Universitat de les
Illes Balears

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OPPORTUNISTIC VERTEBRATES AS MEDIATORS OF THE REPRODUCTIVE SUCCESS OF TWO CANARIAN ENDEMIC PLANTS

Julia Jaca Estepa

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Doctoral Programme of Plant Biology

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I DECLARE:

That the thesis titled ‘Opportunistic Vertebrates as Mediators of the Reproductive Success of two Canarian Endemic Plants’, presented by Julia Jaca Estepa to obtain a doctoral degree, has been completed under my supervision and meets the requirements to opt for an International Doctorate.

For all intents and purposes, I hereby sign this document.

Signature:

Palma de Mallorca, 29th February, 2020

Universitat
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Dr. Manuel Nogales Hidalgo, of University of La Laguna

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Signature:

San Cristóbal de la Laguna, 29th February, 2020

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Abstract

Plant reproductive success depends on the effectiveness of the mutualistic interaction and the context in which it occurs. This thesis investigates and quantifies plant fitness with a particular group of animal mutualists, opportunistic vertebrates, in the context of oceanic islands. Compared to mainland systems, oceanic islands are generally characterized by simpler mutualistic networks, largely as a result of depauperate animal faunas. Because of this, plant species are likely to include new, even novel flower–pollinator and fruit-dispersal interactions after island colonization. In the Canary Islands, passerine birds and lacertid lizards are frequent floral visitors and fruit consumers of native flora, but their effectiveness and relative importance in reproductive success has remained almost unknown. In addition, islands are particularly vulnerable to alien species like rats, honeybees, and goats, which are all successful invaders in the Canary Islands. These interact with native plant species and their potential detrimental effects on plant fitness deserve our attention, in order to preserve this endemic flora.

Our main goal was to gain further insights into the role of vertebrates in plant reproductive success in the archipelago, and the evolution of the bird pollination syndrome in the Macaronesian islands in general. For this, we selected the endemic plant species *Echium simplex* and *Canarina canariensis*, both interacting with native mutualists and alien antagonists.

Chapters 1 and 2 assess the breeding system, identify nocturnal and diurnal flower visitors and compare the pollination effectiveness of different animal guilds (vertebrates vs. insects, and diurnal vs. nocturnal insects) along different inflorescence sections in *Echium simplex*. It is a self-compatible species, but its reproductive success is enhanced by animal pollinators. Bees and beetles were the most common insect visitors, while the African blue tit and the Canarian chiffchaff were the most prevalent vertebrates visiting flowers. Flying diurnal insects increased fruit set whereas small flower dwellers (mostly beetles)

decreased both fruit and seed set; by contrast, vertebrates had a negligible effect on reproductive success. We additionally found that plant reproductive structures are heavily damaged by feral goats, which threaten the conservation of this Canarian endemic species.

Chapter 3 studies the pollination of *Canarina canariensis* in remnants of the former thermosclerophyllous woodland, evaluating how two widespread alien invasive species, the honeybee and the black rat, affect its reproductive success. The Canarian chiffchaff was the most frequent visitor in the early flowering season, whereas the honeybee predominated in the flowers during mid and late flowering periods. Birds increased fruit set, whilst insects had a negligible effect. Besides contributing little to plant reproduction, honeybees might interfere with bird pollination by depleting flowers of nectar. Rats consumed about 10% of the flowers and reduced fruit set to one third. Both alien species can threaten *C. canariensis* reproduction and hence population sustainability in the thermosclerophyllous vegetation.

Chapter 4 investigates the relative effectiveness of two vertebrate groups (birds and lizards) at different stages (pollination and seed dispersal) of the reproductive cycle of *Canarina canariensis* in the thermosclerophyllous habitat. We built three stochastic models (namely ‘pollination’, ‘dispersal’ and ‘pollination + dispersal’) that simulated seedling recruitment. The dispersal stage contributed more than the pollination stage through the recruitment process. Moreover, birds and lizards exhibited a functional complementarity, with birds contributing most in the pollination model and lizards in the dispersal model.

Resumen

El éxito reproductivo de las plantas depende de la eficacia de la interacción mutualista y del contexto en el que ocurre. Esta tesis investiga y cuantifica el ‘fitness’ (éxito biológico) de la planta con un grupo particular de animales mutualistas, los vertebrados oportunistas, en un contexto particular, en islas oceánicas. Comparadas con los sistemas continentales, las islas oceánicas generalmente se caracterizan por redes mutualistas más simples, en gran medida como resultado de su fauna depauperada. A consecuencia de esto, las especies de plantas son proclives a incluir nuevas interacciones de flor-polinizador y fruto-dispersor después de su colonización. En las Islas Canarias, las aves paseriformes y los lagartos (F. Lacertidae) son visitantes florales y consumidores de frutos frecuentes de la flora nativa, pero su eficacia e importancia relativa en el éxito reproductivo es casi desconocida. Por otro lado, las islas son particularmente vulnerables a las especies exóticas. Ratas, abejas de la miel y cabras son invasores exitosos en las Canarias que interaccionan con especies nativas de plantas, muchas de ellas endémicas, y cuyos potenciales efectos perjudiciales en su ‘fitness’ merece nuestra atención con el fin de preservar esta flora endémica.

Con el principal objetivo de aportar más conocimiento en el papel de los vertebrados en el éxito reproductivo de las plantas en el archipiélago canario, y en la evolución del síndrome de polinización por aves en las islas Macaronésicas, seleccionamos los endemismos *Echium simplex* y *Canarina canariensis*, ambos interaccionando con mutualistas nativos, y también con antagonistas exóticos.

Los capítulos 1 y 2 evalúan el sistema reproductivo, identifican los visitantes florales diurnos y nocturnos, y comparan la eficacia de polinización de diferentes gremios de animales (vertebrados vs. insectos, insectos diurnos vs. insectos nocturnos) a lo largo de diferentes secciones de la inflorescencia de *E. simplex.* La planta es auto-compatible, pero su éxito reproductivo es claramente favorecido por animales polinizadores. Las abejas y los escarabajos fueron los insectos visitantes más comunes, mientras que el herrerillo y el mosquitero canarios fueron los vertebrados predominantes. Los insectos voladores diurnos aumentaron el cuajado

de frutos, mientras que los pequeños habitantes de las flores (en su mayoría escarabajos) disminuyeron tanto el cuajado de frutos como de semillas; en cambio, los vertebrados tuvieron un efecto insignificante en el éxito reproductivo. Adicionalmente, encontramos que las estructuras reproductivas son considerablemente dañadas por cabras asilvestradas, lo cual amenaza la supervivencia de esta especie endémica canaria

El capítulo 3 estudia la polinización de *C. canariensis* en fragmentos del antiguo bosque termoesclerófilo, evaluando cómo dos especies exóticas invasoras ampliamente distribuidas, la abeja de la miel y la rata, afectan a su éxito reproductivo. El mosquitero canario fue el visitante más frecuente al principio de la temporada de floración, mientras que la abeja de la miel predominó a mitad y al final de la misma. Las aves aumentaron el cuajado de frutos, mientras que los insectos tuvieron un efecto insignificante. Además de contribuir poco a la reproducción de la planta, la abeja de la miel podría interferir con la polinización por las aves al agotar el néctar de las flores. Las ratas consumieron alrededor del 10% de las flores y redujeron el cuajado de frutos a un tercio. Ambas especies exóticas pueden amenazar la reproducción de *C. canariensis* y, por tanto, el futuro de la sostenibilidad de la población en la vegetación termoesclerófila.

El capítulo 4 investiga la eficacia relativa de diferentes gremios de vertebrados (aves vs. lagartos) en dos etapas diferentes (polinización y dispersión de semillas) del ciclo reproductivo de *C. canariensis* en el hábitat termoesclerófilo. Construimos tres modelos estocásticos (específicamente, 'polinización', 'dispersión', y 'polinización + dispersión') que simulaban el reclutamiento de plántulas. La etapa de la dispersión contribuyó en mayor medida que la etapa de la polinización a lo largo del proceso de reclutamiento. Además, aves y lagartos mostraron una complementariedad funcional, contribuyendo más las aves en el modelo de polinización, mientras que los lagartos influyeron más en el modelo de dispersión.

Resum

L'èxit reproductiu de les plantes depèn de l'efectivitat de la interacció mutualista i del context d'aquesta. Aquesta tesi investiga i quantifica la fitness de les plantes amb un determinat grup de mutualistes animals, els vertebrats oportunistes, dins d'un context en particular, les illes oceàniques. En comparació amb els sistemes peninsulars, les illes oceàniques generalment es caracteritzen per xarxes mutualistes més simples, degut en gran mesura a una fauna animal empobrida. Degut a això, és més probable que les espècies de plantes presentin noves interaccions flor–pol·linitzador i dispersió de fruits després de la colonització de l'illa. A les Illes Canàries, els aucells passeriformes i els sargantanes de la familia dels lacèrtids són visitants freqüents de les flors i consumidors de fruits de la flora nativa, però la seva efectivitat i la importància relativa del seu èxit reproductiu són encara desconeguts. D'altra banda, les illes són particularment vulnerables a les espècies alienes. A les Illes Canàries les rates, les abelles de la mel i les cabres són invasors d'èxit que interactuen amb les espècies natives de plantes i els seus efectes detrimentals a la fitness de les plantes mereix la nostra atenció per a preservar aquesta flora endèmica.

Amb l'objectiu principal d'aportar una visió més profunda del paper dels vertebrats en l'èxit reproductiu de les plantes de l'arxipèlag, i de l'evolució del síndrome de l'aucell pol·linitzador a les illes Macaronèsiques, hem seleccionat les endèmiques *Echium simplex* i *Canarina canariensis*, de cara a la interacció d'ambdues amb els mutualistes natius i els antagonistes aliens.

Els capítols 1 i 2 avaluen el sistema reproductiu d'*Echium simplex*, identificant els visitants nocturns i diürns de les flors i comparen l'efectivitat de la pol·linització dels diferents gremis d'animals (vertebrats vs insectes, i insectes diürns vs nocturns) juntament amb seccions d'inflorescència de la planta. Aquesta espècie és auto- compatible però el seu èxit reproductiu millora amb els pol·linitzadors animals. Les abelles i escarabats varen ser els visitants insectes més comuns, mentre que la mallerenga africana i el mosquiter canari foren els

visitants de flors vertebrats més prevalents. Els insectes voladors diürns incrementaren el quallat del fruit, mentre que els habitants de les flors més petites (principalment escarabats) reduïren el quallat del fruit i de les llavors; en contraposició, els vertebrats tingueren un efecte negligible en l'èxit reproductiu. A més, es va confirmar que les cabres ferals fereixen les estructures reproductives de les plantes, la qual cosa amenaça el manteniment d'aquesta espècie endèmica canària

El capítol 3 estudia la pol·linització de *Canarina canariensis* als vestigis del bosc termescleròfil, avaluant com dues espècies invasores alienes esteses, l'abella de mel i la rata negra, afecta al seu èxit reproductiu. El mosquiter canari va ser el visitant més freqüent al inici de l'estació de floració mentre que l'abella de mel va ser predominant durant els mitjans i darrers períodes de floració. Els aucells incrementaren el quallat del fruit mentre que els insectes tingueren un efecte negligible. A més de contribuir poc a la reproducció de les plantes, les abelles de la mel podrien interferir en la pol·linització dels aucells, buidant les flors del seu nèctar. Les rates consumiren devers el 10% de les flores i reduïren el quallat del fruit a una tercera part. Ambdues espècies alienes poden amenaçar la reproducció de *C. canariensis* i per tant el sosteniment de la població a la vegetació termoescleròfila.

El capítol 4 investiga l'efectivitat relativa de grups vertebrats diferents (aucells and sargantanes) a dos nivells diferents (és a dir, pol·linització i dispersió de llavors) del cicle reproductiu de *Canarina canariensis* a l'hàbitat termoescleròfil. Hem construït tres models estocàstics (principalment els models de 'pol·linització', 'dispersió' i 'pol·linització + dispersió') que simularen el reclutament de plàntules. L'etapa de dispersió va contribuir més que l'etapa de pol·linització en el procés de reclutament. A més, els aucells i les sargantanes exhibiren una complementarietat funcional, amb els aucells contribuint més al model de pol·linització i les sargantanes al de dispersió.

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1. PROLOGUE

1.1 General Introduction

Plant reproductive success is the result of the interactions of both biotic and abiotic components with maternal constraints (Lee 1988). Abiotic interactions refer to interaction with non-living components, e.g. temperature, light, moisture levels, etc., whereas biotic interactions refer to interaction with other organisms. The type of biotic relationships ranges from mutualism (e.g. pollination, seed dispersal) to antagonism (e.g. predation, competition, parasitism). These interactions, in turn, are interlinked themselves, thus not having an additive effect, which generates complex patterns of direct and indirect consequences on plant fitness (Strauss and Irwin 2004). Mutualistic interactions are widespread in nature, as virtually any species on Earth is involved in one or more of them (Bronstein *et al.* 2006).

Plant-pollinator mutualism

About 90% of the plants with flowers are pollinated by animals (Ackerman 2000; Ollerton *et al.* 2011) and around 70% of the 57 crop plants that provide 99% of the world's food are favoured by animal pollination (Klein *et al.* 2007). Pollination is thus also considered an ecosystem service (Costanza *et al.* 1998; Hein 2009). Pollinators greatly enhance reproduction in the majority of plant species (Ashman *et al.* 2004) and are essential for obligate outcrossing animal-pollinated plants (Aguilar *et al.* 2006; Potts *et al.* 2010). Moreover, they play a key role in population dynamics, biodiversity maintenance, diversification, species coevolution and community structure (Pellmyr 2002; Waser and Ollerton 2006; Bascompte and Jordano 2007). The majority of animal pollinators are insects (e.g. bees, butterflies, flies, moths and beetles) and nearly 290 000 flower-visiting insect species have been reported worldwide (Nabhan and Buchmann 1997), but vertebrates also constitute a good proportion of the fauna feeding on floral resources such as nectar and pollen (Faegri and van der Pijl 1966; Proctor *et al.* 1996; Fleming and Muchhala 2008).

Floral syndromes, opportunist vertebrates and selection

The concept of pollination syndrome implies specific flower characteristics associated with different pollination mechanisms within an evolutionary framework (Faegri and van der Pijl 1966; Stebbins 1970; Proctor and Yeo 1973; Johnson and Steiner 2000). However, pollination systems are usually more complex than floral traits per se and may attract a large range of visitors, resulting in criticisms of the pollination syndrome theory (Waser *et al.* 1996).

Ornithophily - bird pollination - is a well studied pollination syndrome, and thousands of species of flowering plants depend on birds as pollinators (Proctor *et al.* 1996; Rodríguez-Gironés and Santamaría 2004). Flower features traditionally associated with ornithophily include scarlet, red or orange color, absence of scent and nectar guides, large, funnel-like flowers and abundant and dilute nectar (Faegri and van der Pijl 1966; Johnson and Nicolson 2008). The bird pollination syndrome has evolved independently from bee-pollinated systems in many groups of plants, ornithophily being an evolutionary dead-end, and where turnabout is uncommon and unlikely (Wolfe *et al.* 2006; Wilson *et al.* 2007; Rausher 2008; Tripp and Manos 2008; Ley and Claßen-Bockhoff 2011; Valente *et al.* 2012; Van der Niet and Johnson 2012). Evolutionary dead-ends imply highly specialized interactions, which are more vulnerable to extinction than more generalized ones (Ojeda 2013). Usually, transition in a pollination system starts with the flower pigmentation changing towards red colours, as in *Lotus, Mimulus* and *Ipomoea* (Cronk and Ojeda 2008).

Three families of birds have arisen as flower specialists: 1) the hummingbirds (Trochilidae), restricted to America but with fossil evidence from Europe (Mayr 2004); 2) the sunbirds and spiderhunters (Nectariniidae) in Southwest-Asia and Mid-south Africa; and 3) the honeyeaters (Meliphagidae) in Oceania; although the icterids, tanagers, honeycreepers, sugarbirds, white-eyes, flower-peckers and lorikeets are also important flower visitors (Carstensen and Olesen 2009). In addition to these groups, up to 50 families of generalist birds act as opportunistic nectar feeders, sometimes while seeking insects concealed in the inflorescences (Proctor and Yeo 1973; Proctor *et al.* 1996). This behaviour has been noticed in Australia (Franklin 1999; Franklin and Noske 1999, 2000), Africa (Pettet 1977), Europe (Kay 1985; Búrquez 1989; Schwilch *et al.* 2001; Merino and Nogueras 2003) and central and south America (Fisk and Steen 1976). However, in Europe, there is only one report of a bird-pollinated native plant, *Anagyris foetida*, in Spain (Ortega-Olivencia *et al.* 2005). Likewise, there are flowers that are typically ornithophilous, but many others visited by birds have more generalized syndromes.

These vertebrates may be more 'trustworthy' pollinators than insects under particular ecological circumstances, specifically when the latter are scarce and have low population densities, e.g. in high altitude ecosystems, bad weather conditions and isolated islands, and for winter-flowering plants (Cronk and Ojeda 2008 and references therein) and are attracted by nectar, and also pollen, floral oil, petals, water and flower visiting insects (Grant 1966; Cecere *et al.* 2011). These rewards can be essential for both specialized and non-specialized nectarivorous birds, especially under food shortage (Búrquez 1989; Cronk and Ojeda 2008; Cecere *et al.* 2011).

