitation may have been an effect of year. However, another explanation for the overall lower germination, survival, and size in the 2013 cohort may be that post-fire succession occurs relatively rapidly in burned heathlands (Ojeda *et al.* 1996; Calvo *et al.* 2002), and the increase in vegetation cover negatively affects the germination and seedling size and survival of a post-fire dweller (Verdú *et al*. 2009). When we quantified seedling emergence in spring 2014, woody vegetation cover in some of our burned subplots had increased two-fold since the spring of 2013, from 30 % to 60 %. As a result, germination and survival of *Drosophyllum* may have been impeded. This conclusion seems reasonable when we consider that the temperature and relative humidity, measured daily with HOBO data loggers, remained stable across the two years of the experiment (Online Resource 3). Other studies in fire-prone systems have also shown that post-fire facilitative species interactions are transient and change throughout succession (Vilà and Terradas 1995; Bullock 2009).

It is notable however that, although our study detected facilitation only in the first year after fire and primarily acting on seedling survival, juvenile size, and subsequent probability of flowering of established individuals, none of the measured vital rates in either the 2012 or 2013 cohort were significantly negatively affected by shrub cover in the first two-three years of post-fire succession. These results are in accordance with several other studies in burned Mediterranean ecosystems where competition between resprouting shrubs and post-fire seeder species was reported to be low (Vilà and Sardans 1999; Calvo *et al.* 2002).

In long-unburned, mature heathland patches, the negative effects of biomass accumulation on post-fire dwellers appear predominant. In the case of *Drosophyllum*, individuals trapped fewer insects under mature shrubs compared with resprouting ones. At the same time, prey capture was not affected by microhabitat in unburned patches, with plants catching few insects regardless of whether they were located close to shrubs or in open microhabitats (Fig. 4a). Our results therefore suggest that a high density of mature heathland shrubs may both directly and indirectly interfere with prey capture and therefore survival of *Drosophyllum* individuals. For carnivorous plants, the direct interference of mature shrubs with light and prey capture has been identified as one important reason why some taxa cannot survive in late post-disturbance habitat states (Schulze *et al.* 2001). At the same time, along with decreasing plant species diversity (Ojeda *et al*. 1996), insect diversity and abundance has also been shown to decrease with time-since fire in Mediterranean ecosystems (Potts *et al*. 2003; Mateos *et al*. 2011). Marure heathlands may therefore indirectly affect prey capture rates of *Drosophyllum* by decreasing the overall availability of prey. *Drosophyllum* efficiently attracts prey (chapter 3), and one may hypothesize that low numbers of prey insects caught in unburned heathland patches (even in open microhabitats within these patches) indicate low availability of prey. However, detailed studies on insect (prey) abundance and diversity in distinct heathland habitat patches are required to corroborate this hypothesis.

The ecological niche of *Drosophyllum* as a short-lived, post-fire dweller in Mediterranean heathlands may directly affect the role of facilitation in the demography of this rare carnivorous species (Maestre *et al.* 2009). Demographic census data of *Drosophyllum* populations show that most individuals in natural heathlands perish after one or two reproductive events, and that populations persist mostly in the seed bank after the third year after fire (chapter 5). Such a “weedy” life-history strategy, typical of an early successional species, may avoid severe effects of (apparent) competition with growing shrub neighbors (Bazzaz 1979). Because seed input into the seed bank, ensuring mass germination after fires, is a key life history strategy for many post-fire dwelling species (Quintana-Ascencio *et al.* 2003; Menges and Quintana-Ascencio 2004), including *Drosophyllum* (chapter 2), shrub facilitation of reproduction in early post-fire stages when the majority of individuals reproduce is likely to significantly affect viability of populations. With post-fire succession, reproductive *Drosophyllum* individuals may show plastic responses to the interference with prey capture exercised by shrubs, such as reduced leaf size and reproductive structures, as has been demonstrated for pitcher plants (Brewer

2003; M. Paniw, pers. obs.). Indirect competition via interference with nutrient acquisition through prey capture may therefore be a price worth paying because the facilitative effects of the surrounding community in initial post-disturbance stages can outweigh these future costs.

Despite the novelty of our results, we must emphasize that they should be expanded on by future studies across the range of *Drosophyllum.* Working with a threatened species, our study was limited by the amount of seeds we could collect in any given year. This, in turn, limited the number of treatments we could perform. For example, the assumption that the identity of the resprouting neighbor did not affect demographic parameters may not hold if different neighboring species are considered and should be tested by including plant neighbor as a fixed effect in future studies. In addition, more exhaustive *in-situ* seed sowing experiments in unburned in addition to burned patches will be able to dientanlge the role of direct vs. indirect fire-related germination cues in natural populations.

8.6 Acknowledgements

We thank the Spanish military *Campo de Adiestramiento de la Armada* in Sierra del Retín (Barbate, Cádiz) for allowing access to the Monte Retin sites and facilitating the study. This study was supported by project BREATHAL (CGL2011-28759/BOS; Spanish Ministerio de Ciencia e Innovación). M.P. was supported by a *Subprograma de Formación de Personal Investigador* (FPI) fellowship (BES-2012-053075).

8.7 References

Adamec L. 2009. Ecophysiological investigation on *Drosophyllum lusitanicum*: why doesn't the plant dry out? *Carnivorous Plant Newslett*etter **38**:71-74.

Adlassnig W, Peroutka M, Eder G, Pois W, Lichtscheidl IK. 2006. Ecophysiological observations on *Drosophyllum lusitanicum*. *Ecological Research* **21**:255–262.

Amarasekare P. 2003. Competitive coexistence in spatially structured environments: a synthesis. *Ecology Letters* **6**:1109-1122.

Andrés C, Ojeda F. 2002. Effects of afforestation with pines on woody plant diversity of Mediterranean heathlands in southern Spain. *Biodiversity and Conservation* **11**:1511–1520.