Reptiles, specifically lizards and geckos, are one of the oldest biotic pollen vectors (Williams and Adam 2010) and also visit flowers in search of nectar, pollen and insects. However, they were ignored before the nineties because most were regarded as carnivorous (Greene 1982; van Damme 1999; Cooper Jr and Vitt 2002). Indeed, only ca. 1% are truly herbivorous (Cooper Jr and Vitt 2002), but many lizards have a broad diet (including flowers, fruit, nectar and pollen besides small animals). At least 37 lizard species visit/pollinate flowers in seven families: Iguanidae, Gekkonidae, Lacertidae and Teiidae being the most common. Saurophily seems to be an island phenomenon (Olesen and Valido 2003b) and has been reported on islands in the Pacific, Atlantic and Indian Oceans, even if their continental ancestors are typical insectivorous species (Olesen and Valido 2003b). In fact, the first time lizards were seen to be effective pollinators was in the Balearic Islands (Traveset and Sáez, 1997).

Islands, biodiversity importance and threats

Oceanic island ecosystems possess a unique evolutionary history as a result of their small size and geographic isolation (Warren *et al.* 2015). Their isolation restricts colonization and dispersal processes, which leads to depauperate and disharmonic faunas and floras (MacArthur and Wilson 1967; Carlquist 1974; Abbott 1976; Tanaka and Tanaka 1982; Becker 1992; Barrett 1996; Gillespie and Roderick 2002; Patiny 2012). Successful colonization and establishment on islands for any group of organisms is influenced by their life histories and reproductive systems (Gillespie and Roderick 2002). For instance, there is a scarcity of insects and mammals on oceanic islands (Carlquist 1974; Gillespie and Roderick 2002), and the requirement of specialized animal pollinators for some plant species may be an impediment to their establishment (Barrett 1996). The usually high incidences of wind-pollinated plants on oceanic islands actually led to the hypothesis of the benefits of wind pollination on islands (Carlquist 1974; Crawford *et al.* 2011) and there is evidence that several originally specialist insect-pollinated plant lineages switched to wind or generalist pollination after island colonization (Crawford *et al.* 2011 and references therein). In spite of this, island floras possess a high number of endemic species in relation to their small area. The most important factors driving such extensive speciation are radiation into diverse habitats and genetic drift (Crawford *et al.* 1987; Baldwin *et al.* 1998; Emerson 2002).

Pollination networks on islands

As a consequence of their depauperate and disharmonic fauna and flora, island plant pollinator networks are also simpler and poorer in species compared to continental ones (Olesen and Jordano 2002; Dupont *et al.* 2003; Philipp *et al.* 2006; Traveset *et al.* 2015). Moreover, oceanic island networks often feature supergeneralist species, i.e. those that interact with a disproportionate number of species (Olesen *et al.* 2002). Such supergeneralist species are important constituents of island ecosystems and are those favouring novel mutualistic interactions.

Vertebrate pollination on islands

Novel pollination interactions between plants and opportunistic non-typical vertebrate pollinators, such as birds and lizards, are particularly prevalent in island systems (Grant and Grant 1981; Vogel *et al.* 1984; Olesen 1985;

Whitaker 1987; Traveset and Sáez 1997; Valido *et al.* 2002; Olesen and Valido 2003a, 2003b, 2004). The occurrence of novel pollinator assemblies might be consequence of the phenomenon of density compensation undergone by some species in islands (Olesen and Valido 2003a, 2003b, 2004). According to the classic niche theory, a lower species density in islands involves weaker interspecific competition, leading to a niche shift or expansion and hence an increase in density (MacArthur *et al.* 1972). Density compensation has been documented in both bird (Grant 1966; MacArthur *et al.* 1972; Wright 1980) and lizard populations (Case 1979; Rodda and Dean-Bradley 2002) but not in insects (Janzen 1973; Connor *et al.* 2000; Ruesink and Srivastava 2001). To sum up, 1) high abundance of individuals, 2) scarcity of insects (as pollinators and food), both these reasons giving 3) a surplus of floral food, and in addition to 4) a reduced risk of predation will ultimately drive vertebrates to consume novel plant resources such as pollen and nectar (Olesen and Valido 2003a, 2004; Valido and Olesen 2010).

This phenomenon has been especially documented in the tropics (Anderson 2003), although more recently also in temperate regions (da Silva *et al.* 2014). Moreover, opportunistic nectarivory by generalist birds and lizards is considered casual in mainland systems, but is a relatively common phenomenon on oceanic islands (Grant and Grant 1981; Olesen 1985; Traveset and Sáez 1997; Hansen *et al.* 2002; Olesen and Valido 2003a; Rodríguez-Rodríguez *et al.* 2013). Examples among birds include Darwin finches in the Galápagos (Grant and Grant 1981), Madagascar fody (Safford and Jones 1998), red-whiskered bulbul (Olesen *et al.* 1998), Mauritius grey and olive white-eyes in Mauritius (Hansen *et al.* 2002) or Japanese white-eye (Pimm and Pimm 1982; Lammers *et al.* 1987) and Hawaiian crow in the Hawaiian islands (Cox 1983); and within lizards the pacific Duvaucel´s geckos in New Zealand (Whitaker 1987), Lilford´s wall lizard in Balearic Islands (Traveset and Sáez 1997) or the southern snow skink in Tasmania (Olsson *et al.* 2000).

Still, there is no proof that island opportunist birds exert a selective pressure strong enough to lead towards an evolution of traits related to plant-bird interactions. Regarding lizards, despite being shown to play a central role in the reproductive ecology of plants from different families (Olesen and Valido 2003b, 2004; Valido and Olesen 2007; Hansen and Müller 2009), there is little evidence that lizards can effect selective pressures on floral traits, although coloured nectar seems to have evolved as a response to saurophily (Hansen *et al.* 2006, 2007).

Seed dispersal

Frugivory constitutes another outstanding interaction between animals and plants. Fruits are a source of food for animals, whereas fleshy fruits encourage animal-mediated seed dispersal (Jordano 2000; Herrera 2002). Frugivorous animals, notably birds and mammals, can process fruits in several ways: typically either (1) swallowing entire fruits and dispersing their seeds (legitimate seed dispersers) or (2), pecking/biting fruits for their pulp or seeds (seed or pulp predators, Jordano and Schupp 2000). Hence, plant fitness can be strongly influenced by the relative frequency of each type of interaction with frugivores (Jordano 2000). Besides this, the spatial pattern of seed dispersal may shape post dispersal processes such as seed survival or seedling establishment (e. g. Jordano and Schupp 2000; García *et al.* 2005) and fruit predation may override other pre-dispersal processes such as pollination (e.g. Traveset 1994; Wang and Smith 2002).

Double mutualisms on islands

Most mutualistic interactions between animals and plants are generalized, frequently involving dozens of species, especially on islands. Highly specific mutualistic interactions are very rare (Jordano 1987). Moreover, the pollinator and seed disperser fauna for most plants are different from each other (Proctor *et al.* 1996).

There are few cases, however, in which a given animal species acts as both pollinator and seed disperser, so-named a double mutualism (Hansen and Müller 2009). Double mutualists can be lizards, birds, bats and even ants, and are more frequent in habitats poor in animal diversity such as islands (Olesen *et al.* 2012), deserts or semiarid ecosystems (Gomes *et al.* 2014), and mountains and cliffs (García *et al.* 2012). A double mutualism that couples pollination and seed dispersal in the same plant species displays a double positive feedback loop (Olesen *et al.* 2018). In other words, there are two main mutualistic routes in such a system. In the first, if the animal increases pollination it will improve the plant's reproductive output, thus rewarding the animal with more fruit in the fruiting season. In the second route, if the animal disperses more fruit it will increase plant recruitment, which will also produce more flowers in the next generation, thus rewarding the next generation of animals. From the plants' point of view, if the flowers offer more resources they increase animal fitness, translating into more seed dispersers later on; similarly, more fruits will tend to increase animal fitness and increase the density of pollinators for the next flowering season (Fuster *et al.* 2018, Olesen *et al.* 2018). The time delay for this reward can vary from nil (if plants set flowers and fruits simultaneously) to several months (the period between the flowering and the fruiting season), and to years (the period between seed dispersal to the first production of flowers).

In general, positive feedback loops and strong omnivory are unstabilizing for the community (Gellner and McCann 2012), and using the same servicing partner twice may also jeopardize plant and animal species survival. Thus, for instance, the loss of an endemic vertebrate mutualist might trigger an extinction cascade which could be particularly grave if the double mutualism core of an island network is destroyed (Anderson *et al.* 2011) or if one species declines as a result of disturbances. In addition, double mutualists might increase each others' risk of coextinction. However, potential benefits include mutually sustained population size, efficient use of resources and increased fitness.

Invasive species

Currently, the introduction of non-native species is one of the main problems for conservation and is considered the second cause of biodiversity loss, after the destruction of habitats (Williamson 1996; Vitousek *et al.* 1997; Soulé 2000, UICN 2000). Most of the planet's ecosystems are affected by the presence of invasive species (Wilcove *et al.* 1998; Levine and D'Antonio 2003). In addition, the effects of biological invasions can interact with the effects of climate change (Walther *et al.* 2009; Mainka and Howard 2010), probably intensifying impacts on ecosystems and increasing the need to effectively manage non-native species (Hellmann *et al.* 2008; Vorsino *et al.* 2014). Although there is some confusion in the terminology used, we can consider that an invasive species is one that is far from its original distribution range, either by the voluntary action of man or by accident; it is capable of reproducing itself and presents stable populations with a tendency towards expansion within the ecosystems in which it has managed to establish itself (Richardson *et al.* 2000).

Biological invasions may imperil native species through direct (e.g. aggression, predation, infection, competition) or indirect effects (altering their mutualistic interactions or ecosystem processes, by hybridizing, introgression, vectoring diseases) (Vitousek *et al.* 1996; Mooney and Cleland 2001 and references therein). These impacts of biological invasions are becoming a major concern, given that in many countries the proportion of non-native flora and fauna is more than 20 per cent of the total number (Vitousek *et al.* 1996).

Islands and invasive species

Spatially restricted environments such as islands and lakes are particularly vulnerable to alien invasions (Worthington and Lowe-McConnell 1994; Sax *et al.* 2002; Hofman and Rick 2018). Islands harbour a considerable portion of global biodiversity and global biota (Kier *et al.* 2009) and at the same time are the recipients of the largest proportional numbers of invaders (Vitousek *et al.* 1996; Mooney and Cleland 2001). On islands there is a greater proportion of endemic species that have evolved with low levels of interspecific competition. They are thus less able to compete with species that arrive from the mainland, more vulnerable to the presence of invasive species and have a higher probability of extinction (Cox and Elmqvist 2000; Simberloff 2000; Gritti *et al.* 2006). On the other hand, invasive species are often those with higher rates of dispersion and reproduction, greater phenotypic plasticity and greater tolerance to climate changes than endemic species that have evolved in isolated conditions (Harter *et al.* 2015). Hence, the disproportionately successful biological invasions in island biotas results from the reduced competition, predation and disease, together with the disharmony of functional groups, lack of diversity, relatively small populations and lack of natural disturbance in the evolutionary histories (Vitousek 1988).

Most islands show a higher percentage of alien than endemic species, many of them naturalized (Vitousek *et al.* 1987, Traveset and Santamaría 2004). Despite this, there is a lack of information about the varied consequences of these new species, despite important effects of the same problem being observed in other ecosystems (Levine *et al.* 2003).

Rats, honeybees and goats on islands

Among the most dangerous invaders worldwide, rats (*Rattus* spp.) prey upon native animals and also consume and destroy plant matter, affecting reproductive, photosynthetic and supporting parts, ranging from flowers, fleshy fruits, seeds and seedlings, to roots, rhizomes, buds, leaves and bark (Cuddihy and Stone 1990; Sugihara 1997; Harper and Bunbury 2015). Indeed, plant material constitutes an important fraction of the diet of rats, making up 73-99% of their stomach contents (Traveset *et al.* 2009 citing Cheylan 1982). Rats have reached about 90% of the world´s islands and are among the most successful invasive mammals (Martin *et al.* 2000; Towns *et al.* 2006 and references therein). Introduced rats threaten native plants and animals (Hernández *et al.* 1999; Martin *et al.* 2000; Towns *et al.* 2006), ecosystem functioning (Towns *et al.* 2006) and plant-animal mutualistic interactions (Traveset and Richardson 2006; Traveset *et al.* 2009).

On the other hand, the honeybee (*Apis mellifera*) originated in Africa or Asia and naturally expanded into the Old World (Whitfield *et al.* 2006; Han *et al.* 2012). It has been introduced in most parts of the world to produce honey and improve crop pollination (Moritz *et al.* 2005). Traditionally, the introduced honey bee has been considered an effective flower pollinator and thus beneficial for plants in particular, and for the ecosystem in general. However, from the seventies up to now, many studies have pointed out that the massive presence of honey bees disrupts native plant-animal interactions by means of exclusive competition with the native pollinators, and by reduction of fitness in plants (Roubik 1978; Schaffer *et al.* 1983; Sugden and Pyke 1991; Hansen *et al.* 2002).

Finally, the feral goat (*Capra hircus*), native to Asia, has been introduced in most parts of the world to produce milk and meat. They can have a devastating impact in island ecosystems, causing wholesale changes to plant communities (Donlan *et al.* 2002) and threatening the populations of vulnerable plants (Gurevitch and Padilla 2004). They are also responsible for the rarefaction and extinction of several endemic plants (Turbott 1948; Coblentz 1978; Parkes 1993), for defoliation and erosion, affecting the breeding burrows of seabirds (McChesney and Tershy 1998) and for the impoverishment of vertebrate and invertebrate fauna due to overgrazing (Hamann 1975; Brook 2002).

The Canary Islands

This archipelago lies off the northwest coast of Africa and includes seven main islands together with a number of smaller islets. They are also older, larger and closer to the mainland than the other Macaronesian archipelagos. This may explain their habitat diversity, number of species (ca. 1,400 plant and animal species, Sundseth 2005), and percentage of endemic taxa (ca. 45% of the Canarian vascular flora, Caujapé-Castells *et al.* 2010). For this reason, Canary Islands are considered a hotspot of plant diversity within the Mediterranean global diversity hotspot (Médail and Quézel 1997). They also possess the highest percentages of endemic plants in Europe (González and Fuertes 2011) and their endemic flora and fauna make the archipelago one of the biodiversity hotspots of the planet (Sundseth 2005). However, it has been estimated that 113 of the Canary Islands endemic taxa are endangered (UICN red list 2014).

Ornithophily and saurophily in the Canary Islands

Many plant species in this archipelago possess flowers ‘adapted’ to bird pollination. However, specialist nectar feeders are absent from the islands, both nowadays and in the fossil record (Valido *et al.* 2004) and this vacant mutualistic service is currently occupied by opportunistic passerine birds and lizards (Valido *et al.* 2004). The origin of the ornithophilous floral traits present in Canary plants is uncertain and seems to depend on their group. Some species evolved ornithophily after island colonization under the influence of opportunistic vertebrates (e.g. *Lotus* sect. Rhyncholotus: Ojeda *et al.* 2012; *Scrophularia* *calliantha*: Navarro-Pérez *et al.* 2013). In contrast, the ancestors of some lineages were already adapted to bird pollination before their arrival on the islands (e.g. *Canarina canariensis* Olesen *et al.* 2012; Mairal *et al.* 2015a).

This opportunistic pollination in the Canary Islands began to attract interest in the 1980s, when researchers listed potential native plant species that fitted with an ornithophilous syndrome and could be pollinated by opportunistic birds (Vogel *et al.* 1984; Olesen 1985). Later, researchers also started to pay attention to opportunistic flower visitation by Canarian Lacertidae (Speer 1994; Valido *et al.* 2002; Nelson 2010). Generalist passerine birds, which habitually visit flowers for nectar include the Eurasian blackcap (*Sylvia atricapilla*), spectacled warbler (*S. conspicillata*) and Sardinian warbler (*S. melanocephala*) from Sylvidae, the Canary Islands chiffchaff (*Phylloscopus canariensis*) from Phylloscopidae, the Eurasian blue tit (*Cyanistes caeruleus*) from Paridae, and the Atlantic canary (*Serinus canaria*), the common chaffinch (*Fringilla coelebs*) from Fringillidae, whereas the endemic lizards are *Gallotia galloti*, *G. atlantica*, *G. stehlini* and *G. caesaris* from Lacertidae (Olesen 1985; Valido *et al.* 2002; Olesen and Valido 2003b; Rodríguez-Rodríguez and Valido 2008, 2011; Nelson 2010; Valido and Olesen 2010; Fernández de Castro *et al.* 2017).

These vertebrates are flower visitors of the classical Canarian ornithophilous plant genera such as *Isoplexis*, *Echium*, *Canarina*, *Aeonium*, *Lotus*, *Navaea*, *Euphorbia*, *Teucrium*, *Rhamnus* or *Scrophularia* (Vogel *et al.* 1984; Olesen 1985; Valido *et al.* 2002; Olesen and Valido 2003b; Valido and Olesen 2010).

Invasive species in the Canary Islands

In this archipelago, rats (*Rattus* sp.) are known to prey upon several species of land- and sea- birds, lizards and gasteropod species (Nogales *et al.* 2006; Traveset *et al.* 2009 and references therein) and have been interacting with the Canarian native plants probably since the arrival of Europeans (Traveset *et al.* 2009). The abundance of rats shows a patchy distribution on the islands in terms of habitat and localization. For example, on Tenerife, rats are more abundant in Anaga compared with Teno (Hernández *et al.* 1999; Rodríguez-

Rodríguez and Valido 2011), and in the laurel forests compared with the pine forests (Delgado *et al.* 2001).