Baraza E, Zamora R, Hódar JA. 2006. Conditional outcomes in plant–herbivore interactions: neighbours matter. *Oikos* **113**:148-156.

Bartolomé J, Plaixats J, Fanlo R, Boada M. 2005. Conservation of isolated Atlantic heathlands in the Mediterranean region: effects of land-use changes in the Montseny biosphere reserve (Spain). *Biological Conservation* **122**:81–88.

Bates D, Maechler M, Bolker B, Walker S. 2014. *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-7 <http://CRAN.R-project.org/package=lme4>.

Bazzaz FA. 1979. The physiological ecology of plant succession. *Annual Review of Ecology and Systematics* **10**:351-371.

Bertness MD, Callaway R. 1994. Positive interactions in communities. *Trends in Ecology and Evolution* **9**:191–193.

Brewer JS. 2003. Why don't carnivorous pitcher plants compete with non-carnivorous plants for nutrients? *Ecology* **84**:451-462.

Bullock JM. 2009. A long-term study of the roles of competition and facilitation in the establishment of an invasive pine following heathland fires. *Journal of Ecology* **97**:646-656.

Calvo L, Tárrega R, De Luis E. 2002. The dynamics of Mediterranean shrubs species over 12 years following perturbations. *Plant Ecology* **160**:25-42.

Callaway RM. 1995. Positive interactions among plants. *The Botanical Review* **61**:306-349.

Callaway RM. 2007. *Positive Interactions and Interdependence in Plant Communities.* Springer.

Cavieres LA, Brooker RW, Butterfield BJ, Cook BJ, Kikvidze Z, Lortie CJ, Pugnaire FI, Schöb C, Xiao S, Anthelme F, Björk RG, Dickinson KJM, Cranston BH, Gavilán R, Gutiérrez-Girón A, Kanka R, Maalouf J-P, Mark AF, Noroozi J, Parajuli R, Phoenix GK, Reid AM, Ridenour WM, Rixen C, Wipf S, Zhao L, Escudero A, Zaitchik BF, Lingua M, Aschehoug ET, Callaway RM. 2013. Facilitative plant interactions and climate simultaneously drive alpine plant diversity. *Ecology Letters* **17**:193-202.

Céspedes B, Torres I, Pérez B, Luna B, Moreno JM. 2014. Burning season does not affect post-fire regeneration but fire alters the balance of the dominant species in a seeder-dominated Mediterranean shrubland. *Applied Vegetation Sci*ence **17**:711-725.

Correia E, Freitas H. 2002. *Drosophyllum lusitanicum*, an endangered West Mediterranean endemic carnivorous plant: threats and its ability to control available resources. *Botanical Journal of the Linnean Society* **140**:383–390.

Dickie IA, Schnitzer SA, Reich PB, Hobbie SE. 2005. Spatially disjunct effects of co-occurring competition and facilitation. *Ecology Lett*ers **8**:1191–1200.

Donovan LA, Richards JH. 2000. Juvenile shrubs show differences in stress tolerance, but no competition or facilitation, along a stress gradient. *Journal of Ecology* **88**:1-16.

Garrido B, Hampe A, Marañón T, Arroyo J. 2003. Regional differences in land use affect population performance of the threatened insectivorous plant *Drosophyllum lusitanicum* (Droseraceae). *Diversity and Distributions* **9**:335–350.

Gómez-Aparicio L, Zamora R, Gómez JM, Hódar JA, Castro J, Baraza E. 2004. Applying plant facilitation to forest restoration in Mediterranean ecosystems: a meta-analysis of the use of shrubs as nurse plants. *Ecological Applications* **14**:1128–1138.

He Q, Bertness MD, Altieri AH. 2013. Global shifts towards positive species interactions with increasing environmental stress. *Ecology Letters* **16**:695-706.

Holmgren M, Scheffer M. 2010. Strong facilitation in mild environments: the stress gradient hypothesis revisited. *Journals of Ecology* **98**:1269–1275.

Holmgren M, Scheffer M, Huston MA. 1997. The interplay of facilitation and competition in plant communities. *Ecology* **78**:1966-1975.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* **50**:346-363.

Keeley JE, Bond WJ, Bradstock RA, Pausas JG, Rundel PW. 2012. *Fire in Mediterranean Ecosystem: Ecology, Evolution and Management*. Cambridge University Press.

Maestre FT, Bautista S, Cortina J. 2003. Positive, negative, and net effects in grass-shrub interactions in Mediterranean semiarid grasslands. *Ecology*, **84**:3186-3197.

Maestre FT, Callaway RM, Valladares F, Lortie, CJ. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology* **97**:199-205.

Maestre FT, Valladares F, Reynolds, JF. 2005. Is the change of plant–plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *Journal of Ecology* **93**:748-757.

Mateos E, Santos X, Pujade-Villar J. 2011. Taxonomic and functional responses to fire and post-fire management of a Mediterranean Hymenoptera community. *Environmental Management* **48**:1000-1012.

Moreno JM, Zuazua E, Pérez B, Luna B, Velasco A, Resco de Dios V. 2011. Rainfall patterns after fire differentially affect the recruitment of three Mediterranean shrubs. *Biogeosciences* **8**:3721-3732.

Menges ES, Quintana-Ascencio PF. 2004. Population viability with fire in *Eryngium cuneifolium*: deciphering a decade of demographic data. *Ecological Monographs* **74**:79-99.

Müller J, Deil U. 2001. Ecology and structure of *Drosophyllum lusitanicum* (L.) Link populations in the south-western of the Iberian Peninsula. *Acta Botanica Malacitana* **26**:47-68.

Ojeda F, Marañón T, Arroyo J. 1996. Postfire regeneration of a Mediterranean heathland in southern Spain. *International Journal of Wildland Fire* **6**:191-198.

Ojeda F, Pausas JG, Verdú M. 2010. Soil shapes community structure through fire. *Oecologia* **163**:729-735.

Ortega-Olivencia A, Carrasco JP, Devesa JA. 1995.. Floral and reproductive biology of *Drosophyllum lusitanicum* (L.) Link (Droseraceae). *Botanical Journal of the Linnean Society* **118**: 331-351.

Peñuelas J, Prieto P, Beier C, Cesaraccio C, De Angelis P, de Dato G, Emmett BA, Estiarte M, Garadnai J, Gorissen A, Kovács Láng E, Kröel-Dulay G, Llorens L, Pellizzaro G, Riiz-Nielsen T, Schmidt IK, Sirka C, Sowerby A, Spano D, Tietema A. 2007. Response of plant species richness and primary productivity in shrublands along a north–south gradient in Europe to seven years of experimental warming and drought: reductions in primary productivity in the heat and drought year of 2003. *Global Change Biology* **13**:2563-2581.

Potts SG, Vulliamy B, Dafni A, Ne'eman G, O'Toole C, Roberts S, Willmer P. Response of plant- pollinator communities to fire: changes in diversity, abundance and floral reward structure. *Oikos* **101**:103-112.

Quintana-Ascencio PF, Menges ES, Weekley CV. 2003. A fire-explicit population viability analysis of *Hypericum cumulicola* in Florida rosemary scrub. *Conservation Biology* **17**:433–449.

Raffaele E, Veblen TT. 1998. Facilitation by nurse shrubs of resprouting behavior in a post-fire shrubland in northern Patagonia, Argentina. *Journal of Vegetation Science* **9**:693-698.

Schulze W, Schulze ED, Schulze I, Oren A. 2001. Quantification of insect nitrogen utilization by the Venus fly trap *Dionaea muscipula* catching prey with highly different isotope signatures. *Journal of Experimental Botany* **52**:1041-1049.

Sedláková I, Chytrý M. 1999. Regeneration patterns in a Central European dry heathland: effects of burning, sod-cutting and cutting. *Plant Ecology* **143**:77-87.

Soliveres S, Maestre FT, Berdugo M, Allan E. 2015. A missing link between facilitation and plant species coexistence: nurses benefit generally rare species more than common ones. *Journal of Ecology* **103**:1183-1189.

Verdú M, Rey PJ, Alcantara JM, Siles G, Valiente-Banuet A. 2009. Phylogenetic signatures of facilitation and competition in successional communities. *Journal of Ecology* **97**:1171-1180.

Ver Hoef JM, Boveng PL. 2007. Quasi-Poisson vs. negative binomial regression: How should we model overdispersed count data? *Ecology* **88**:2766 – 2772.

Vilà M, Sardans J. 1999. Plant competition in Mediterranean-type vegetation. *Journal of Vegetation Science* **10**:281-294.

Vilà M, Terradas J. 1995. Effects of competition and disturbance on the resprouting performance of the Mediterranean shrub *Erica multiflora* L. (Ericaceae). *American Journal of Botany* **82**:1241-1248.

Vuong HQ. 1989. Likelihood ratio tests for model selection and non-nested hypotheses. *Econometrica* **57**:307-333.

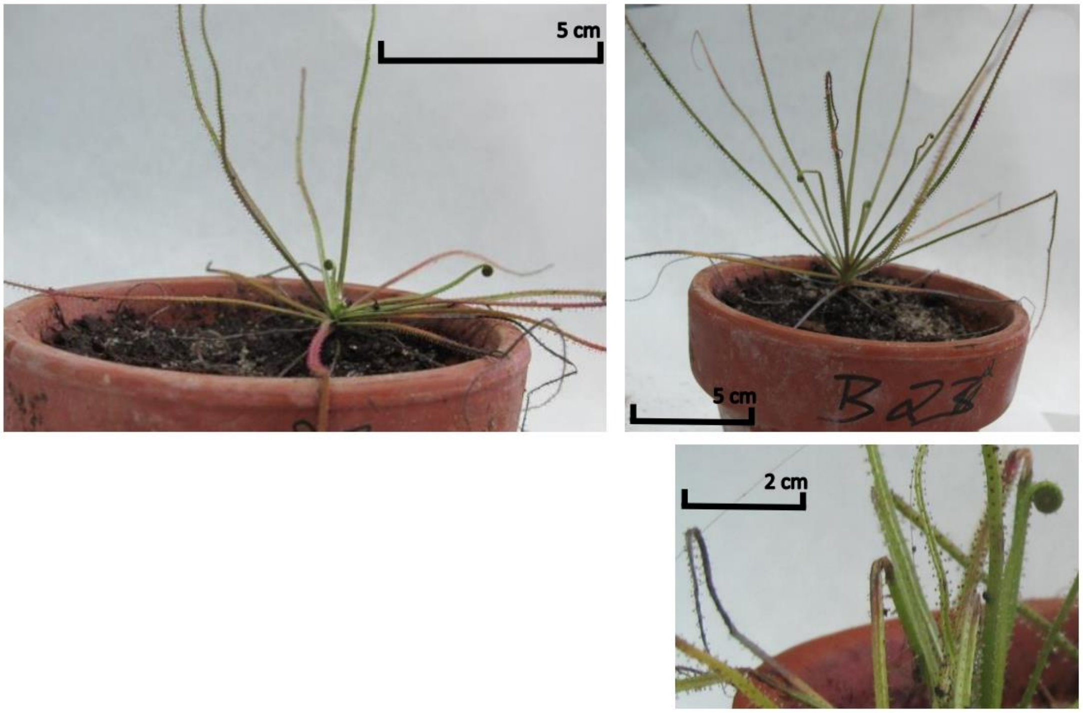
Wright A, Schnitzer SA, Reich PB. 2014. Living close to your neighbors: the importance of both competition and facilitation in plant communities. *Ecology* **95**:2213-2223.