Apis mellifera is present on all the Canary Islands except Lanzarote and Fuerteventura. Since honey-bees have been domesticated for millennia, their native range and taxonomic status (native vs. introduced) is still controversial (Hohmann *et al.* 1993; Arechavaleta *et al.* 2010). The most plausible option is that it has been introduced by humans as on most other oceanic islands around the world (Michener 1979; Moritz *et al.* 2005). Moreover, the number of managed beehives has dramatically increased in the last 20 years, from 20,293 beehives in 1997 to 38,699 beehives in 2017, ca. half of them are situated on Tenerife (Dirección General de Ganadería 2006; Subdirección General de Productos Ganaderos 2017), causing dramatic changes in pattern visitation of native pollinators (Valido *et al.* 2019).

Capra hircus was introduced into the Canary archipelago since pre-Hispanic times, ca. 2,000 years BP. In the last 40 years, the number of feral goats has decreased due to changes in farming practices, but some animals have remained and reproduced on the steep slopes of the islands. Feral goats affect native vegetation in both high mountains and pine forests (Nogales *et al.* 2006, citing Sventenius 1946 and Ceballos and Ortuño 1976) and facilitate the establishment of introduced and ruderal plants (Dickson *et al.* 1987).

1.2 General Objectives of the Thesis

The effectiveness of generalist vertebrates as pollinators has been confirmed so far in the Canary Islands only in a few studies for *Isoplexis canariensis* (Rodríguez-Rodríguez and Valido 2008), *Canarina canariensis* in laurel forest (Rodríguez-Rodríguez and Valido 2011) and *Navaea phoenicea* (Malvaceae) (Fernández de Castro *et al.* 2017). With the main goal of providing further insights into the role of vertebrates in plant reproductive success in the archipelago, and on the evolution of the bird pollination syndrome in the macaronesian islands, I selected the endemic *Echium simplex* and *Canarina canariensis*, both belonging to the Macaronesian bird-flower element (Vogel *et al.* 1984; Olesen 1985; Valido *et al.* 2004; Valido and Olesen 2010). There was no information for *Echium simplex* on its breeding and pollination system, whereas for *Canarina canariensis* previous studies in laurel forest showed effective bird pollination, fruit predation by rats (Rodríguez-Rodríguez and Valido 2011), and flower visitation by *Apis mellifera* (Ollerton *et al.* 2009b; Valido and Olesen 2010).

Below I outline the particular objectives addressed within each chapter:

Chapter 1. Reproductive success of the Canarian endemic *Echium simplex* (Boraginaceae), mediated by vertebrates and insects

- To assess the breeding system of *Echium simplex*.
- To determine the diurnal flower visitors of *Echium simplex*, both vertebrate and invertebrate.
- To evaluate the effectiveness of vertebrates compared to insects as pollinators of *Echium simplex*, in terms of fruit and seed set, seed weight and germination.
- As naturalized goats were observed feeding on reproductive *Echium simplex* plants, to quantify the level of herbivory on this endemic plant.

Chapter 2. Effect of diurnal *vs*. nocturnal pollinators and flower position on the reproductive success of *Echium simplex*

- To determine the nocturnal flower visitors of *Echium simplex*, both vertebrates and invertebrates.
- To investigate the pollination effectiveness of diurnal and nocturnal insects in *Echium simplex*.
- To study reproductive success of within inflorescence spatial positions in *Echium simplex*.

Chapter 3. Impact of alien rats and honeybees on the reproductive success of an ornithophilous endemic plant in Canarian thermosclerophyllous woodland relicts

- To describe the flower visitors of *Canarina canariensis*, both vertebrate and invertebrate.
- To evaluate the effectiveness of vertebrates compared to insects as pollinators of *Canarina canariensis*, in terms of fruit and seed set, seed weight and germination.
- To assess the levels of floral damage and its consequences on fruit production in *Canarina canariensis*.

Chapter 4. Pollination and seed dispersal effectiveness of birds and lizards in a double mutualism system

- To predict seedling recruitment by means of three stochastic models in *Canarina canariensis*.
- To evaluate the relative importance of each mutualistic process (pollination and seed dispersal) as drivers of the natural regeneration of *Canarina canariensis*.
- To establish the relative contribution of birds and lizards to both pollination and seed dispersal processes in *Canarina canariensis.*

2. MAIN CHAPTERS

2.1 Chapter 1

Reproductive success of the Canarian Echium simplex (Boraginaceae) mediated by vertebrates and insects

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Abstract

Oceanic island ecosystems favor the appearance of novel interactions as a consequence of their depauperate and disharmonic flora and fauna. *Echium simplex*, endemic to the Anaga Biosphere Reserve in NE Tenerife, Canary Islands, belongs to the Canarian bird–flower element. Along two flowering seasons, we studied its breeding system, identified the floral visitors, and compared the pollination effectiveness of different animal guilds (insects vs. vertebrates) by means of selective exclosures. The plant showed to be self-compatible but selfing significantly reduced fruit set. The flowers were visited by five bird species (mostly *Phylloscopus canariensis* and *Serinus canarius,* but also *Cyanistes teneriffae*, *Sylvia atricapilla* and *Sylvia melanocephala*), a lizard species (*Gallotia galloti*), and over a hundred insect species (mainly hymenopterans and coleopterans). Flying insects increased fruit set whereas small flower dwellers (mostly beetles) decreased both fruit and seed set; by contrast, vertebrates had a negligible effect on reproductive success. We conclude that despite the floral resources provided by *E. simplex* may be important to some vertebrate species, these do not appear to notably contribute to increase the fitness of the plant, which show to be more dependent upon flying insects to set fruits and seeds. We

additionally found that plant reproductive structures are heavily damaged by feral goats, which threaten the maintenance of this Canarian endemic species.

2.1.1 Introduction

Due to their geographic isolation and relatively small size compared to inland areas, oceanic island ecosystems possess unique evolutionary histories (Warren *et al.* 2015), often bearing a high number of endemic species (Carlquist 1974; Kier *et al.* 2009). The isolation hinders colonization and dispersal processes, what leads to depauperate and disharmonic biota (Gillespie and Roderick 2002). Successful colonization and establishment on islands for any group of organisms is influenced by their life histories and reproductive systems (Gillespie and Roderick 2002). Thus, for instance, the requirement of specialized animal pollinators for some plant species may be an impediment for their establishment (Barrett 1996). The usually high incidence of wind-pollinated plants on oceanic islands actually led to postulate the hypothesis on the benefits of wind pollination on islands (Carlquist 1974; Crawford *et al.* 2011 and references therein), and there is evidence that several originally specialist insect-pollinated plants lineages switched to wind- or generalist pollination after island colonization (Crawford *et al.* 2011 and references therein). As a consequence of the depauperate and disharmonic flora and fauna, island plant-pollinator networks are much poorer in species and less complex than those in mainland areas, especially oceanic islands (Traveset *et al.* 2015). Moreover, oceanic island networks often bear supergeneralist species, i.e. those that interact with a disproportionate number of species (Olesen *et al.* 2002). Such supergeneralist species are important elements of island ecosystems and are those favouring novel mutualistic interactions.

Novel pollination interactions between plants and opportunistic, food generalist vertebrates, such as birds and lizards, are particularly prevalent in island systems (e. g. Grant and Grant 1981; Olesen 1985; Traveset and Sáez 1997; Olesen and Valido 2003b). This phenomenon has been especially documented from the tropics (Anderson 2003), although more recently also from temperate regions (da Silva *et al.* 2014), and it has been interpreted as the response of such vertebrates to density compensation and trophic niche expansion undergone in both bird (Wright 1980 and references therein) and lizard populations (Rodda and Dean-Bradley 2002 and references therein) in island areas. Under food shortage, flower rewards such as nectar, pollen, floral oil, petals, water and flower-visiting insects attract potential vertebrate pollinators (Cecere *et al.* 2011), and can be essential for both specialized and non-specialized nectarivorous species (Cronk and Ojeda 2008; Cecere *et al.* 2011).

These vertebrates may be more ‘trustworthy’ pollinators than insects under particular ecological circumstances, specifically when the latter are scarce and have low population densities, e.g. in high altitude ecosystems, bad weather conditions, isolated islands, and for winter-flowering plants (Cronk and Ojeda 2008 and references therein). Typical ornithophilous (bird-visited) flowers, in particular, possess a set of features (bird pollination syndrome), such as scarlet, red or orange colour, absence of scent and nectar guides, large, funnel-like flowers, and abundant and dilute nectar (Faegri and van der Pijl 1966; Rausher 2008). This syndrome has evolved independently in many groups of plants from bee-pollinated systems, being ornithophily a dead evolutionary end, and where turnabout is uncommon and unlikely (Rausher 2008; Van der Niet and Johnson 2012; Ojeda 2013). Usually, transition in a bird pollination system starts with the flower pigmentation changing towards red colors, as in *Lotus*, *Mimulus* and *Ipomoea* (Cronk and Ojeda 2008). Such evolutionary dead end implies highly specialized interactions, which are more vulnerable to extinction than more generalized ones (Ojeda 2013).

The Macaronesian islands have received much attention regarding flower visitation by generalist passerines since the late XIX century and early last century (Porsch 1924 citing observations made by Lowe in 1896; Schmucker 1936; Ojeda 2013). Nearly 20 plant species, most of them endemic, from different families have been reported to be ornithophilous. The assemblage of such species is actually referred as the Macaronesian bird flower element which includes plant genera such as *Isoplexis, Scrophularia, Echium, Canarina, Aeonium, Lotus, Anagyris, Lavatera, Euphorbia, Teucrium, Rhamnus*, *Scrophularia* and *Navaea* (Valido and Olesen 2010). Generalist vertebrates that often visit flowers for nectar include bird species in the genera *Phylloscopus, Cyanistes, Sylvia, Serinus* and *Fringilla* species among birds, and lizard species belonging to the genus *Gallotia*. Most ornithophilous plants in the Canary Islands, in particular, have shown to descend from continental ancestors that had entomophilous flower traits (Valido *et al.* 2004). One exception is, however, the *Canarina* genus in which ornithophily already evolved in continental ancestors in response to specialized nectarivorous sunbirds (Olesen *et al.* 2012). No evidence of sunbirds, present in the past in the mainland (Mayr and Wilde 2014), exists from the fossil record of the Canarian archipelago (Valido *et al.* 2004).

The effectiveness of generalist vertebrates as pollinators has been confirmed so far only in a few studies in the Canarian archipelago for *Isoplexis canariensis* (Rodríguez-Rodríguez and Valido 2008), *Canarina canariensis* (Rodríguez-Rodríguez and Valido 2011) and *Navaea phoenicea* (Malvaceae) (Fernández de Castro *et al.* 2017). With the main goal of providing further insights on the role of vertebrates in plant reproductive success in the Canarian archipelago, and on the evolution of the bird pollination syndrome in Macaronesia, we selected the endemic *Echium simplex* (Boraginaceae) which belongs to the Canarian bird-flower element (Valido and Olesen 2010) and for which there was no information on its breeding and pollination system. The main objective of our study was to experimentally determine the effectiveness of vertebrates (birds and lizards, specifically) as pollinators and compare it to that of insects. We first assessed the breeding system of the plant and then evaluated the relative effectiveness of the different groups of flower visitors as pollinators in terms of fruit and seed production and seed germination. In addition, as naturalized goats were observed feeding on reproductive *E. simplex* plants, at different stages of inflorescence development and often causing important plant damages, we wanted to quantify the level of herbivory in this endemic plant whose most abundant populations are found in the study area.

2.1.2 Materials and Methods

Study species—

The giant rosette plant *Echium simplex* DC. (Boraginaceae), locally known as ‘tajinaste blanco’, is endemic to the Anaga Biosphere Reserve in NE Tenerife (Canary Islands). This area encompasses one of the most recent zones of the island, a 4.9-3.9 million-year-old basaltic massif (Guillou *et al.* 2004). It is considered a vulnerable species in the red list of Spanish vascular flora (Moreno, 2008), with very few, reduced and isolated populations. The species is one of the three monocarpic *Echium* species in the Canary Islands, together with *Echium wildpretti* in La Palma and Tenerife, and *Echium pininana* in La Palma, and it grows for 5-9 years before producing a single inflorescence (Stöcklin and Lenzin 2013). Reproductive individuals reach a height of up to 3 m, of which the prolonged inflorescence - composed of scorpioid cymes - can contribute up to 1.5 m. The inflorescence height is directly proportional to the rosette diameter. The cymes are double coiled and the largest plants may show 3-4 branches per cyme. After a successful pollination event, a flower develops into a fruit which consists of a maximum of four nutlets. The number of cymes and flowers per wrap increases much along the inflorescence. The smallest of our examined plants had an average of 12 flowers per wrap whereas the largest had 51. The number of mature subfruits per flower (from one to three, on average) also increased along the length of the inflorescence. Hence, the number of potential seeds produced increases enormously with the size of the inflorescence, ranging from 4,560 to 234,000 (Stöcklin and Lenzin 2013). Preliminary observations on the flower visitors of *E. simplex* pointed out insect species, mostly honeybees and wild bees, beetles and ants (Stöcklin and Lenzin 2013) and also some passerine birds (Valido and Olesen 2010), but neither quantitative nor qualitative data existed whatsoever previously to this study.

Study area—

The study site is located at the north-west of Chamorga village, northeastern Tenerife (Canary Islands). The population of *E. simplex* is found at an altitude of ca. 250 m a.s.l. and occupies an area of ca. 1 Km^2. There are also scattered individuals along the trail of the north coast. The location has a warm coastal climate with average temperatures between 17 and 19 °C in winter and between 20 and 25 °C in summer (Aemet 2016). The summer is very dry and most rain falls in winter, but only in small quantities (Marzol-Jaén 1988). The area is exposed to the northeastern wind, which is responsible for the lush green vegetation of Anaga mountains. The vegetation is shrubby-herbaceous, dry-Mediterranean and characterized by numerous endemic species such as *Artemisia thuscula, Descurainia millefolia, Aeonium canariense, Asphodelus tenuifolius, Achyranthes aspera* and *Galactites tomentosa.* Fieldwork was conducted during the reproductive season of *E. simplex*, between April and June of 2015 and 2016.

Plant breeding system —

Hand-pollination experiments were performed on a total of 30 randomly selected plants. In each individual plant, we randomly assigned flowers to five different treatments: (1) *autogamy*: flowers were bagged (with muslin) before anthesis and left until fruits were produced to evaluate the autonomous selfing capacity; (2) *apomixis:* flowers were emasculated and bagged to assess their capacity to produce fruits without pollen; (3) *anemogamy:* flowers were emasculated and bagged with a nylon mesh allowing pollen but not insects to pass through; (4) *xenogamy*: a fresh pollen mix source (from 2-3 plants in the same population) was applied to the stigmas of the flowers which had been also previously emasculated, and (5) *open pollination*: a group of flowers from each plant were left as the control treatment, leaving them open to natural pollination.

The apomixis and anemogamy treatments were performed only in 2015 on one flower per plant. By contrast, the autogamy and xenogamy treatments were conducted both in 2015 (on three flowers per plant) and 2016 (on four flowers per plant). Each year, the control flowers were marked simultaneously as pollination treatments were performed.

Flowers used for the autogamy and xenogamy treatments were kept in muslin bags to exclude any animals or potential airborne pollen grains. Bags were removed and the fruits collected when ripen. Fruit set was calculated as the proportion of flowers that became fruit, whereas seed set as the amount of viable seeds produced per fruit. Seeds were regarded as non-viable (aborted) based on a characteristic greyness and smaller size. A germination experiment was conducted in order to test the accuracy of this assumption. A total of 313 seeds were randomly selected from a pool that had been classified as aborted and were left to germinate. Only 4.15% of those seeds germinated successfully in contrast to the 77.02% of the seeds considered as viable, thus we considered our classification as valid.

***Flower visitors and visitation frequency*—**

To identify flower visitors and determine their visitation frequency, a total of 143 individual plants, hapharzardly chosen, were observed during focal censuses. Vertebrates (birds and lizards) were monitored for 30 min per census from a place located 5-10 m away from the plant and by means of binoculars. Flying insects were observed for 10-15 min per census at a shorter distance (0.5 m) from the plant. Insects of all species or morphospecies were captured and taken to the lab for their identification. Flower dweller insects on each censused plant were counted after the flying insects' observations. These direct observations were supplemented by video recording and photographs. Animals were considered as flower visitors whenever they touched the flower, as sexual organs are exerted from the corolla. For each flower visitor, we recorded: species identity (sometimes family or order level for insects), number of flowers visited (for insects and birds when possible), and time on the inflorescence (for birds and lizards). For the vertebrate censuses, we monitored 41 plants during 33.75 h of diurnal observations (N = 72; 15-30 min periods) in 2015 and 41 plants during 104.58 h of diurnal observations (N = 217; 10-30 min periods) in 2016. For the insect censuses, we monitored 35 plants during 10.84 h of diurnal observations (N = 65; 10-min periods) throughout the flowering season of 2015 and 31 plants during 31 h of diurnal observations (N = 120; 15-30 min periods) in 2016. All censuses began on early April and lasted until early June.

***Relative effectiveness of flower visitors as pollinators*—**

Based on the observations of flower visitors, we conducted experiments to study the pollination importance of the different guilds of pollinators (passerine birds, lizards, flying insects and flower dwellers). Five randomly assigned exclusion treatments were performed on each individual plant: (1) *total exclusion*: the whole inflorescence was bagged (with muslin bags) to exclude any type of flower visitor; (2) *bird-exclusion*: the plants were entirely covered by a 5 x 2 cm plastic mesh to avoid birds accessing the flowers; (3) *lizard-exclusion*: a 30 cm diameter acetate funnel was placed at the base of the inflorescence to avoid lizards climbing it; (4) *vertebrate (bird + lizard) exclusion*: plants were covered with the cage and a basal funnel was placed at their base; and (5) *only access by flower dwellers*: plants were covered by a nylon mesh bag with 3 x 3 mm openings that allowed small insects (mainly small beetles and ants) to go through and crawl between flowers. Another group of plants were used as controls, i.e. they were left open to all flower visitors. A total of 10-15 individuals were used for each treatment. The plants were inspected daily to guarantee that the enclosures did not interfere with the flower visitors while foraging.