Yates CJ, Ladd PG. 2010.. Using population viability analysis to predict the effect of fire on the extinction risk of an endangered shrub *Verticordia fimbrilepis* subsp. *fimbrilepis* in a fragmented landscape. *Plant Ecology* **211**:305–319.

Zeppel MJ, Harrison SP, Adams HD, Kelley DI, Li G, Tissue DT, Dawson TE, Fensham R, Medlyn BE, Palmer A, West AG, McDowell NG. 2015. Drought and resprouting plants. *New Phytologist* **2**:583–589.

8.8 Appendix S1

Figure S1 Typical plant damage (red, desiccated, and/or broken leaves) encountered on potted juvenile plants of *Drosophyllum lusitanicum* used in the prey-capture experiment described in the main text.



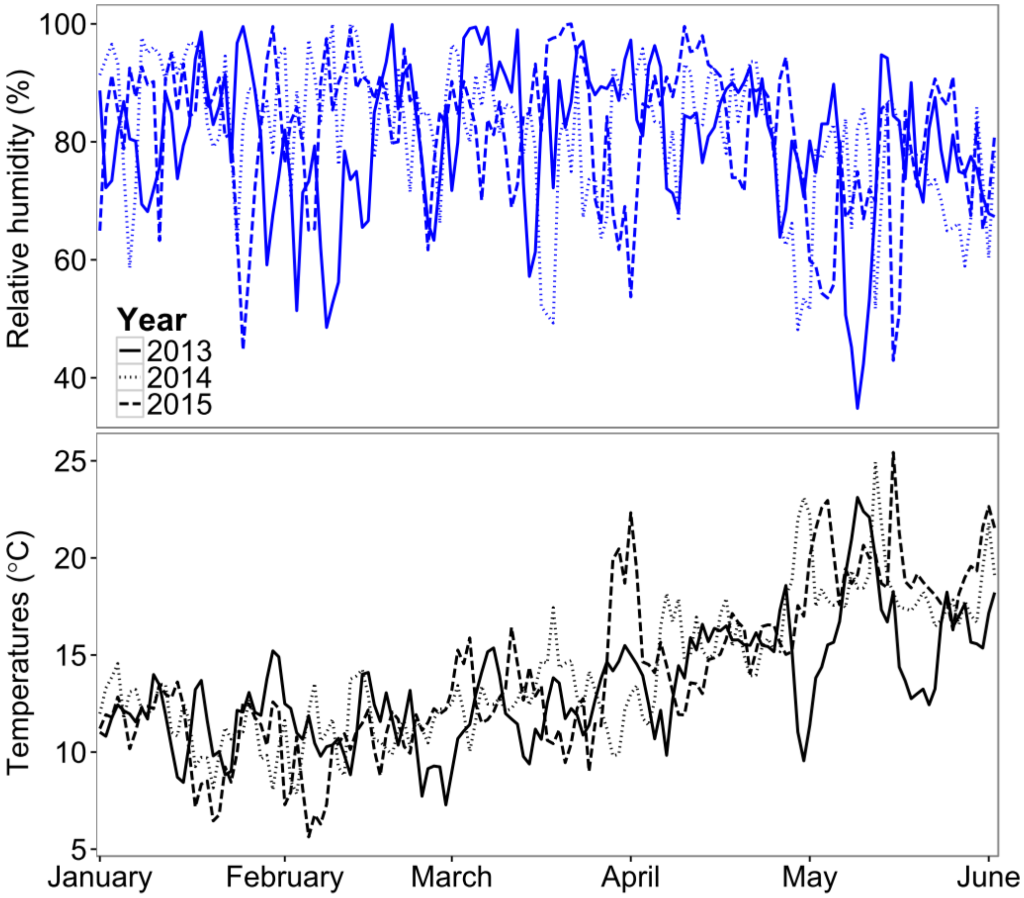
8.9 Appendix S2

Table S2 Diversity of prey insects shown as mean (± 1 SE) number of insect taxa per individual of *Drosophylum lusitanicum* in distinct post-fire habitat states in the prey-capture experiment performed during two years at Sierra Retin. A Chao-Jaccard diversity index, quantifying differences in species composition between burned and unburned sites, was calculated for each year separately (Chao *et al*. 2006, *Biometrics* 62: 361-371). Index values > 0.98 indicated a strong overlap of prey fauna between post-fire habitat states in both years.

	Post-fire habitat state		
Order	**Year**	**Burned**	**Unburned**
Diptera	2013	29.00 (2.20)	16.00 (1.20)
	2014	21.00 (2.50)	13.00 (0.90)
Lepidoptera	2013	1.50 (0.30)	0.20 (0.07)
	2014	2.00 (0.90)	0.80 (0.03)
Aranaea	2013	0.10 (0.05)	0.10 (0.05)
	2014	0.00	0.00
Orthoptera	2013	0.00	0.10 (0.07)
	2014	0.00	0.00
Hymenoptera	2013	0.03 (0.03)	0.00
	2014	0.01 (0.01)	0.02 (0.01)
Coleoptera	2013	0.03 (0.03)	0.10 (0.05)
	2014	0.00	0.00

8.10 Appendix S3

Figure S3 Average daily relative humidity and temperature values measured during the months of seedling emergence in two years at Sierra Carbonera (Figure 1) where the seed-sowing experiment was performed. Measurements were obtained with HOBO data loggers (Onset Corporation, 2013) positioned at the site 1 m above ground.



CHAPTER 9

Discussion and Conclusions

CHAPTER 9

Discussion and Conclusions

9.1 Discussion

Natural disturbances regularly affect a large part of the globe (CHRR 2005) and have contributed to life-cycle adaptations in numerous species to cope and even profit from disturbance regimes such as fires, floods, or windstroms (Benton and Grant 1996; Smallegange and Coulson 2013; Cayuela *et al*. 2016). However, human activities increasingly interact with natural disturbance regimes, with potentially severe consequences for disturbance-adapted species (Paine *et al*. 1998; Duwyn and MacDougall 2015). At the same time, information on consequences of disturbance interaction on population dynamics of disturbance-adapted species remains scarce (Ehrlén *et al*. 2016). This is worrisome because the viability of species that reach highest population densities after disturbance events may be seriously jeopardized if such regimes are changed, as this thesis has highlighted from various angles.

This doctoral thesis focused largely on life-cycle dynamics of the fire-adapted carnivorous plant species *Drosophyllum lusitanicum* (Drosophyllaceae) increasingly affected by human activities. Field censuses across the species' range demonstrated that most extanct populations persist in habitats where small-scale human disturbances have replaced the role of fires of vegetation clearance (chapter 6). These habitat associations have resulted in the omission of the role of fires in the ecology of the species (Garrido *et al*. 2003; Adlassnig *et al*. 2006; but see Correia and Freitas 2002). However, much of the biological and ecological characteristics quantified in this work through *in-situ* and greenhouse experiments (chapters 2-4) can best be described in the light of population dynamics cued to recurrent fires. The costly maintenance of large flowers in *Drosophyllum* despite high autogamy and relatively modest increases in seed set due to pollinating insect attraction may be explained as a life-cycle adaptation to early-succesional habitats (chapter 2). That is, for an early-successional fire-adapted species, reproduction largely occurs in a short post-fire window, in which producing seeds to replenish the seed bank is critical (Quintana-Ascencio *et al*. 2003; Menges and Quintana-Ascencio 2004). Similarly, despite being strongly carnivorous, roots of *Drosophyllum* are able to uptake soil nutrients (chapter 4), which may allow individuals to profit from soil nutrient flushes after fires. The nutrients are however rapidly leached from the soil (Keeley *et al*. 2012), and both the strong carnivorous syndrome and ecological rarity of *Drosophyllum* (chapter 4) are adaptatitions to largely nutrient-poor soils combined with seasonal drought in natural heathland habitats (Adlassnig *et al*. 2006). Lastly, and perhaps most importantly, the stochastic integral projection models developed here demonstrate (chapter 5) that the persistent seed bank strongly affects population growth in natural heathland habitats – typical of fire-adapted species (Adams *et al*. 2005).

Adaptations to fire regimes do not necessarily mean extinction when such regimes change, particularly not in the Mediterrean Basin, where humans have for millennia shaped landscapes and species' responses to disturbances (Keeley *et al*. 2012). *Drosophyllum* is one in a number of documented case studies demonstrating that populations of a fire-adapted plant species can persist under human disturbance regimes (Bartolomé *et al*. 2005; Quintana-Ascencio *et al*. 2007; Velle *et al*. 2014; Tye *et al*. 2016). It has been shown that large-scale habitat degradation by human activity negatively affects viability of many species (Wayne *et al.* 2006; Yasué 2006; Brunbjerg *et al.* 2014). However, small-scale human disturbances that do not fundamentally change the habitat requirements of disturbance-adapted species can indeed prevent the local extinction of populations where natural disturbance regimes are not maintained. This is not only true for *Drosophyllum,* but for many other plant and animal species that may positively respond to various sources of disturbances (McKinney and Lockwood 1999; Quintana-Ascencio *et al.* 2007; Hendry *et al.*, 2008).

Maintaining populations of disturbance-adapted species in human-disturbed habits, however, does not equate to long-term viability and may simply reflect a slow progression to local extinction (González-Varo *et al*. 2015). Human disturbances not only alter and replace but increasingly interact with natural disturbance regimes, and this thesis demonstrates that such interactions, if occurring at high frequencies, may pose serious risk to population viability of *Drosophyllum* (chapter 7). This is fundamentally due to incompatible selection pressures posed by fires *vs*. chronic small-scale vegetation removal, selecting for seed-bank dynamics *vs*. continuous recruitment, respectively. When disturbance interactions are moderate, viability of populations may be achieved even under lengthening fire return intervals because vegetation removal can increase plant survival and create suitable patches for recruitment (Vilà and Terradas 1995; Sánchez-Velásquez *et al*. 2002). In turn, moderate interacting disturbances may increase local biodiversity (Solar *et al*. 2015). However, high frequencies of both fires and repeated vegetation removal, common across the range of *Drosophyllum* and other species (Lawson *et al*. 2010; Mandle *et al*. 2015) will put populations at serious risk of extinction. This is an important finding because it implies that the conservation of disturbance-adapted species must consider the detailed disturbace history populations have been exposed to (Bonebrake *et al*. 2014). Precisely, fires may be detrimental to a fire-adapted species if populations have persisted under different disturbance regimes (chapter 7).

Another critical finding of this thesis is that frequent human disturbances do not promote increases in population sizes, and that may exacerbate the effects of environmental variability leading to local extinction, despite some populations thriving in such habitats. Chronic human disturbances have been known to cause local extinctions and impoverish ecoystems (Turner 2010; Ribeiro *et al*. 2015). In the case of *Drosophyllum*, chronic vegetation removal increases the risk of plants being trampled or exposes them to adverse microhabitat conditions such as increased solar radiation (chapter 7). Such adverse habitat conditions may result in plants not being able to maintain the water balance in their trapping structures (Adlassnig *et al*. 2006). Vegetation removal may also increase seedling mortality as shrubs showed nurturing effects on several vital rates of *Drosophyllum* (chapter 8). Increased variability in survival and reproduction in human-disturbed habitats has been documented for numerous plant and animal species (Vieira-Neto *et al*. 2016; Tye *et al*. 2016) and decreases population growth if this variability is assumed to be random (Tuljapurkar 1990).