Fruits were collected when ripe and taken to the laboratory, where seed viability was evaluated using the procedure described above. In 2015, all treatments were performed, but in 2016 we only repeated treatments 1 (total exclusion) and 5 (only flower dwellers exclusion), plus the control group. For each treatment, we assessed again fruit and seed set.

In order to test for differences in germination patterns (germinability and germination rate) among treatments, we carried out germination trials in a greenhouse in Tenerife. A total of 2,245 viable seeds were sown in early October (both in 2015 and 2016) into trays filled with a 1.2.1 mixture of peat, common agricultural soil and ravine sand. Trays were periodically watered every two days to ensure that the soil was constantly moist, and seedling emergence was registered every five days for three months until January, when the germination experiment concluded after no seed germination for more than 25 days. Germinability refers to the fraction of seeds that germinated, whereas germination rate is the number of days elapsed since seed sowing to seedling emergence. Sown seeds of each treatment were previously weighted to the nearest 0.1 mg.

Herbivory levels—

Most reproductive individuals in the population were checked for herbivore damage in late June in 2015 and 2016 and, when this was present, it was classified into four types depending on the stage at which it was produced and on the extent of the damage: (1) at early stage with total or partial herbivory of the flower meristem, causing total lack of flowers, or delayed and ball-shaped flowering; (2) at intermediate stage with apical herbivory of the growing inflorescence stem, causing short/square-shaped flowering; and (3) at final flowering stage, causing well-shaped but with some grazed parts of the inflorescences. In the latter case, the percentage of the plant that was affected was also recorded.

Statistical analyses—

We used generalized linear mixed models (GLMM) in R software version 3.3.3 (R Core Team 2018) followed by a Tukey test of multiple comparisons. For the hand-pollination, plant exclusion and germination experiments, each estimate of plant reproductive success (i.e. fruit set, seed set, seed weight, germinability, and germination rate) was analyzed separately as a dependent variable. Treatment and year were used as fixed factors, and seed weight and inflorescence height were also included in the models as covariables; these were removed from such models when non-significant. Individual plant was used as random effect to control for lack of independence among flowers on the same individual plant. Differences in fruit set and germinability were estimated using a binomial error distribution and logit link function whereas a Poisson family was used to test for differences in seed set and germination rate (as the data was a discrete count of seeds or days, respectively). Seed weight was normally distributed and, for this variable, we thus adjusted errors to a Gaussian distribution.

To test for differences in flower visitation rates, we built a model including as response variable the number of flower visits per unit time and per flower, whereas the predictor variables were: animal group, year, day, time of the day, and number of flowers per plant. Besides differences between the animal groups, we thus aimed at detecting differences in the pattern of flower visitation between years, within the season and along the day; we further included individual flower crop as this might be an important factor determining the visits of a given guild of pollinators. We performed separate analyses considering the different types of censuses carried out, i.e., for insects and for vertebrates. We evaluated colinearity by means of the variance inflation factor (VIF), and variables with VIF value > 3 were removed from the model (Zuur *et al.* 2009). To find the best model, we used model selection with the dredge function in the package *MuMIn* (multimodel inference) and adjusted data to a gamma error distribution. Again, observed plant was used as random effect. The VIF analysis showed that all predictors had a low colinearity (< 3) and thus were included in the models

Pollination effectiveness (PE) was calculated and pollination landscape built using the methodologies developed by Reynolds and Fenster (2008) and Schupp *et al.* (2010). Only the major groups of flower visitors (i.e. birds, lizards, flower insect dwellers and flying hymenoptera) were considered in this analysis. The quantitative component (QNC) was considered as the number of visits per 500 flowers per hour, whereas the qualitative component (QLC) was the percentage of fruit set. Mean and standard error of each component was estimated using 500 simulations on boostrap resamples of 80 empirical data in order to combine estimates of QNC and QLC subcomponents obtained in different sets of field observations and experiments. The final QNC and QLC subcomponents were obtained as the average across resampling trials to evaluate the stability of the estimation, and pollination effectiveness was calculated as the product of QNC and QLC subcomponents. Throughout the paper, all means are accompanied with their standard errors unless otherwise indicated.

2.1.3 Results

Flower characteristics—

Flowers are protandrous and open during two to three days (N = 90). The carpel elongates and splits becoming longer than the anthers during the female phase. The flowers open successively from the proximal to the distal part of the cyme. The total flowering time of a plant is 3-5 weeks. We studied the nectar standing crop in 20 plants, finding that it varied significantly during flower ontogeny ($\chi^2 = 6.53$, df = 2, P = 0.04), with male and transitional flowers producing more nectar (2.05 ± 0.46 µl, N = 23; and 1.89 ± 0.48 µl, N = 28, respectively) than females flowers (1.09 ± 0.24 µl, N = 37).

However, we did not detect significant differences ($\chi^2 = 4.72$, df = 2, P = 0.09) in sugar concentration between flower phases; it was 15.85% ± 0.93 in male flowers (N = 22), 18.18% ± 0.74 in transitional flowers, (N = 23) and 16.71% ± 0.73 in female flowers (N = 28).

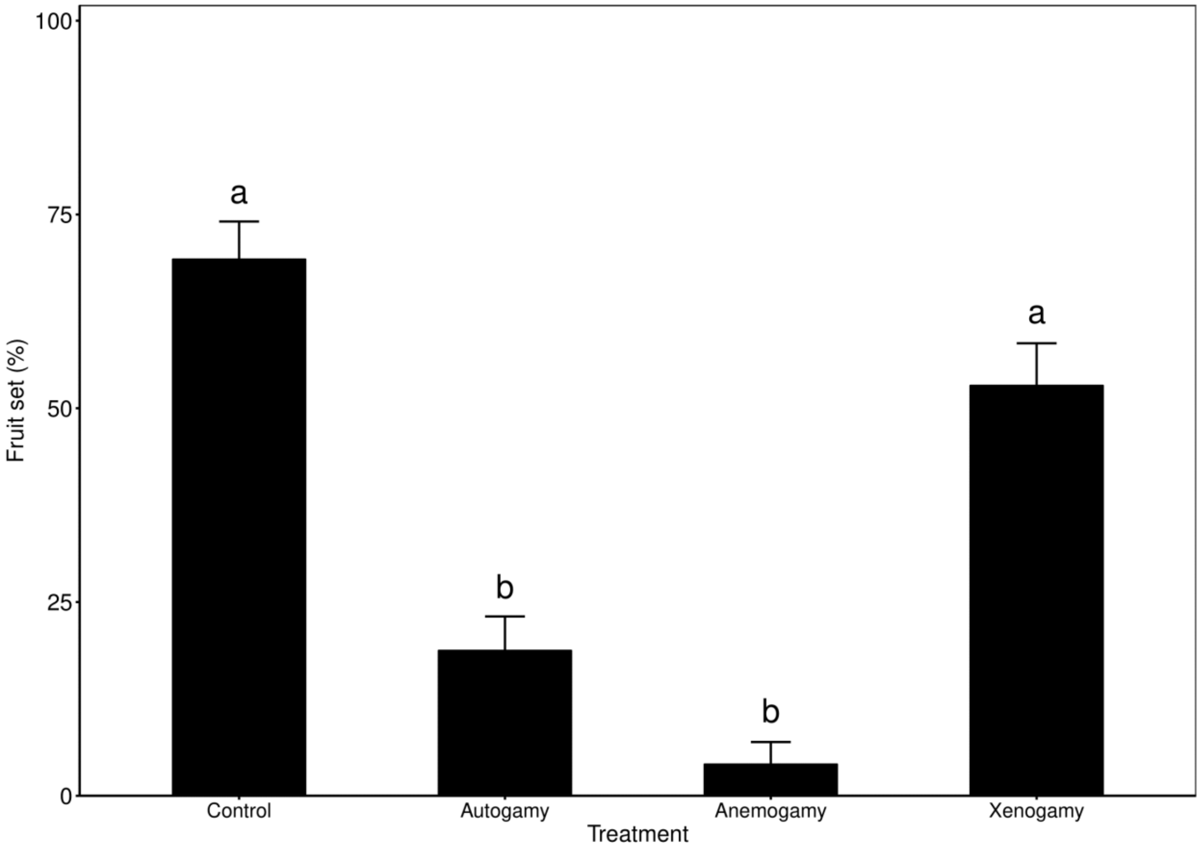

***Figure 1** Mean (+ SE) percentage of flowers that set fruit for hand-pollination treatments (Control, N = 91 flowers; Autogamy, N = 80 flowers; Anemogamy, N = 49 flowers; Xenogamy, N = 85 flowers) in E. simplex. Different letters indicate significant differences among treatments using Tukey´s test after GLM.*

Breeding system—

No fruits were obtained by apomixis and thus those flowers were removed from subsequent analyses. Likewise, fruit set resulting from anemogamy was negligible (4%) and might be due to accidental pollen contamination or even pollination by tiny insects, such as thrips or tiny ants (Figure 1). Fruit set varied with the hand-pollination treatment, and the effect was consistent the two study years. An average of 70% of flowers open to pollinators produced fruits, and this was not significantly different from the xenogamy treatment, suggesting no pollen limitation. By contrast, autogamous flowers produced significantly less fruits (ca. 20%) than controls, showing the benefit of animal pollination. Fruit set showed to be independent of inflorescence height (Table 1).

Regarding seed set, it was similar across treatments, and this was also consistent in time (Table 1). Hence, fruits from autogamous flowers produced similar number of seeds per fruit than either xenogamous or control flowers. The mean number of seeds per fruit was 1.25 ± 0.07 (N = 125 fruits).

Floral visitors and visitation rates—

Five species of passerine birds were observed visiting the flowers of *E. simplex,* in order of importance: *Phylloscopus canariensis* Hartwig*, Serinus canarius* L*., Cyanistes teneriffae* Lesson*, Sylvia atricapilla* L. and *Sylvia melanocephala* Gmelin. The mean number of flowers contacted by birds per unit time was 0.66 ± 0.02 visitis · h^{-1} · $flower^{-1}$ (N = 14). Although much more rarely and only in 2016, a lizard species - *Gallotia galloti* Oudart- was also seen lapping the nectar of this species (Figure 2).

Table 1 Mixed model analysis of the effects of year, pollination, and inflorescence height on fruit set and seed set in E. simplex.

	Fruit set			**Seed set**		
Source of variation	*df*	χ^2	P	*df*	χ^2	P
Year	1	1.142	0.285	1	1.552	0.213
Treatment	3	88.903	**<0.001**	3	3.630	0.304
	Treatment	Mean ± SE	N			
	Control	69.23±4.86	91			
	Autogamy	18.75±4.39	80			
	Anemogamy	4.08 ± 2.86	49			
	Xenogamy	52.94±5.45	85			
Inflorescence height	1	0.493	0.493	1	0.868	0.352
Year*Treatment	3	2.4132	0.4827	3	4.868	0.182

Vertebrate species and year had an interactive effect in flower visitation rate (χ^2 = 109.21, df = 4, P < 0.001). Data of both *Sylvia* species were pooled for the analysis as identification to species level was not always possible. Flower visitation rate was consistent in time within species except for *P. canariensis*, with more visits on 2016 compared to 2015 (χ^2 = 12.22, df = 1, P < 0.001) (Table 2). *Cyanistes teneriffae* was the most frequent visitor in 2015 whereas *Phylloscopus canariensis* was it in 2016. Only one observation of *Gallotia galloti* on the flowers was recorded during the censuses in 2016 (Table 2), although this interaction was also observed in several occasions outside census periods during June 2016.

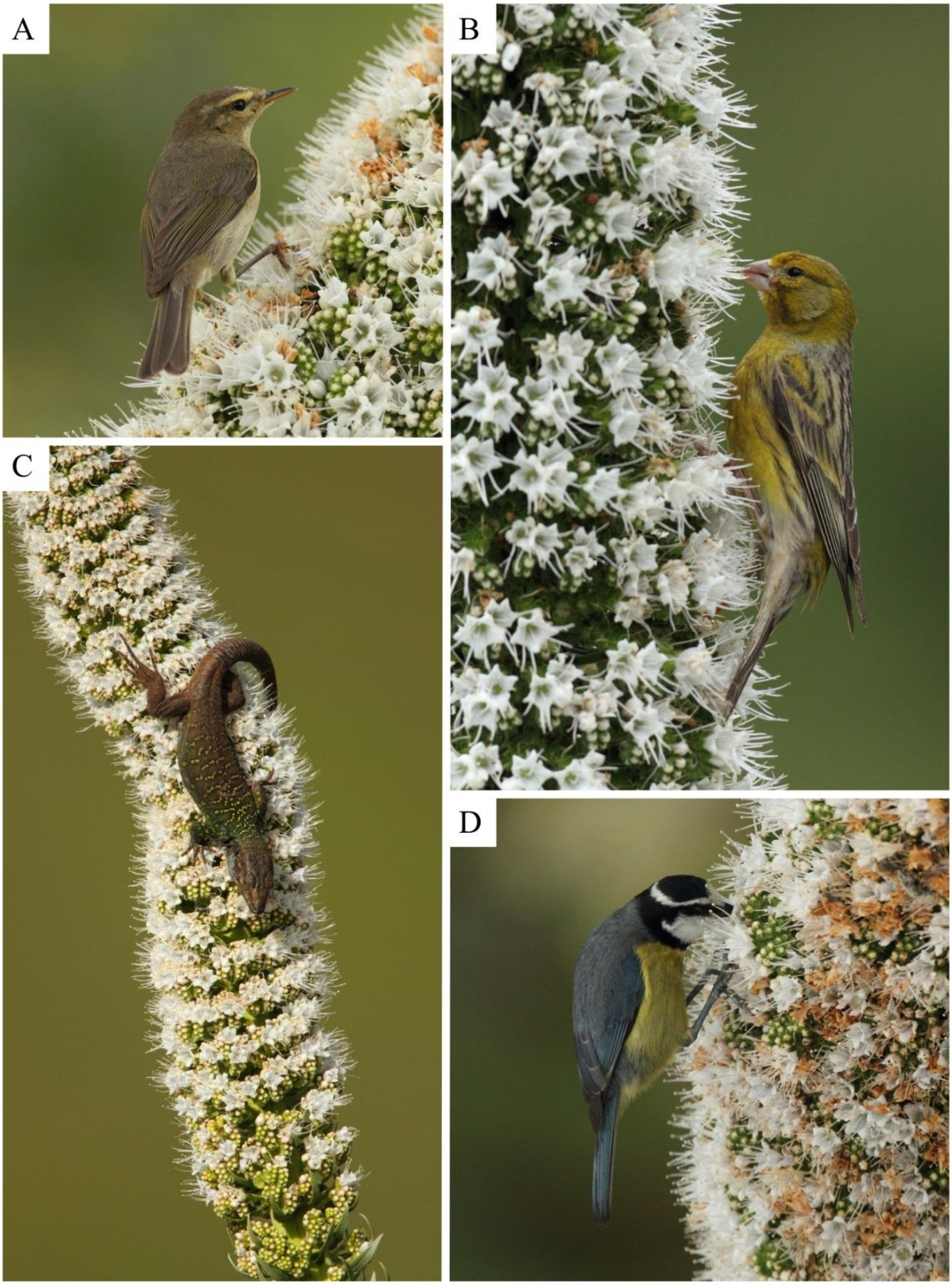

Figure 2 Images of different vertebrate flower-visitors of E. simplex: (A) Phylloscopus canariensis; (B) Serinus canarius; (C) Gallotia galloti; (D) Cyanistes teneriffae. Photo credits: Beneharo Rodríguez.

Table 2 Flower visitation rate (visits · h^{-1} · $flower^{-1}$) in 2015 and 2016 for each animal group or species. Asterisks indicate significant differences between years in the visitation rate. Hymenoptera group is Hymenoptera other than Formicidae

	2015	**2016**
Invertebrates		
Coleoptera *	0.28 ±0.037	0.017±0.0027
Diptera	0.015 ±0.0040	0.022±0.0049
Formicidae *	0.044 ±0.0086	0.021±0.0026
Hemiptera	0.003 ±0.0010	0.003 ± 0.0006
Hymenoptera*	0.46 ±0.065	0.23 ±0.030
Lepidoptera	0.0002 ±0.0001	0.0006±0.0003
Other	0.0003±0.002	0.0002±0.0001
Vertebrates		
Serinus	0.015 ±0.007	0.017±0.002
Sylvia	0.011 ± 0.006	0.005 ±0.001
Cyanistes	0.036 ±0.021	0.012 ± 0.03
*Phylloscopus**	0.008 ±0.05	0.03±0.005
Gallotia	0	0.0003 ±0.0003

Regarding insects, all floral visitors were clustered in seven groups: (1) Coleoptera (29 species), (2) Diptera (25 species), (3) Formicidae (7 species), (4) Hymenoptera (23 species; Formicidae excluded), (5) Hemiptera (12 species), (6) Lepidoptera (3 species), and (7) Other (including anecdotic visits of different species of Thysanoptera, Araneae, Blattaria and Dermaptera species) (Figure 3). The list of all identified species of insect floral visitors is given in the supplementary material Table 9. The most frequent insect groups were hymenopterans and coleopterans, especially in 2015, followed by dipterans and ants (Table 2). There was an interactive effect of group and year (χ^2 = 182.69, df =

6, P < 0.001). Overall, coleopterans, ants, and flying hymenopterans predominated on the flowers ($\chi^2 = 77.24$, df = 1, P < 0.001, $\chi^2 = 10.74$, df = 1, P < 0.001, and $\chi^2 = 58.71$, df = 1, P < 0.001, respectively) (Table 2).