This thesis contributes with critical findings to previous studies assessing vital-rate variability and population vability by showcasing potentially detrimental effects of chronic vegetation removal on a rare plant species found in one of the most biodiverse ecosystems in Europe. Current management practices that replace fire by small-scale, chronic vegetation removal – widely applied across Spain (Valdecantos *et al*. 2008) – are therefore not appropriate for the conservation of the study species and likely many other post-fire recruiting seeder species. Ultimately, the ongoing loss of *Drosophyllum* populations from Mediterranean heathland habitats can be seen as a warning sign for biodiversity loss across other disturbance-adapted systems. Mediterranean heathlands in southwestern Spain and northern Morocco are biodiversity hotspots in which floristic diversity is closely tied to recurrent fires (Ojeda *et al*. 1996, 2000). However, strict fire-suppression policies have resulted in other types of disturbances dominating these systems (Bartolomé *et al.* 2005). Such shifts are not unique to these systems and must be quantified wherever they occur because they are likely to have significant – negative – effects on biodiversity.

Lastly, this doctoral work demonstrates that any assessment of changes in population dynamics and viability due to changes in disturbance regimes must consider uncertainty in the estimates of model parameters (chapter 5). This is particularly true for rare or threatened species where data on certain vital rates may be limited, therefore increasing the uncertainty in the estimates of vital-rate responses to environmental drivers (Elderd and Miller 2016). In the case of *Drosophyllum*, only limited experimental data could be obtained on seed-bank dynamics (chapter 5). At the same time, sample sizes of above-ground individuals in human-disturbed habitats, for which demographic data were particularly variable, were also limited given the small sizes of most populations and the limited number of populations overall (chapter 7). These data limitations and resulting uncertainty around estimates of vital-rate parameters translated into high uncertainty in the inference on the effect of disturbance interactions on population dynamics (chapters 5 and 7). Increasingly sophisticated ecological models, reflecting complex interactions of environmental drivers, combined with decreasing population sizes of many species will require researchers to include detailed accounts of uncertainties implicit in their models for a more robust interpretation of results (Evans *et al*. 2010; Hunter *et al*. 2010).

9.2 Conlusions

1. Several biological and ecological characteristics of *Drosophyllum* were investigated in using greenhouse and *in-situ* experiments. The results not only add to the research on carnivorous plant biology but also indicate that fire regimes shaped the evolution of the

species. The production of large, showy flowers despite high autogamy rates may be linked to life-history adaptations of an early-successional species, which uses a short post-disturbance (here fire) window to maximize input into the seed bank and, in turn, benefits from the seed bank for an effective post-fire colonization.

2. The reliance on insects to increase seed set (likely by assisting self-pollination) is further supported by the separation of prey and pollinator fauna in the species. This separation in ensured by complex carnivorous structures, an adaptation to the extreme nutrient poverty in Mediterranean heathlands. The unique habitat characteristics have also contributed to the ecological uniquess of *Drosophyllum*, which is one of a handful species to have evolved a strong carnivorous character in seasonally dry habitats.

3. Likely, the most important adaptation to recurrent fire regimes is seed dormancy. *Drosophyllum* produces a persistent seed bank, critical for population dynamics in fire-disturbed habitats as assessed by stochastic integral projection models in this thesis. The models showed that populations may persists, largely in the seed bank, for > 40 years after fires. Including uncertainty in the estimates of seed-bank related vital rates, e.g. seed-bank stasis, can give robust information on the certainty of estimates of population viability at various fire return intervals.

4. Despite adaptations to recurrent fires, most extant populations of *Drosophyllum* are found in human-disturbed habitats. While large-scale human disturbances such as the conversion of heathlands to pine and eucalypt plantations may pose serious threats to populations, the effect of human disturbances is much stronger, and positive, at a local spatial scale. Small-scale vegetation removal mimics fires and may significantly positively affect species occurrence and abundance of several life-cycle stages.

5. Although large populations of *Drosophyllum* can be found in human-disturbed habitats, local extinctions across the species' range are frequent. Stochastic integral projection models showed that such extintions may be explained by negative interactions of fire and human disutrbances. Such interactions may prove fatal for population viability because the the two different disturbance types pose opposing selection pressures – fires and small-scale, chronic vegetation removal selecting for traits ensuring seed-bank stasis and

continuous reproduction, respectively. In addition, frequent human disturbances, even in the absence of fire, do not favor population increases and may pose servere theats to the viability of existing populations by exacerbating the effects of variability in vital rates (survival, growth, and reproduction) and thereby decreasing population growth rates.

6. One major way in which human disturbances may negatively affect vital rates is by increasing plant mortality due to fundamental changes to community structure. In habitats marked by chronic vegetation removal, *Drosophyllum* populations are found in less diverse communities, with fewer if any shrub neighbors. This work showed that shrub neighbors in post-fire heathland habitats may positively affect several vital rates of *Drosophyllum* throughout post-fire habitat succession. The absence of such nursing effects in human-disturbed habitats may explain the higher seedling and adult mortality of *Drosophyllum* individuals.

9.3 Conclusiones

1. Se han estudiado varias características biológicas y ecológicas de *Drosophyllum* mediante experimentos *in-situ* y en invernadero. Los resultados constituyen un avance en el conocimiento sobre plantas carnívoras e indican que los regímenes de fuego moldean la evolución de las especies. La producción de flores grandes y vistosas, a pesar de las altas tasas de autogamia, puede estar relacionada con adaptaciones del ciclo vital de la especie a etapas tempranas de la sucesión, restringida a una corta ventana temporal tras las perturbaciones (fuego) para maximizar su contribución al banco de semillas: De esta forma, se beneficia del banco de semillas para una regeneración por reclutamiento más eficiente tras el fuego.