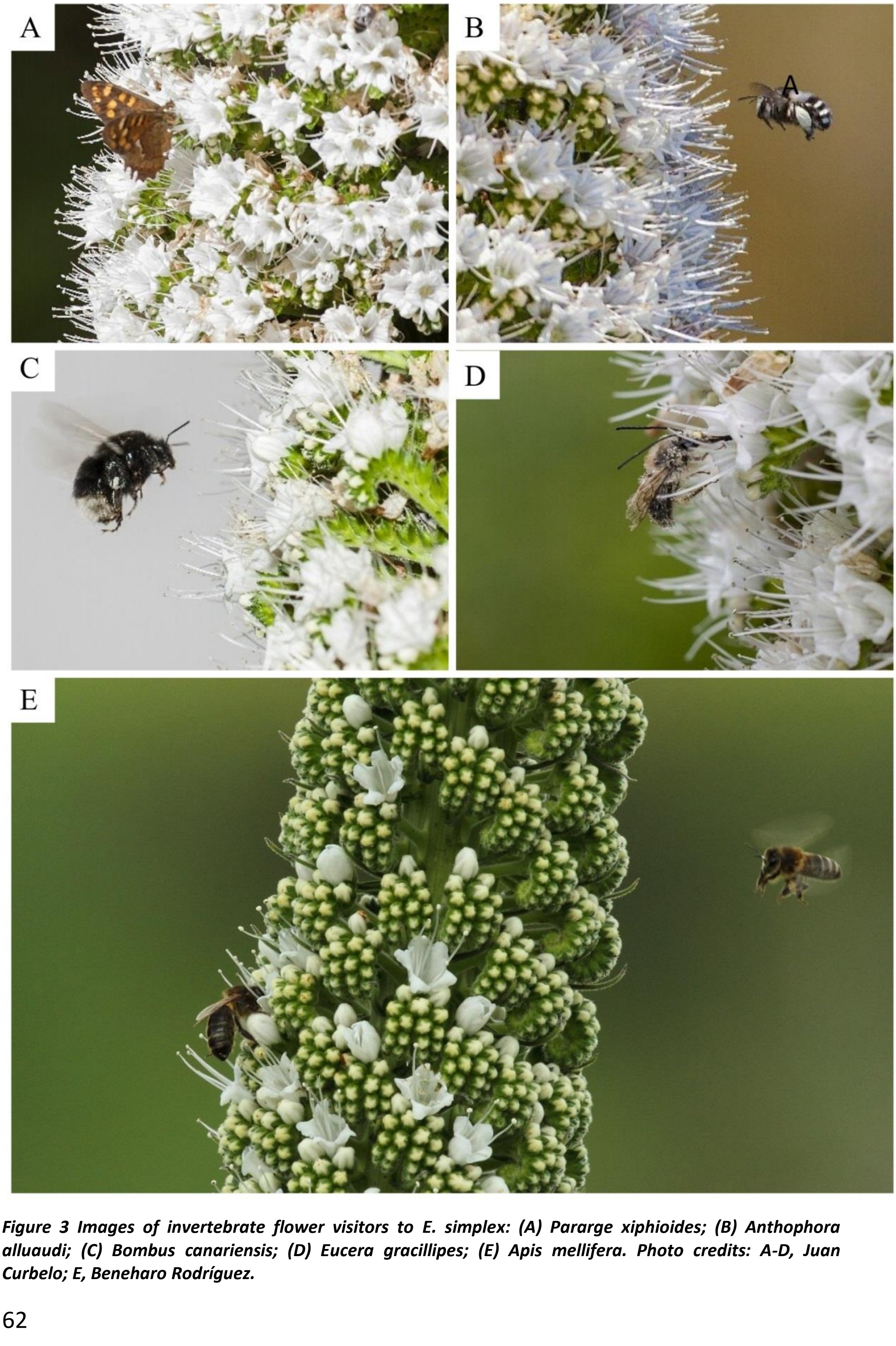

***Figure 3** Images of invertebrate flower visitors to E. simplex: (A) Pararge xiphioides; (B) Anthophora alluaudi; (C) Bombus canariensis; (D) Eucera gracillipes; (E) Apis mellifera. Photo credits: A-D, Juan Curbelo; E, Beneharo Rodríguez.*

Pollination effectiveness of the different flower-visitor groups—

Data on fruit set from treatments performed both years (i.e. total exclusion, only flower dwellers and control) were pooled, as no significant effect of year (χ^2 = 0.02, df = 1, P = 0.96) and no interaction between treatment and year (χ^2 = 0.56, df = 2, P = 0.75) were found. Although the exclusion of both birds and lizards produced a slightly lower fruit set than control flowers, differences were not significant (Figure 4A). When both vertebrates and insects were excluded (i.e. flowers could only be self-fertilized), however, fruit set was much lower, consistent with the breeding system data. On the other hand, insect flower dwellers showed to play a negligible role as pollinators; fruit set in this treatment was similar to that observed when all flower visitors were excluded (Figure 4A). Hence, flying insects showed to be the most effective pollinators contributing to fruit set.

Contrasting results between years were found regarding seed set. In 2015, both, the lizard exclusion and the flower dwellers' treatments produced fruits with less seeds than the other treatments (χ^2 = 19.95, df = 5, P < 0.01; Figure 4B). In 2016, by contrast, seed set was not affected by treatment (χ^2 = 1.96, df = 2, P = 0.38), but a significant negative effect of inflorescence height on this variable was detected (χ^2 = 4.11, df = 1, P = 0.04), i.e. longer inflorescences set less seeds per fruit than shorter ones. Control flowers set slightly more seeds in 2015 than in 2016 (1.54 ± 0.03 vs. 1.33 ± 0.03 seeds/fruit, respectively; χ^2 = 4.31, df =1, P = 0.04); the 'flower dwellers' treatment, however, showed the opposite pattern (1.30 ± 0.08 vs. 1.58 ± 0.04 seeds/fruit, respectively; χ^2 = 7.92, df = 1, P < 0.01). Flowers totally excluded from visitors set a similar number of seeds per fruit the two years, an average of 1.67 ± 0.04 seeds per fruit (Figure 4B).

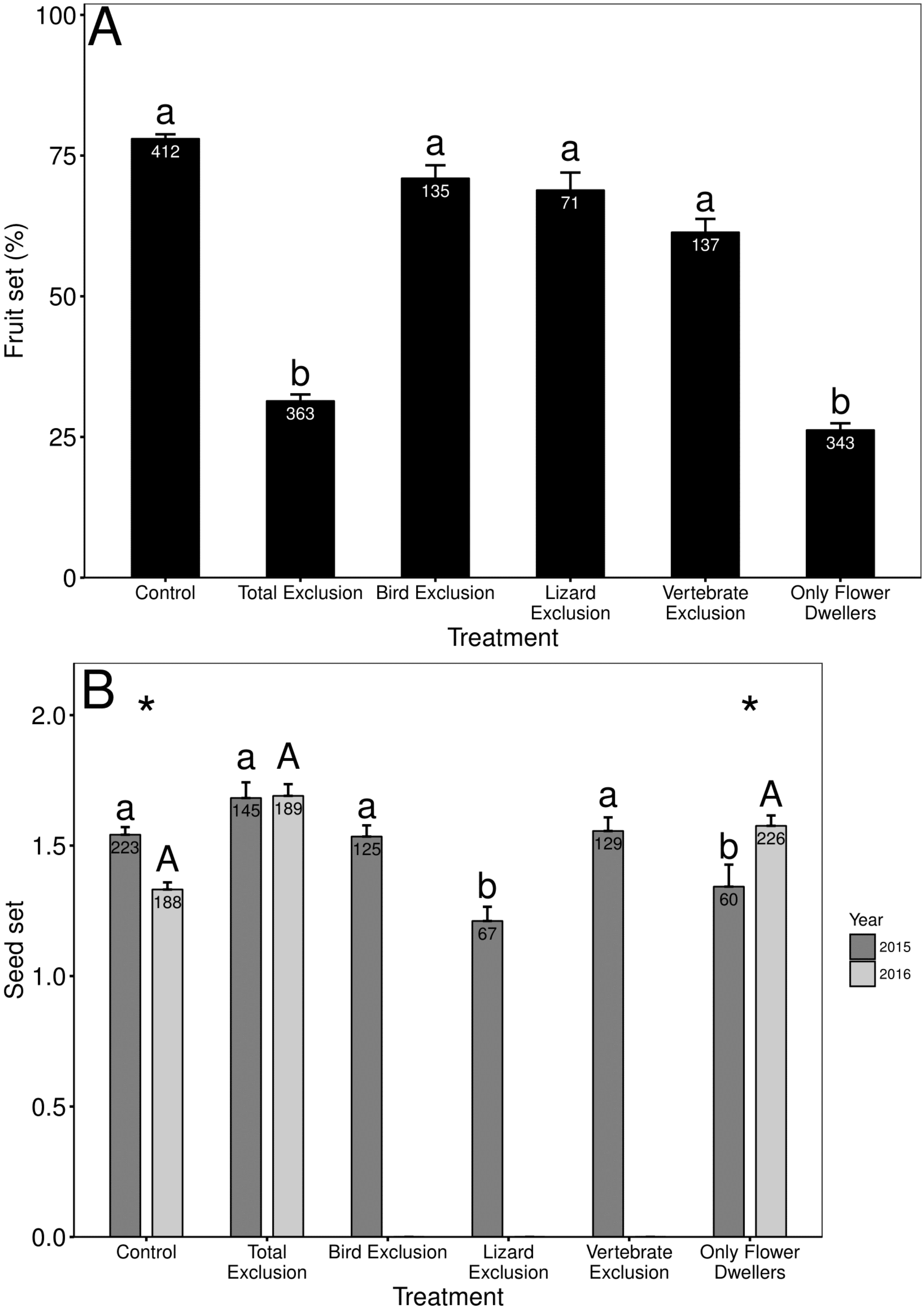

Figure 4 Mean (+ SE) (A) percentage of flowers that set fruit for exclusion experiments and (B)number of viable seeds per fruit for exclusion experiments in 2015 and 2016. Numbers in each bar are samples sizes. For each year, letters indicate significant differences between treatments; and for each treatment, asterisks indicate significant differences among years using Tukey´s test after GLM

Flying hymenopterans were, by far, the most effective pollinators (PE = 5,662.78) whereas birds, lizards and insect flower dwellers had a PE close to 0 (PE = 7.04; 0.86 and 2.17, respectively; Figure 5). Flying hymenopterans showed both high QNC and QLC but relative strengths of the components depended on pollinator identity on the other groups. Regarding lizards, fruit set was the major determinant (7.6%), whereas for insect flower dwellers the visitation frequency was the dominant factor (34.73 visits · h^{-1} · 500 $flowers^{-1}$) and the other components were null. The higher PE of birds compared to lizards and insect flower dwellers was explained by both modest QNC and QLC.

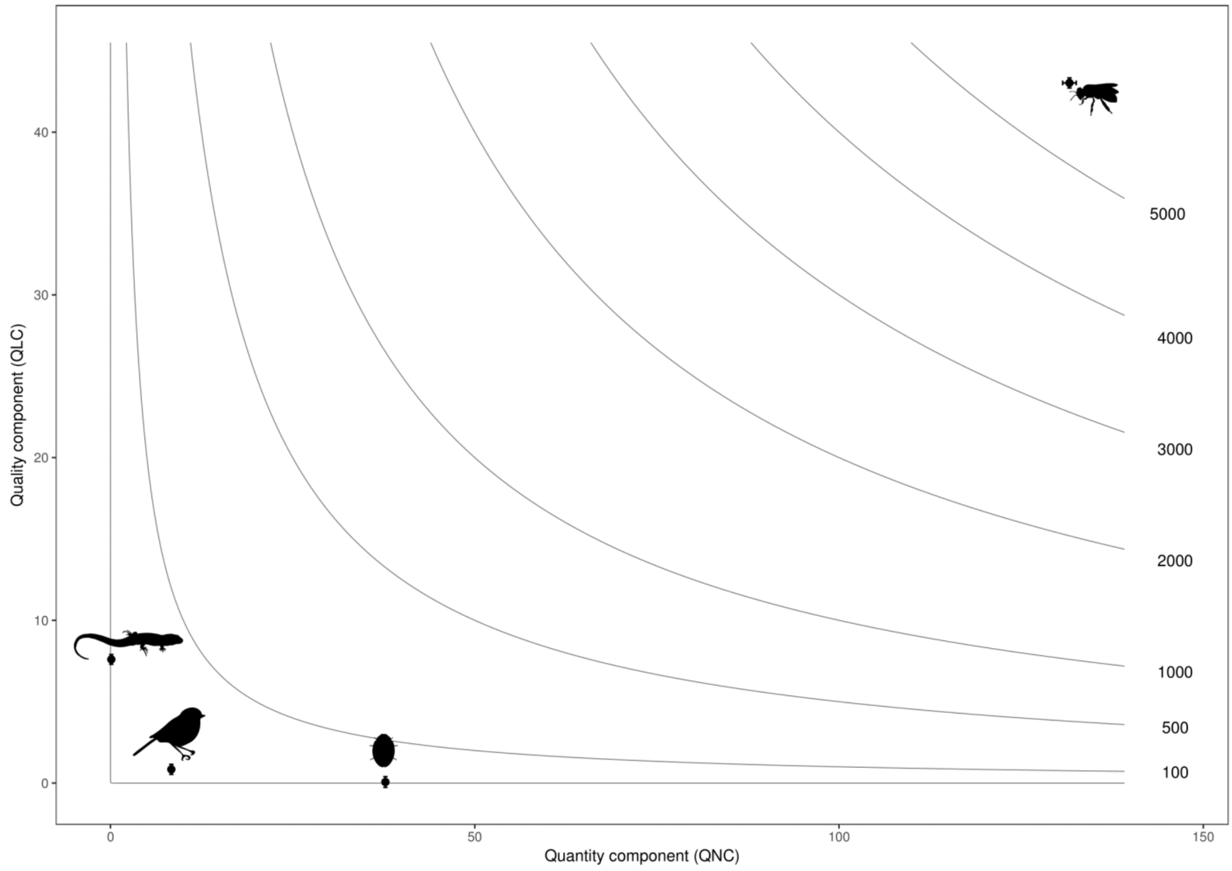

***Figure 5** Mean (± SE) pollination efficiency (PE) values for the pollinator guilds for the quantitative (x axis) and the qualitative (y axis) components. Isoclines represent all combinations of quantity and quality components with the same PE measured as visit rate (visits·h^{-1}·$flower^{-1}$) and fruit set, respectively. Images depict: flying insects; insect flower dwellers; Gallotia galloti lizards; and birds.*

Seed weight varied significantly among treatments ($\chi^2 = 22.59$, df = 5, $P < 0.001$). Interestingly, the 'total exclusion' and 'flower dwellers' treatments produced heavier seeds (with thicker seed walls) than control and bird-excluded plants. By contrast, seeds resulting from vertebrate exclusions had similar weight to those from the other treatments (Figure 6A); for this analysis, data from the two years were pooled, as there was no effect of year ($\chi^2 = 0.02$, df = 1, $P = 0.88$) and no interaction between treatment and year ($\chi^2 = 2.72$, df = 1, $P = 0.26$).

Regarding germination patterns, the effect of exclusion treatment was highly significant ($\chi^2 = 12.85$, df = 5, $P = 0.02$), but varied depending on seed weight, both in 2015 ($\chi^2 = 13.80$, df= 5, $P = 0.017$) and 2016 ($\chi^2 = 10.75$, df = 2, $P = 0.004$). Given that there was a significant triple interaction (treatment x seed weight x year) on germinability ($\chi^2 = 6.62$, df = 2, $P < 0.05$), seed germination data were separately analyzed for each year (see supplementary material Figure 22). In 2015, control seeds germinated more than selfed seeds whereas in 2016, all treatments showed similar germinability (Figure 6B).

When comparing only the treatments performed in the two years (i.e. control, total exclusion and flower dwellers), germinability showed to be higher in 2015 than in 2016 (78.67 ± 0.02% and 70.78 ± 0.01%, respectively; $\chi^2 = 4.79$, df = 1, $P < 0.05$) (Figure 6B). Nevertheless, germination rate showed no significant differences among treatments, either in 2015 ($\chi^2 = 7.31$, df = 5, $P > 0.05$) or 2016 ($\chi^2 = 3.36$, df = 2, $P > 0.05$). Interestingly, there was an interaction effect of seed weight x treatment: $\chi^2 = 39.90$, df =2 , $P < 0.001$ and $\chi^2= 21.47$, df = 5, $P < 0.001$, respectively), i.e. the effect of treatment depended again on seed weight (see supplementary material Figure 22). Germination rate was higher in 2015 (12.20 ± 0.31 days) than in 2016 (16.12 ± 0.49 days) ($\chi^2 = 17.73$, df = 1, $P < 0.001$), and this was so both for control and selfed seeds ($\chi^2 = 7.68$, df = 1, $P = 0.006$, and $\chi^2 = 8.17$, df = 1, $P = 0.004$, respectively).

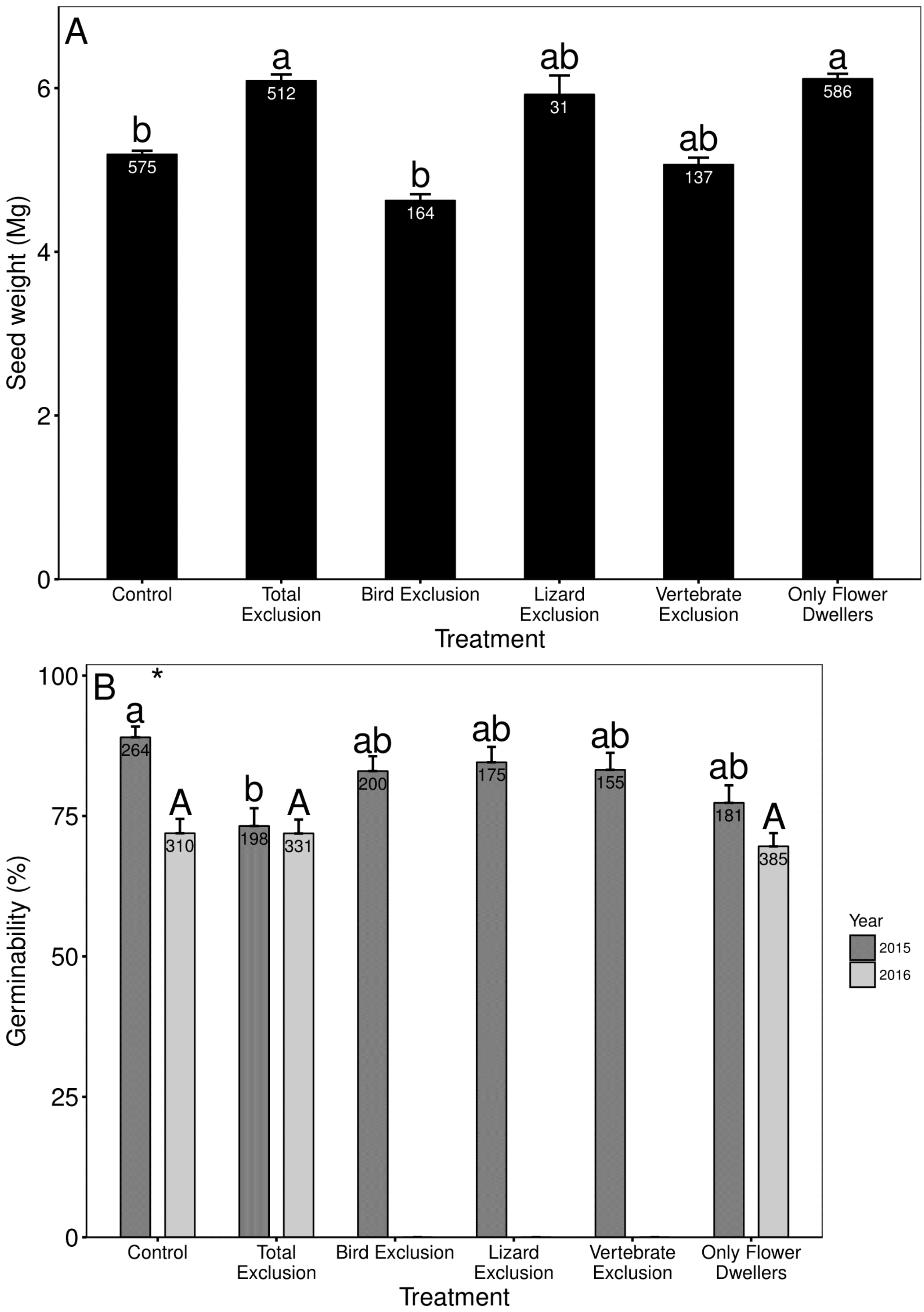

Figure 6 Mean (+ SE) (A) seed weight per treatment in 2015 and 2016 and (B) seed germinability percentage per treatment and year. Numbers in each bar are sample sizes. For each year, letters indicate significant differences between treatments; and for each treatment, asterisks indicate significant differences among years using Tukey´s test after GLM.

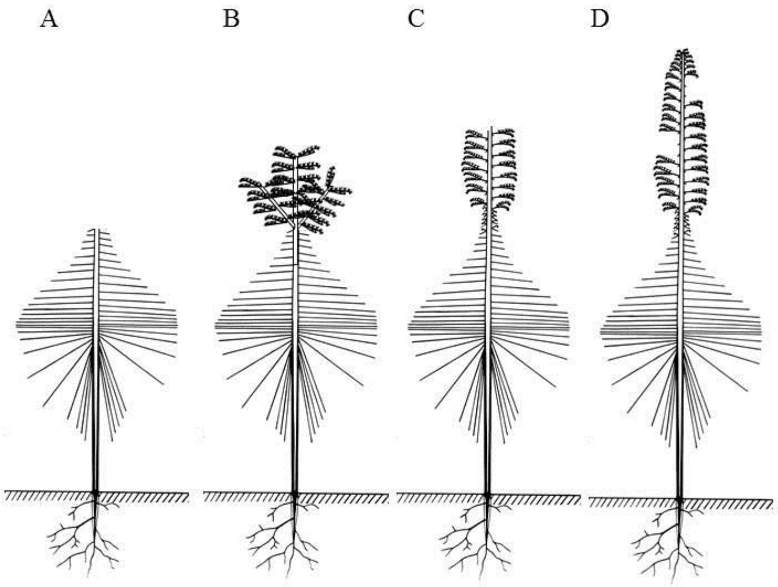

Figure 7 Classification of the different types of herbivory that feral goats can exert on the inflorescences of E. simplex (A) lack of flowers, (B) ball-shaped inflorescence, (C) short inflorescence and (D) well-shaped but with some grazed parts inflorescence.

Herbivory by goats—

In 2015, ca. 50% of the 464 reproductive plants examined to assess herbivory by goats was consumed by these animals to some extent. In 2016, the level of herbivory was even higher: ca. 99% of the 835 adult plants examined were damaged. This year, at least 30% of the inflorescences were totally consumed and thus no fruits and seeds were produced. Table 3 shows the number of plants affected for each category of damage (Figure 7). Both years, most damaged plants exhibited a ball-shaped inflorescence, thus losing also a high proportion of the flowers.

Table 3 Types of herbivory damage by feral goats recorded in 2015 and 2016 in E. simplex.

Damage Type	**Year**	
	2015	**2016**
No flowers	31 (13.25%)	247 (29.94%)
Short inflorescence	58 (24.79%)	5 (0.61%)
Ball-shape	130 (55.55%)	569 (68.94%)
Some grazed parts (mean ± SE, range)	15 (6.41%) (44.67% ± 7.21, 10 - 100%)	4 (0.48%) (85% ± 9.57, 60 - 100%)

2.1.4 Discussion

Echium simplex has been only anecdotically reported as ornithophilous species (Valido and Olesen 2010; Ojeda 2013; Mittelbach *et al.* 2015), but in this study we show that it is visited by a high number of bird species and even, though rarely, by the lizard *Gallotia galloti*. Despite the flowers can self-fertilize, the plant seems to rely mostly on insects for its reproductive success, although birds represent a non-irrelevant number of the total visits to flowers. Our findings indicate that the flowers of *E.simplex* constitute a food and water source for birds and lizards whereas the plant is not benefitted as much by the visits of these vertebrates to its flowers.

Breeding system—

Echium simplex can self-fertilize although it is not agamospermic. The role of wind for seed production is irrelevant, too. Selfed flowers produce much less fruits than flowers open to pollination, as reported for other *Echium* species (Bramwell 1972; Sedlacek 2009); this might be due to the existence of self-incompatibility systems or inbreeding depression, as suggested by Bramwell (1972), and/or due to protandry. Male flowers open before female ones, as in *Echium wildpretti* (Olesen 1988), though both phases coexist during day 2 of anthesis and this is why autogamy is possible. Xenogamy produced as many fruits as the open pollination treatment which suggests that pollen is not limiting, even though we cannot discard an outbreeding phenomenon or some imperceptible damage to the flower during hand-pollination. On the other hand, the fact that seed set was similar among treatments indicates that seed formation is controlled by resource-based constraints rather than by pollen limitation (Yang *et al.* 2005).

The nectar concentration and volume vary among flower phases, population and time of the day in *Echium* species (Olesen 1988; Kraemer and Schmitt 1997; Dupont *et al.* 2004b; Mittelbach *et al.* 2015). In general, male flower phases produce more nectar than female flowers, and during the third day no nectar is produced (Olesen 1988; Kraemer and Schmitt 1997). Mittelbach *et al.* (2015) found higher nectar volumes and lower sugar concentration in *E. simplex* than us. Differences in nectar composition are usual among populations (Mittelbach *et al.* 2015), whereas differences in nectar standing crop varies depending on the nectar consumed by flower visitors (Kraemer and Schmitt 1997); on the other hand, sugar concentration depends heavily on ambient relative humidity and the rate of photosynthesis (Corbet and Delfosse 1984).

Flower visitors—

The extremely high diversity of flower visitors found in this study contrasts with that found in other *Echium* species (but see Bramwell 1972). For *E. wildpretti,* Valido *et al.* (2002, 2004) reported three bird species (*Cyanistes caeruleus*, *Serinus canarius* and *Phylloscopus canariensis*), one lizard (*Gallotia galloti*), and 16 species of bees, butterflies and flies visiting its flowers. The previuos observations done in *E. simplex* reported only a few insects (Dupont and Skov 2004; Stöcklin and Lenzin 2013) all of which have been recorded in our study, and three bird species (*Serinus canarius, Phylloscopus canariensis* and *Cyanistes caeruleus* (Valido and Olesen 2010; Mittelbach *et al.* 2015). *P. canariensis* is the most common visitor in ornitophilous plant species in the Canary Islands (Valido *et al.* 2004), and is widely distributed in Tenerife, especially in Anaga (Carrascal and Palomino 2005).

Insects outnumbered birds as flower visitors of *E. simplex*. Hymenopterans, in particular, were up to 31 times more frequent than birds. However, at least for certain species in both years, flower visitation rate was higher for birds than for dipterans species. Bird visitation rate in *E. simplex* was lower than that found by Ollerton *et al.* (2009b) in *Canarina canariensis*, but higher than that recorded by Rodríguez-Rodríguez and Valido (2011) in the same species and in others like *Isoplexis canariensis, Lotus berthelotii* (Rodríguez-Rodríguez and Valido 2008; Ollerton *et al.* 2009b), and even in the same *E simplex* (Mittelbach *et al.* 2015).

Relative contribution of the different floral visitors to plant reproductive success—

Contrary to our expectations, we found no evidence for an increase in reproductive success of the flower visits by vertebrates, as reported in other studies (Ratto *et al.* 2018 and references therein) that include also species of the bird-flower Canarian element (Rodríguez-Rodríguez and Valido 2008, 2011; Fernández de Castro *et al.* 2017). Hence, vertebrates show to play a minor role in the reproduction of *E. simplex*.

Flying insects, especially bees, were the most frequent pollinators and the most effective ones, i.e. those contributing most to the reproductive success of *E. simplex*. By contrast, insect flower dwellers, mainly ants and small beetles did not increase fruit set and indeed reduced seed set relative to selfed flowers, suggesting that they consume pollen and by doing so they reduce final reproductive success (Kevan and Baker 1983). Pollination by beetles and ants has mainly been documented in some tropical plant families, and in pollinator-depauperated and environmentally stressful areas (Bawa 1990; Gómez *et al.* 1996).

Annual differences in seed set found are attributed to the lower insect visitation rates –mainly of bees and flies- found in 2016, probably due to a lower insect abundance, or to a higher flower availability. Fluctuations in insect populations are usual and often associated to inter-annual variation in climatic conditions, as these can have a substantial effect on insect survival or overwintering (Chown and Terblanche 2007 and references therein).

Seeds of *E. simplex* coming from selfed flowers were heavier than those resulting from cross-pollination. The reason is that the former have a thicker coat, whilst embryo size is similar between the two treatments. Such difference is consistent with that found in another study of *Echium vulgare* (Melser *et al.* 1997) and also with studies of other species (Kudo and Maeda 1998; Hudewenz *et al.* 2014). However, it contrasts with many other studies that find either the opposite (Galen *et al.* 1985; Navarro and Guitián 2002) or no differences in seed weight between selfed and crossed flowers (Eckert and Barrett 1994; Abdelgadir *et al.* 2009). Regarding germination, crossed and lighter seeds of *E. simplex* germinated more and faster than selfed ones; moreover, within the control seeds, heavier seeds performed better than lighter ones, in agreement with findings from other studies (Schemske 1983; Navarro and Guitián 2002). The thicker coat of the seeds coming from selfed flowers is probably what slows germination (Crocker 1906; Miyoshi and Mii 1988). This has indeed been previously reported in at least one species, *Sinapis arvensis* (Paolini *et al.* 2001).

Impact of feral goats on plant performance—

Introduced mammals are major drivers of extinction (Elton 1958; Gurevitch and Padilla 2004 and references therein). Those mammal species acting as herbivores are known to have strong deleterious effects on plant growth, reproduction and even survival (Crawley 1989; Marquis 1992). Feral goats (*Capra hircus*), in particular, can have a devastating impact in island ecosystems (Donlan *et al.* 2002; Gurevitch and Padilla 2004). The intense damage that goats showed to produce to *E. simplex*, particularly grave the second year of the study, not only causes a delayed flowering but also reduces the recruitment probabilities of this endemic species. A high incidence of herbivory by feral cattle has been reported for other plant species in some plant communities, where up to 96% plants were consumed. Reproduction may be totally impeded in many individuals, and initial herbivore damage might also weaken individuals and increase their susceptibility to other stress agents (Chynoweth *et al.* 2013 and references therein).

Damage by herbivores can modify flower characteristics and decrease overall plant attractiveness to pollinators (McCall and Irwin 2006), greatly reducing the opportunity for pollinators to select between plants (Strauss and Zangerl 2002). In general, plants have evolved a wide variety of rapid, inducible responses to herbivory that allow their survival (Strauss and Agrawal 1999). However, oceanic island ecosystems usually have evolved under very low pressure from herbivory (e.g. Van Vuren and Bowen 1999). Hence, endemic plants such as *E. simplex* are not expected to have evolved any compensatory mechanism to efficiently couple with mammalian herbivores. Further studies are needed to quantify to what extent herbivory upon *E. simplex* is altering its population dynamics in the long term, but we foresee that feral goats can intensely alter this isolated, fragile, and exclusive island habitat, in which *E. simplex* is one of the dominant species in the community. We argue that only with effective control campaigns that minimize the effects of this invasive herbivore can this endemic species be maintained in the most natural state possible.

Concluding remarks —

Despite the abundant bibliography on endemic Canarian flora visited by vertebrates, very few studies have yet examined their role as legitimate pollinators. Our study contributes to fill this gap of information. We have focused on a narrow endemic, *E. simplex*, finding that despite its floral resources might be important to some bird and lizard species, these do not appear to notably contribute to increase the fitness of the plant, which is on the other hand effectively pollinated by a wide assemblage of flying insects. We additionally found that feral goats represent a serious threat to the survival of this endemic species, as it damages a large fraction of the reproductive structures. We thus argue that these alien animals should be controlled in this highly valuable Biosphere Reserve.

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2.1.6 Acknowledgments

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2.2 Chapter 2

Effect of diurnal vs. nocturnal pollinators and flower position on the reproductive success of Echium simplex

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This chapter has been pusblished as:

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[Available from https://link.springer.com/article/10.1007/s11829-020-09759-4]

Abstract

Nocturnal pollination plays an important role in sexual plant reproduction but has been overlooked, partially because of intrinsic difficulties in field experimentation. Even less attention has received the effect of within-inflorescence spatial position (distal or proximal) on nocturnal pollinators of columnar plants, despite there have been numerous studies examining the relationship between such position and reproductive success. Woody endemic *Echium simplex* possesses large erect inflorescences bearing thousands of flowers which are visited by a wide array of diurnal and nocturnal animals. In this study, we identified nocturnal visitors and compared their pollination effectiveness with that of diurnal pollinators in different inflorescence sections by means of selective exclosures in NE Tenerife (Canary Islands). Nocturnal visitors included at least ten morphospecies of moths (such as *Paradrina rebeli* and *Eupithecia* sp.), two coleopteran species (mainly *Alloxantha* sp.), neuropterans (*Chrysoperla carnea*), dictyopterans (*Phyllodromica brullei*), dermapterans (*Guanchia* sp.) and julidans (*Ommatoiulus moreletii*). In general, plants excluded from pollinators set less fruits than open-pollination (control) plants which set fruits homogeneously across sections. Diurnally-pollinated

plants set more fruit in their upper parts whereas nocturnally-pollinated plants set fruit in both upper and bottom sections. We conclude that although the frequency and diversity of diurnal pollinators is far higher than that of nocturnal pollinators, both exhibit different foraging behaviour that generates complementary effects on the reproductive success of *E. simplex*.

2.2.1 Introduction

Plant reproductive success is the result of the interactions of both biotic (e.g. pollination, herbivory, disease) and abiotic (e.g. resource availability, physical environment) components of the ecological context with maternal constraints (Lee 1988). Mutualistic interactions between plants and their pollinators are of particular interest. Although most studies have focused on diurnal pollinators, nocturnal pollination plays a more important role in sexual plant reproduction than previously suspected, since pollen is carried over greater distances by moths than by diurnal insect pollinators (Macgregor *et al.* 2015 and references therein; Macgregor *et al.* 2019). Nocturnal pollination has been overlooked partially because of the intrinsic difficulty of field experimentation at night; moreover, such process may easily be affected by artificial light at night (Knop *et al.* 2017).