2. La dependencia de las plantas sobre los insectos para incrementar la cantidad de semillas (mayormente mediante autopolinización asistida) se confirma debido a la separación entre las presas y los polinizadores en esta especie. Esta separación está asegurada por la complejas estructuras carnívoras que probablemente han evolucionado en *Drosophyllum* debido a las características únicas de los brezales, tales como extrema escasez de nutrientes y condiciones microclimáticas que permiten que se mantenga su capa mucilaginosa incluso en veranos secos. Las características únicas de este hábitat también contribuyen a la

singularidad ecológica de *Drosophyllum*, una de las pocas especies carnívoras sobre suelos secos.

3. Probablemente la adaptación más importante a los regímenes de fuego recurrente es la latencia de las semillas. *Drosophyllum* produce un banco de semillas persistente, el cual es crítico para la dinámica de sus poblaciones en hábitats perturbados por el fuego según los modelos estocásticos de proyección integral usados en esta tesis. Los modelos muestran que las poblaciones pueden persistir, en gran parte en forma de banco de semillas, hasta más de 40 años tras episodios de fuego. La incorporación de la incertidumbre en la estimación de las tasas vitales de los bancos de semillas (ej.: estasis de los bancos de semillas) permite dar información más robusta sobre la viabilidad de las poblaciones en relación a distintos intervalos temporales de episodios de fuego.

4. A pesar de las adaptaciones a fuegos recurrentes, la mayoría de las poblaciones de *Drosophyllum* existentes se encuentran en hábitats perturbados por el hombre. Aunque las perturbaciones antropogénicas a gran escala como la transformación de brezales en plantaciones de pinos y eucaliptos puedan suponer una seria amenaza para las poblaciones, los efectos de estas perturbaciones son mucho más fuertes y positivos a una escala espacial local. La eliminación de vegetación a pequeña escala simula el efecto del fuego de eliminación de biomasa vegetal y puede afectar de forma significativamente positiva a la presencia y abundancia de la especie en varias etapas de su ciclo vital.

5. Aunque se pueden encontrar grandes poblaciones de *Drosophyllum* en hábitats perturbados por el hombre, las extinciones locales de esta especie son frecuentes. Los modelos estocásticos de proyección integral muestran que estas extinciones pueden explicarse por interacciones negativas entre el fuego y las perturbaciones antropogénicas. Estas interacciones pueden ser fatales para la viabilidad de las poblaciones debido a que los dos tipos diferentes de perturbaciones ejercen presiones selectivas opuestas (los fuegos y eliminación recurrente de la vegetación a pequeña escala favorecen el estasis de los bancos de semillas y la reproducción continua, respectivamente). Además, las perturbaciones antropogénicas frecuentes, aunque no haya fuego, no favorecen que se incrementen las poblaciones y pueden suponer una amenaza severa a la viabilidad de las poblaciones existentes ya que agravan los efectos de la variabilidad en las tasas vitales (supervivencia,

crecimiento y reproducción) y, por tanto, hacen disminuir las tasas de crecimiento de las poblaciones.

6. Una de las principales formas en la que las perturbaciones antropogénicas pueden afectar negativamente a las tasas vitales es incrementando la mortalidad de las plantas debido a cambios fundamentales en la estructura de la comunidad. En hábitats donde la eliminación de la vegetación es crónica, las poblaciones de *Drosophyllum* se hallan en comunidades menos diversas, con pocos o ningún arbusto vecino. Este trabajo muestra que los arbustos vecinos en brezales previamente afectados por el fuego pueden afectar de forma positiva a varias tasas vitales de *Drosophyllum* a través de la sucesión que tenga lugar en esos hábitats tras los fuegos. La ausencia de estos efectos positivos en hábitats perturbados por el hombre se puede explicar por la mayor mortalidad de las plántulas e individuos adultos de *Drosophyllum*.

9.4 References

Adams VM, Marsh DM, Knox JS. Importance of the seed bank for population viability and population monitoring in a threatened wetland herb. 2005. *Biological Conservation* **124**:425-436.

Adlassnig W, Peroutka M, Eder G, Pois W, Lichtscheidl IK. 2006. Ecophysiological observations on *Drosophyllum lusitanicum*. *Ecological Research* **21**:255-262.

Bartolomé J, Plaixats J, Fanlo R, Boada M. 2005. Conservation of isolated Atlantic heathlands in the Mediterranean region: effects of land-use changes in the Montseny biosphere reserve (Spain). *Biological Conservation* **122**:81–88.

Benton TG, Grant A. 1996. How to keep fit in the real world : Elasticity analyses and selection pressures on life histories in a variable environment. *The American Naturalist* **147**:115–139.

Bonebrake TC, Syphard AD, Franklin J, Anderson KE, Akçakaya HR, Mizerek T, Winchell C, Regan HM. 2014. Fire management, managed relocation, and land conservation options for long-lived obligate seeding plants under global changes in climate, urbanization, and fire regime. *Conservation Biology* **28**: 1057-1067.

Brunbjerg AK, Svenning JC, Ejrnæs R. 2014. Experimental evidence for disturbance as key to the conservation of dune grassland. *Biological Conservation* **174**:101-110.

Cayuela H, Boualit L, Arsovski D, Bonnaire E, Pichenot J, Bellec A, Miaud C, Léna JP, Joly P, Besnard A. 2016. Does habitat unpredictability promote the evolution of a colonizer syndrome in amphibian metapopulations? *Ecology*, in press.

Correia E, Freitas H. 2002. *Drosophyllum lusitanicum*, an endangered West Mediterranean endemic carnivorous plant: threats and its ability to control available resources. *Botanical Journal of the Linnaean Society* **140**:383–390.

Duwyn A, MacDougall AS. 2015. When anthropogenic-related disturbances overwhelm demographic persistence mechanisms. *Journal of Ecology* **103**:761–768.

Ehrlén J, Morris WF, Euler T, Dahlgren JP. 2016. Advancing environmentally explicit structured population models of plants. *Journal of Ecology* **104**:292-305.

Elderd BD, Miller TE. 2016. Quantifying demographic uncertainty: Bayesian methods for Integral Projection Models (IPMs). *Ecological Monographs* **86**:125-144.