Nocturnal pollinators include a variety of taxa including insects, bats, birds, and even rodents (Baker 1961; von Helversen and Winter 2003; Knop *et al.* 2017). Some floral traits are usually associated with nocturnal pollination and form a particular pollination syndrome (Faegri and van der Pijl 1966; Fenster *et al.* 2004; Reynolds *et al.* 2009). This idea has been a central theme in pollination biology for many years (Faegri and van der Pijl 1966) and suggests that certain floral traits enhance the pollination efficiency of a particular pollinator type, leading to specialization in that pollination type. The flower characteristics traditionally associated with nocturnal pollination syndrome include: opening at dusk/night (Baker 1961; Van Doorn and Van Meeteren 2003), pale colour or white (Baker 1961; Lunau and Maier 1995), attracting scent (Jürgens *et al.* 2002; Raguso 2008) and copious nectar (Fenster *et al.* 2004). However, most plants are visited by a broad range of morphologically and taxonomically diverse species

(Waser 1982; Elam and Linhart 1988; Haber and Frankie 1989; Thompson and Pellmyr 1992; Sahley 1996; Nassar *et al.* 1997), indicating that flower morphology may not be an accurate predictor of the type of animal visiting the flowers. Moreover, further observations and experiments addressed at evaluating the contribution of pollination to plant fitness are needed in order to differentiate pollinators from other visitors, since many species are nectar and/or pollen thieves (Schemske and Horvitz 1984; Waser *et al.* 1996).

In plants in which the flowers are grouped in inflorescences, numerous studies have examined the relationship between reproductive success and flower anthesis (early or late) and/or within-inflorescence spatial position (distal or proximal) (for a review, see Stephenson 1981; Wyatt 1982; Lee 1988; or Diggle 1995). For example, in species with columnar inflorescences with acropetal flower opening, higher fruit and seed set are often found in proximal flowers (Solomon 1988; Herrera 1991; Ehrlén 1992, 1993; Karoly 1992; Guitian 1994; Guitián and Navarro 1996; Navarro 1996) than in intermediate flowers (Sutherland 1987) or distal flowers (Goldingay and Whelan 1993). Three non-exclusive hypotheses have been proposed to explain these patterns of within-inflorescence variation regarding reproductive success:

1) The ‘resource competition hypothesis’, focused on abiotic components, postulates that the ovaries compete for a limited amount of resources (Stephenson 1981 and references therein; Klein *et al.* 2015).
2) The ‘architectural effects hypothesis’, related to maternal constraints, postulates that there is a constraint on the translocation of nutrients to reproductive organs due to the inherent structural features of an inflorescence, such as the waning of the vasculature in distal structures or the variation in the diameter of supporting structures (Diggle 1995 and references therein).
3) The ‘non-uniform pollination hypothesis’, with biotic components, postulates that there is a variation in pollen receipt along the inflorescence and differences may be attributable to insufficient quantity or quality of pollen (Lee 1988; Thomson 1989a; Berry and Calvo 1991; Goldingay and Whelan 1993; Kudo *et al.* 2001).

Woody endemic *Echium* species in the Canary Islands, both candelabra shrubs and monocarpic rosette 'trees', possess large erect inflorescences often carrying thousands of flowers visited by a wide range of animals. The patterns of female reproductive success within inflorescences have never been assessed. Previous studies with *Echium simplex* revealed that despite being visited by diurnal insects, birds and lizards, flying insects were responsible for most of the pollination (Jaca *et al.* 2019). However, *E. simplex* might also be visited at night, as its flowers possess traits associated with the moth pollination syndrome (phalaenophily): they open at night, produce pale-coloured or white flowers with a heavy scent, offering rewards (nectar and pollen) in tubular corollas (Baker 1961; Kevan and Baker 1983).

In the present study, we aimed to investigate the reproductive success of both nocturnal and diurnal pollinators in different inflorescence sections. Our specific questions were: (1) what are the nocturnal pollinators of *E. simplex* in each inflorescence section and how frequent are they relative to diurnal pollinators? (2) what is the pollination effectiveness of nocturnal and diurnal pollinators in each inflorescence section, in terms of fruit and seed set, seed weight, and germination?

2.2.2 Materials and Methods

Study species—

The giant rosette plant *E. simplex* DC. (Boraginaceae), locally known as 'tajinaste blanco', is endemic to the Anaga Biosphere Reserve in NE Tenerife (Canary Islands). This area encompasses a 4.9-3.9 million-year-old basaltic massif (Guillou *et al.* 2004). It is considered a vulnerable species in the red list of Spanish vascular flora (Moreno, 2008), with very few, reduced and isolated populations. The species is one of the three monocarpic *Echium* species in the Canary Islands, together with *E. wildpretii* on La Palma and Tenerife, and *E. pininana* on La Palma, and it grows for 5-9 years before producing a single large inflorescence (Stöcklin and Lenzin 2013). Reproductive individuals reach a height of up to 3 m, of which the prolonged inflorescence - composed of scorpioid cymes - can contribute up to 1.5 m. The inflorescence height is directly proportional to the rosette diameter and it flowers acropetally (from bottom/proximal to upper/distal parts). The cymes are double-coiled and the largest plants may show 3-4 branches per cyme. After a successful pollination event, a flower develops into a fruit which consists of a maximum of four nutlets. The number of cymes and flowers per cyme increases along the inflorescence. The smallest of our examined plants had an average of 12 flowers per cyme whilst the largest had 51. The number of mature subfruits per flower (from one to three, on average) also increased along the inflorescence. Hence, the number of potential seeds produced increases enormously with the size of the inflorescence, ranging from 4,560 to 234,000 (Stöcklin and Lenzin 2013).

Flowers are protandrous and are open for two to three days. The carpel elongates and splits, becoming taller than the anthers during the female phase. The flowers open successively from the proximal to the distal part of the cyme. The total flowering time of an individual plant is 3-5 weeks. Nectar standing crop varies during flower ontogeny with male and transitional flowers producing more nectar than in the female phase (approx. 2 µl vs. 1µl) but sugar concentration remains constant (~17%) (Olesen 1988; Stöcklin and Lenzin 2013; Jaca *et al.* 2019).

Study area—

The study site is located at the north-west of Chamorga village, northeastern Tenerife (Canary Islands).The population of *E. simplex* is found at an altitude around 250 m a.s.l. and occupies an area of about 1 km^2. There are also scattered individuals along the north coast trails. The location has a warm coastal climate with average temperatures between 17 and 19 °C in winter and 20 and 25 °C in summer. The summer is very dry and most rain falls in winter, but only in small quantities. The area is exposed to the moist northeastern trade-wind, which is responsible for the lush green vegetation of Anaga mountains. The vegetation is shrubby-herbaceous, dry-Mediterranean and characterized by numerous endemic species such as *Artemisia thuscula, Descurainia millefolia, Aeonium canariense, Asphodelus tenuifolius, Achyranthes aspera* and *Galactites tomentosa.* Fieldwork was conducted once a week during a five-week period at the peak of the flowering season of *E. simplex*, between 10th May and 8th June 2016.

Flower visitors and visitation frequency—

Data on diurnal visitors and visitation frequency was available from our previous study on this plant (Jaca *et al.* 2019). To identify nocturnal flower visitors and determine their visitation frequency, a total of 18 haphazardly chosen individual plants were observed during focal censuses for a total of 35 h. Individual plants were observed for 60 min per census (ca. 2 h observation per plant) at a shorter distance (0.5 m) from dusk to midnight. Insects of all species or morphospecies were captured and taken to the lab for identification. Animals were considered as flower visitors whenever they touched the flower, as the sexual organs are exerted from the corolla. For each flower visitor, we recorded species identity (sometimes at family or order level), number of flowers and section of the plant visited (i.e. high, intermediate or low section).

Relative effectiveness of night and day flower visitors as pollinators—

We conducted experiments to study the importance of pollination by diurnal and nocturnal flower visitors. Prior to flowering, the inflorescences of 21 haphazardly selected plants were bagged with muslin cloth to exclude any type of flower visitor and randomly assigned to day ('diurnally pollinated plants') or night ('nocturnally pollinated plants') time exposure treatment. Once per week, diurnally pollinated plants were unbagged during all the hours of the day (from 6:00 am to 9:00 pm), while nocturnally pollinated plants were unbagged all the hours of the night (from 9:00 pm to 6:00 am the next day), and kept bagged the rest of the time. Additionally, 12 plants were permanently bagged to assess the level of autogamy, while 13 individuals were left open to pollinators, i.e. acting as a control group.

Five cymes from upper, intermediate and lower sections of each inflorescence and plant were collected once ripe and taken to the laboratory. Fruit set was calculated as the proportion of flowers that develop into fruits, and seed set as the amount of viable seeds produced per fruit. Seeds were regarded as non-viable (aborted) based on a characteristic smaller size and greyness. Previous germination trials confirmed that such seeds are indeed not viable (Jaca *et al.* 2019).

Germination trials were later carried out to test for differences among treatments (i.e., control, autogamy, diurnal pollination and nocturnal pollination). A total of 1,105 viable seeds (at least 18 seeds per plant, i.e., six seeds per inflorescence section per plant) were sown in early October 2016 into trays filled with a 1.2.1 mixture of peat, common agricultural soil and ravine sand in a greenhouse in Tacoronte (North Tenerife), as in Jaca *et al.* (2019). Trays were watered every two days to ensure that the soil was constantly moist, and seedling emergence was registered every five days for three months until January 2017, when the germination experiment concluded after no seeds germinated during the next 25 days. Germinability (fraction of seeds that germinate) and germination rate (days to germination) were recorded for each seed (although we use the term germination we actually refer to the seedling time emergence). Seeds sown under each treatment were previously weighed to the nearest 0.1 mg.

Statistical analyses—

We used generalized linear mixed models (GLMM) in R software version 3.5.0 (R Core Team 2018), followed by a Tukey test of multiple comparisons. Census observations were clustered into functional groups of visitors for the analysis. The model was adjusted to a gamma error distribution, using the number of probed flowers per unit time and per flower as response variables and observation ID, nested in individual plant, as random effect. For the diurnal vs. nocturnal pollination and germination experiments, each estimate of plant reproductive success (i.e. fruit set, seed set, seed weight, germinability, and germination rate) was analysed separately as a dependent variable. Differences in fruit set and germinability were estimated using a binomial error distribution and logit link function, whereas a Poisson family was used to test for differences in seed set and germination rate (as the data were a discrete count of seeds or days, respectively). Seed weight was normally distributed and, for this variable, we thus adjusted errors to a Gaussian distribution. In all of these models, individual plant was used as random effect to control for lack of independence among flowers on the same individual plant.

2.2.3 Results

Floral visitors and visitation rates—

Nocturnal insects visiting flowers of *E. simplex* were clustered into 6 groups: (1) moths, at least ten morphospecies, of which only two (*Paradrina rebeli* and *Eupithecia* sp.) could be identified, (2) beetles, mainly *Alloxantha* sp., with one unidentified, (3) neuropterans (*Chrysoperla carnea*, F. Chrysopidae), (4) dictyopterans (*Phyllodromica brullei*, F. Blattellidae), (5) dermapterans, (*Guanchia* sp. F. Forficulidae), and (6) julidans (*Ommatouilus moreletii,* F. Julidae) (Table 4). Visitation rates exhibited differences among insect groups ($\chi^2 = 142.03$, df = 5, P < 0.001). The most frequent insect groups were lepidopterans (Figure 8), visiting higher (distal) sections within the inflorescences, followed by coleopterans at intermediate and low positions, and other species mainly at the low sections (Table 4).

Table 4 Number of visits per inflorescence section by each visitor group

Visitor group	No. Visits	Position within the inflorescence		
		Upper	Intermediate	Bottom
Lepidoptera (at least 10 moth morphospecies)	69	41	23	5
Coleoptera (mainly *Alloxantha* sp.)	19	0	8	11
Chrysoperla carnea (Neuroptera)	1	0	1	0
Phyllodromica brullei (Blattaria)	1	0	0	1
Guanchia sp. (Dermaptera)	4	0	1	3
Ommatouilus moreletii (Julidae)	3	0	0	3

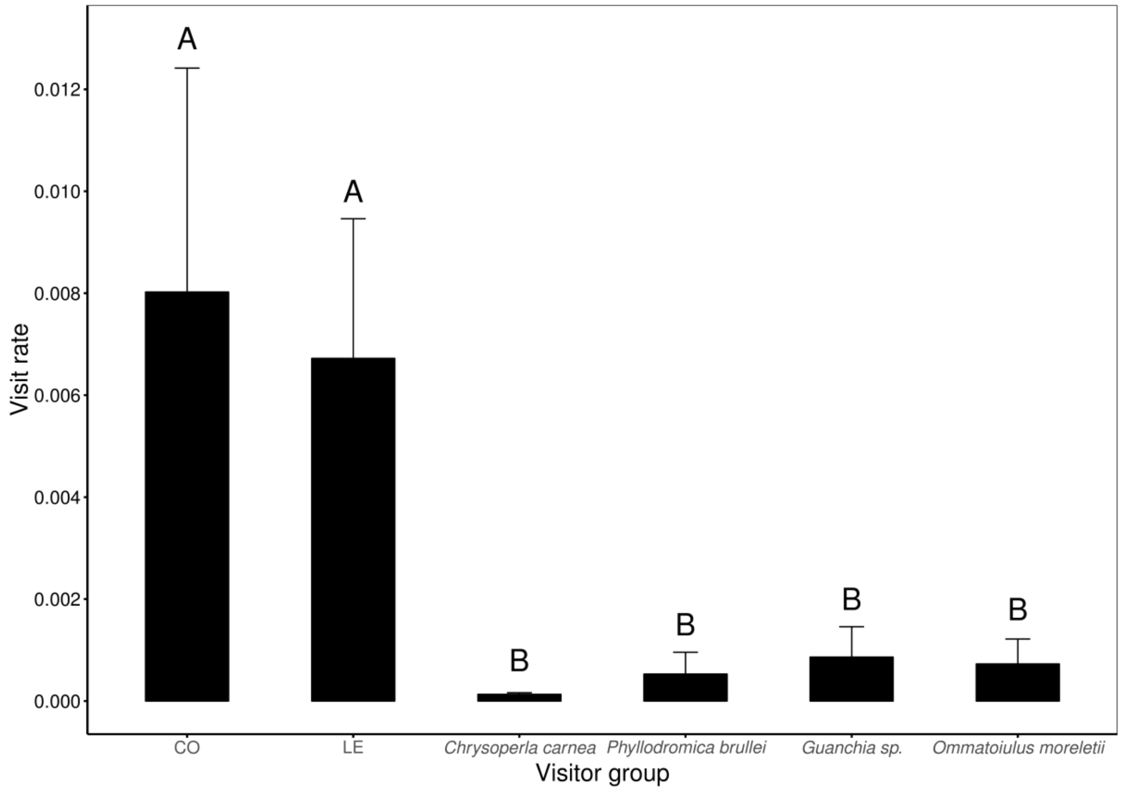

Figure 8 Flower night visitation rate (visits h^{-1} flower^{-1}) of E. simplex by different insect groups (CO: coleoptera, mainly Alloxantha sp., LE: lepidoptera). Letters indicate significant differences using Tukey´s test after GLM

Comparative reproductive effectiveness of nocturnal and diurnal pollination in the three inflorescence sections—

Fruit set was affected by pollination treatment and inflorescence section (pollination treatment x section: $\chi^2 = 33.34$, df = 6, $P < 0.001$, Figure 9). The number of fruits produced per flower was higher in the control plants open to pollinators, compared to those excluded from all pollinators and to those only visited by nocturnal pollinators. Within a plant, the number of fruits produced was higher in upper and bottom inflorescence sections in nocturnally-pollinated plants, whereas it was higher in the upper section in diurnally-pollinated plants (Figure 9).

There was no interaction effect of pollination treatment x inflorescence section on seed set ($\chi^2 = 12.38$, df = 6, $P = 0.054$). Seed set was influenced by pollination treatment ($\chi^2 = 17.25$, df = 3, $P < 0.001$, Figure 10) but not by inflorescence section ($\chi^2 = 1.93$, df = 2, $P = 0.38$). Diurnally pollinated plants produced more seeds per fruit than nocturnally pollinated ones and also than control plants (Figure 10).