Evans ME, Holsinger KE, Menges ES. 2010. Fire, vital rates, and population viability: a hierarchical Bayesian analysis of the endangered Florida scrub mint. *Ecological Monographs* **80**:627-649.

Garrido B, Hampe A, Marañón T, Arroyo J. 2003. Regional differences in land use affect population performance of the threatened insectivorous plant *Drosophyllum lusitanicum* (Droseraceae). *Diversity and Distributions* **9**:335–350.

González-Varo JP, Albaladejo RG, Aizen MA, Arroyo J, Aparicio A. 2015. Extinction debt of a common shrub in a fragmented landscape. *Journal of Applied Ecology* **52**:580-589.

Hendry AP, Farrugia TJ, Kinnison MT. 2008. Human influences on rates of phenotypic change in wild animal populations. *Molecular Ecology* **17**:20-29.

Hunter CM, Caswell H, Runge MC, Regehr EV, Amstrup SC, Stirling I. 2010. Climate change threatens polar bear populations: a stochastic demographic analysis. *Ecology* **91**:2883-2897.

Keeley JE, Bond WJ, Bradstock RA, Pausas JG, Rundel PW. 2012. *Fire in Mediterranean Ecosystems: Ecology, Evolution and Management*. Cambridge University Press.

McKinney ML, Lockwood JL. 1999. Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends in Ecology and Evolution* **14**:450-453.

Lawson DM, Regan HM, Zedler PH, Franklin, J. 2010. Cumulative effects of land use, altered fire regime and climate change on persistence of *Ceanothus verrucosus*, a rare, fire-dependent plant species. *Global Change Biology* **16**:2518–2529.

Mandle L, Ticktin T, Zuidema PA. 2015. Resilience of palm populations to disturbance is determined by interactive effects of fire, herbivory and harvest. *Journal of Ecology* **103**:1032-1043.

Menges ES, Quintana-Ascencio PF. 2004. Population viability with fire in *Eryngium cuneifolium*: deciphering a decade of demographic data. *Ecological Monograph*s **74**: 79-99.

Ojeda F, Marañón T, Arroyo J. 1996. Patterns of ecological, chorological and taxonomic diversity on both sides of the Strait of Gibraltar. *Journal of Vegetation Science* **7**:63-72.

Ojeda F, Marañón T, Arroyo J. 2000. Plant diversity patterns in the Aljibe Mountains (S. Spain): a comprehensive account. *Biodiversity and Conservation* **9**: 1323-1343.

Paine RT, Tegner MJ, Johnson EA. 1998. Compounded perturbations yield ecological surprises. *Ecosystems* **1**:535–545.

Quintana-Ascencio PF, Menges ES, Weekley CV, 2003. A fire-explicit population viability analysis of *Hypericum cumulicola* in Florida rosemary scrub. *Conservation Biology* **17**:433–449.

Quintana-Ascencio, P.F., Weekley, C.W., and Menges, E.S. 2007. Comparative demography of a rare species in Florida scrub and road habitats. Biol. Cons. 137, 263-270.

Ribeiro E, Arroyo-Rodríguez V, Santos BA, Tabarelli M, Leal IR. 2015. Chronic anthropogenic disturbance drives the biological impoverishment of the Brazilian Caatinga vegetation. *Journal of Applied Ecology* **52**:611-620.

Sánchez-Velásquez LR, Ezcurra E, Martínez-Ramos M, Álvarez-Buylla E, Lorente R. 2002. Population dynamics of *Zea diploperennis*, an endangered perennial herb: effect of slash and burn practice. *Journal of Ecology* **90**:684-692.

Smallegange IM, Coulson T. 2013. Towards a general, population-level understanding of eco-evolutionary change. *Trends in Ecology and Evolution* **28**:143-148.

Solar RR, Barlow J, Ferreira J, Berenguer E, Lees AC, Thomson JR, Louzada J, Maués M, Moura NG, Oliveira VH, Chaul J. 2015. How pervasive is biotic homogenization in human-modified tropical forest landscapes? *Ecology Letters* **18**:1108-1118.

Tuljapurkar S. 1990. *Population Dynamics in Variable Environments*. Springer, New York, USA.

Turner MG. 2010. Disturbance and landscape dynamics in a changing world. *Ecology* **91**:2833-2849.

Tye MR, Menges ES, Weekley C, Quintana-Ascencio PF, Salguero-Gómez R. 2016. A demographic ménage à trois: interactions between disturbances both amplify and dampen population dynamics of an endemic plant. *Journal of Ecology*, in press.

Valdecantos A, Baeza MJ, Vallejo VR. 2008. Vegetation management for promoting ecosystem resilience in fire- prone Mediterranean shrublands. *Restoration Ecology* **17**:414-421.

Velle LG, Nilsen LS, Norderhaug A, Vandvik V. 2014. Does prescribed burning result in the homogenization of coastal heathlands? *Global Change Biology* **20**:1429-1440.

Vieira-Neto EHM, Vasconcelos H.L., Bruna M. 2016. Roads alter the population growth rates of a native dominant herbivore in Neotropical savannahs. *Journal of Applied Ecology* **53**:983-992.

Vilà M, Terradas J. 1995. Effects of competition and disturbance on the resprouting performance of the Mediterranean shrub *Erica multiflora* L. (Ericaceae). *American Journal of Botany* **82**:1241-1248.

Wayne AF, Cowling A, Lindenmayer DB, Ward CG, Vellios CV, Donnelly CF, Calver MC. 2006. The abundance of a threatened arboreal marsupial in relation to anthropogenic disturbances at local and landscape scales in Mediterranean-type forests in south western Australia. *Biological Conservation* **127**:463-476.

Yasué M. 2006. Environmental factors and spatial scale influence shorebirds' responses to human disturbance. *Biological Conservation* **128**:47-54.