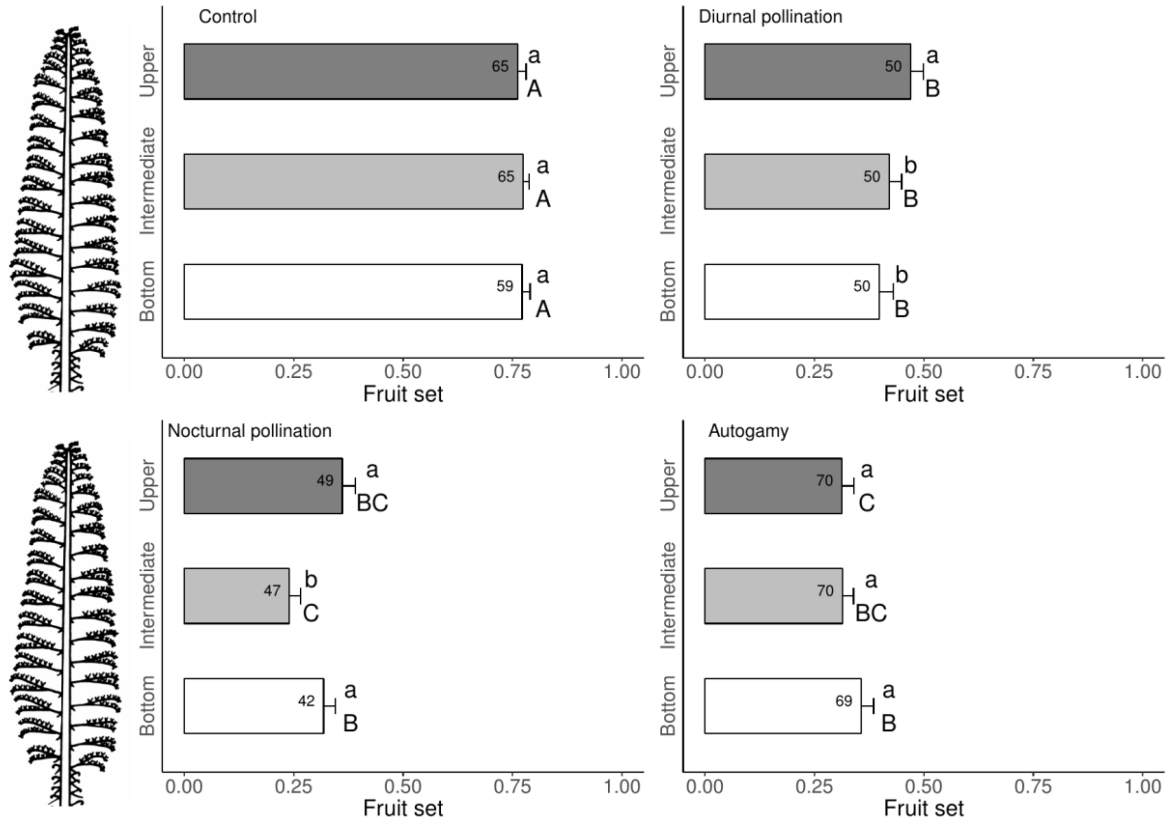

Figure 9 Mean (+SE) proportion of flowers that set fruit per pollination treatment and inflorescence section. Numbers inside each bar are sample sizes (number of cymes). Lower case letters indicate significant differences between inflorescence sections within each treatment, and capital letters indicate significant differences between treatments within inflorescence sections using Tukey´s test after GLM.

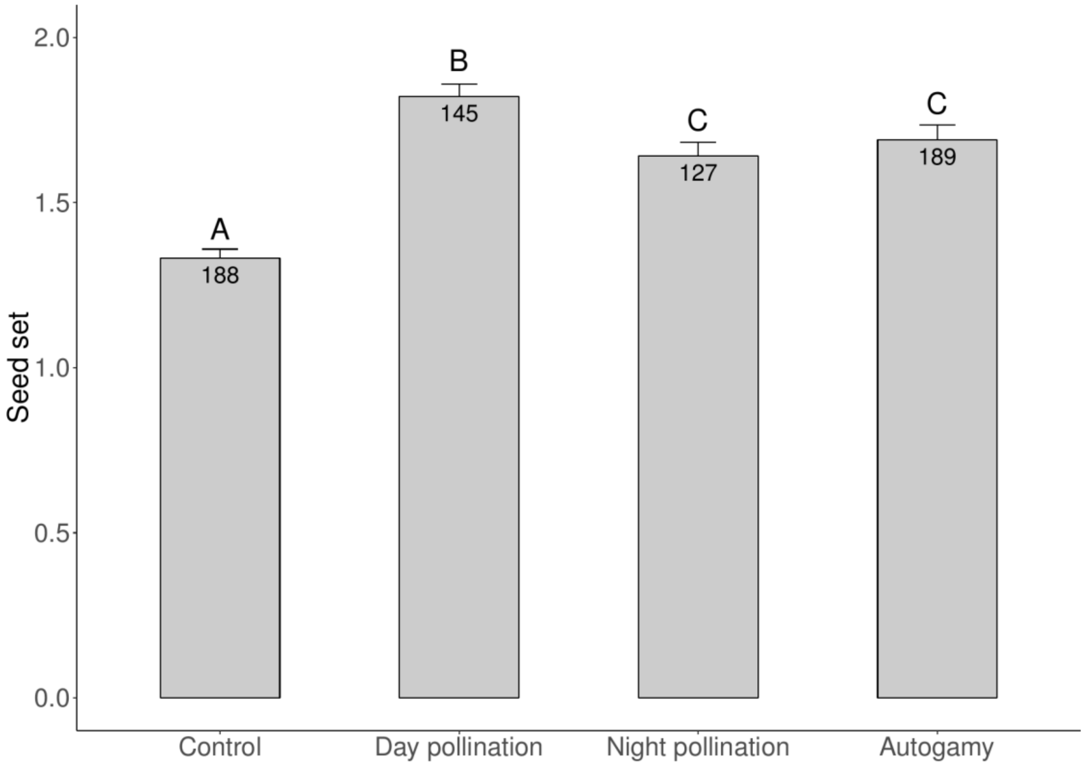

Figure 10 Mean (+SE) number of seeds per fruit for each pollination treatment. Numbers inside each bar are fruit sample sizes. Letters indicate significant differences using Tukey´s test after GLM.

Similarly, there was no interactive effect on seed weight between pollination treatment and inflorescence section ($\chi^2 = 10.67$, df = 6, P = 0.10). Seed weight was affected by both pollination treatment and inflorescence section ($\chi^2 = 8.96$, df = 3, P = 0.03; and $\chi^2 = 24.51$, df = 2, P < 0.01, respectively, Figure 11). Seeds from selfed flowers were significantly heavier than those from control flowers (Figure 11A). Moreover, bottom inflorescence sections produced lighter seeds than upper and intermediate sections (Figure 11B).

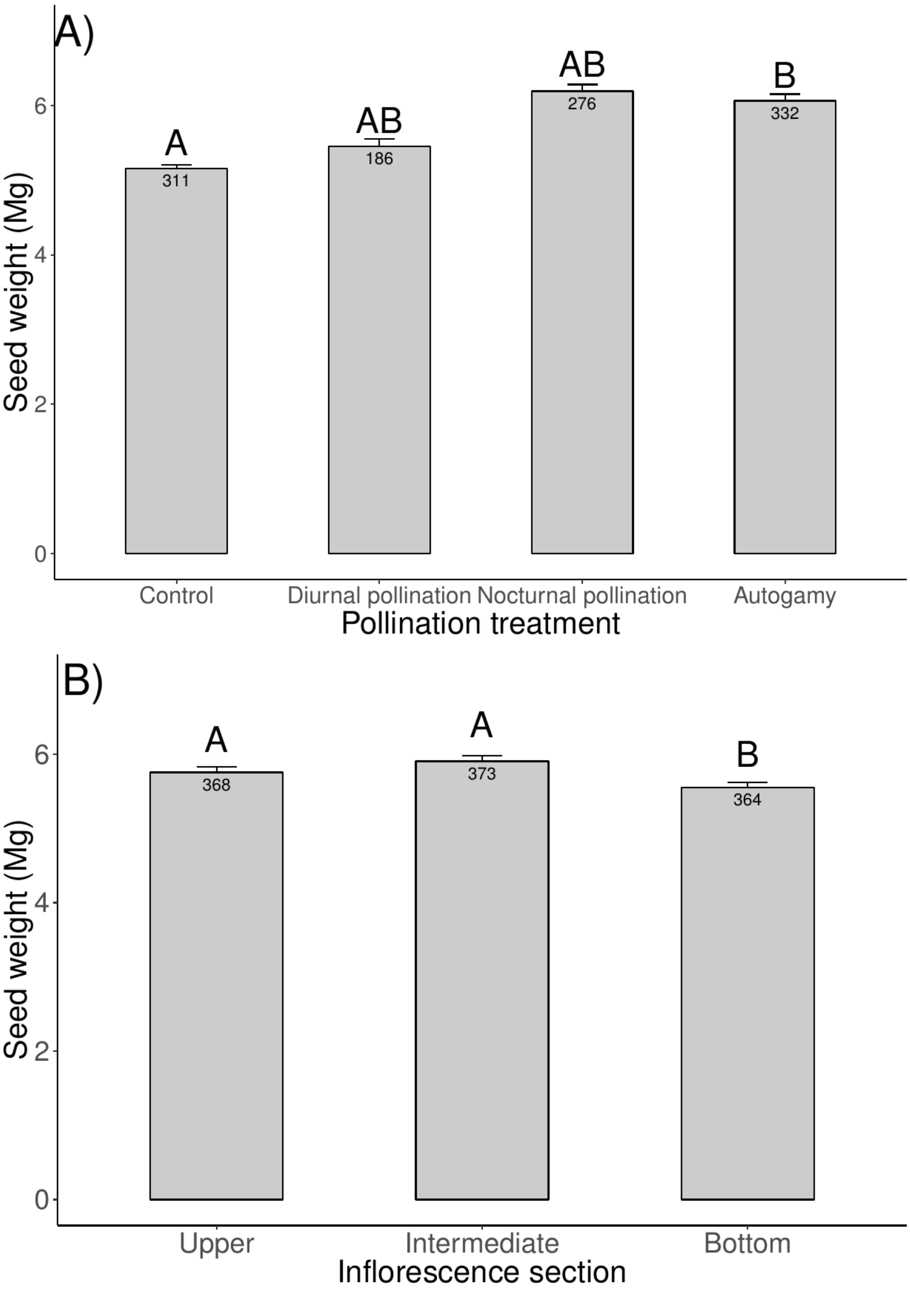

Figure 11 Mean (+SE) seed weight per (a) pollination treatment and (b) inflorescence section. Numbers in each bar are sample sizes. Letters indicate significant differences using Tukey´s test after GLM.

Regarding germination patterns, both germinability and germination rate were influenced by an interactive effect among seed weight, inflorescence section and pollination treatment ($\chi^2 = 16.01$, df = 6, $P < 0.05$, and $\chi^2 = 104.30$, df = 6, $P < 0.001$, respectively, Figure 12 and Figure 13).

In all inflorescence sections, most of the heavier seeds from control plants germinated. However, seeds from other treatments and inflorescence sections behaved differently.

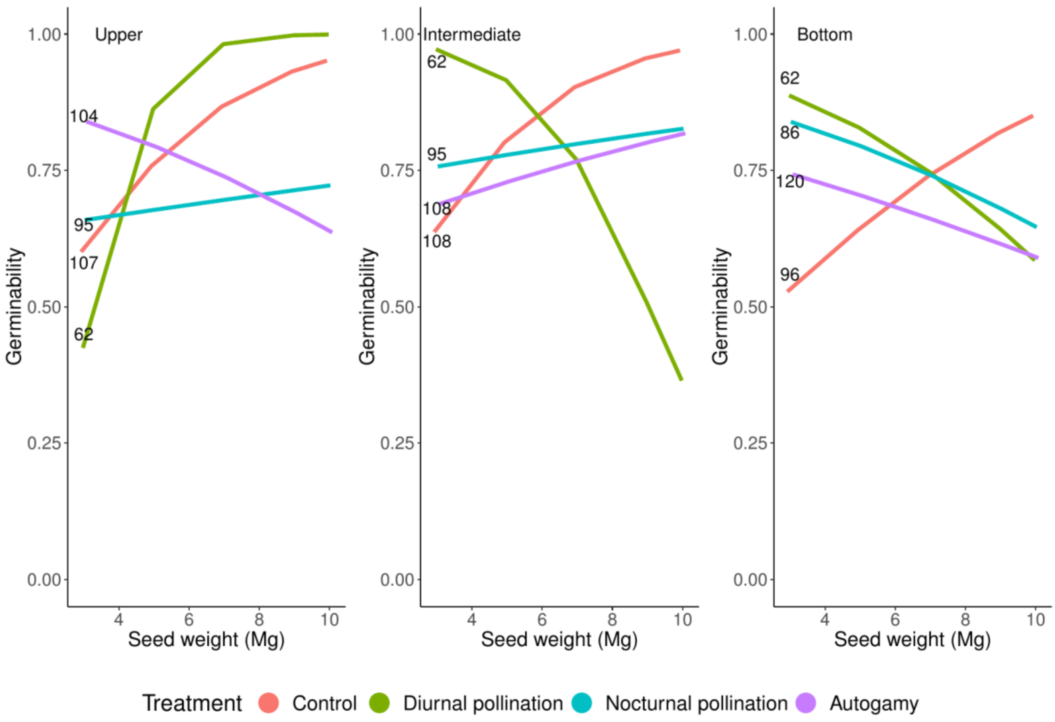

Figure 12 GLMM predicted probabilities of germinability along seed weight for each exclusion treatment and inflorescence section. Numbers besides each line are sample sizes.

The heavier seeds of the diurnally pollinated plants germinated more when seeds were from the high sections of the inflorescence. The opposite occurred with seeds from the intermediate and low inflorescence sections, i.e. heavier seeds germinated less. Furthermore, the heavier seeds of the nocturnally pollinated plants in the high and intermediate sections germinated slightly more than the lighter ones, whereas the opposite happened with seeds from the low sections, i.e. germinated less than lighter ones. Finally, for the autogamy treatment, we found that the heavier seeds had a higher germinability than the lighter ones, but this was only with seeds from the intermediate section and we found the opposite in the low and high sections, i.e. lighter seeds germinated more (Figure 12).

Regarding germination rate, heavier control seeds from the upper and intermediate sections germinated earlier, whereas those from the bottom section were later. The germination rate of seeds in relation to their weight in diurnally vs. nocturnally plants showed the opposite patterns, i.e. heavier seeds from the upper and bottom sections of diurnally pollinated plants germinated faster, but not those from intermediate sections, and heavier seeds from the upper and bottom sections of nocturnally pollinated plants took longer to germinate, while those from intermediate sections germinated faster (Figure 13). Finally, heavier selfed seeds germinated faster than the lighter ones from all sections of the plant.

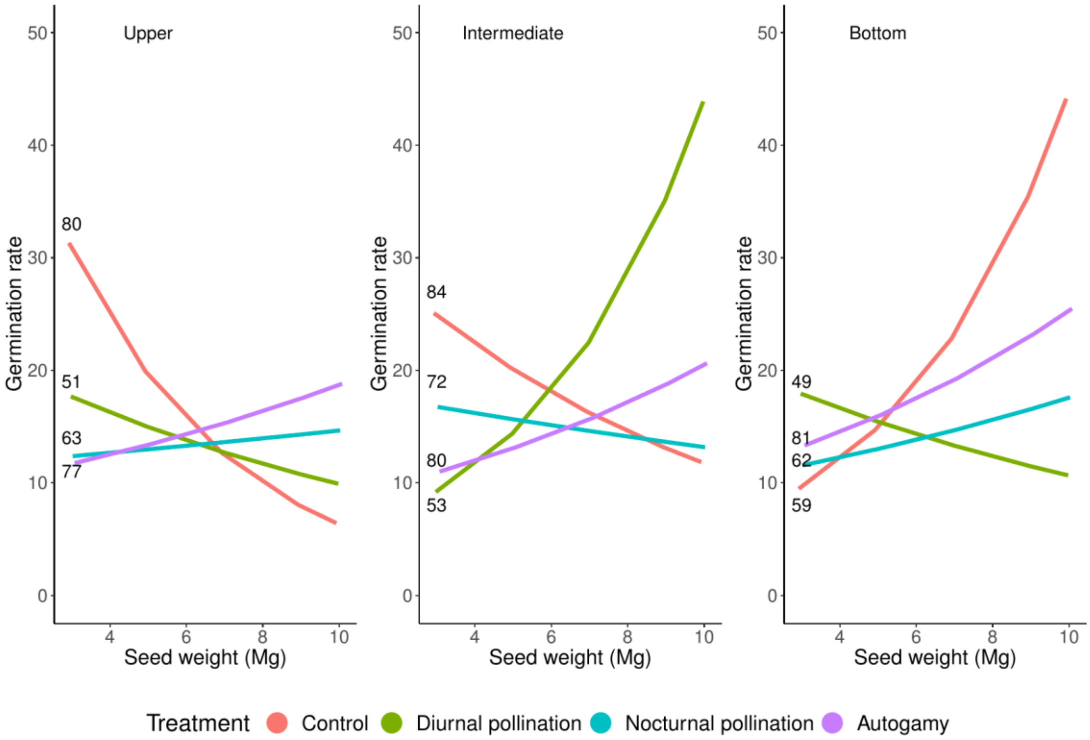

Figure 13 GLMM predicted probabilities of germination rate according to seed weight for each exclusion treatment and inflorescence section. Numbers besides each line are sample sizes.

2.2.4 Discussion

Ours is the first study that combines the effect of type of pollinators (nocturnal vs. diurnal) and inflorescence section on the reproductive success of a plant species. *Echium simplex* exhibited a uniform fruit set along the inflorescence, suggesting absence of competition among sections or maternal constraints, and uniform pollination. Although the species is mostly pollinated during the day, we found that nocturnal and diurnal pollinators displayed a complementary pollination behavior which translated into a complementary reproductive success.

Diversity of flower visitor groups—

At night, *E. simplex* flowers were visited by six different functional groups of animals. This is a higher number than the usually reported in nocturnal pollination studies, where mostly moth visits are reported (Stephenson and Thomas 1977; Jennersten and Morse 1991; Jürgens *et al.* 1996; Ghazoul 1997; Groman and Pellmyr 1999; Martinell *et al.* 2010, but see Brantjes and Leemans 1976). However, the attractiveness of this plant for insect visitors is greater during daytime, with up to 90 species of flower visitors identified (Jaca *et al.* 2019). This pattern of higher species diversity during the day is found in some plants (Jennersten and Morse 1991; Ghazoul 1997), though diversity is higher at night in others (Brantjes and Leemans 1976; Stephenson and Thomas 1977; Jürgens *et al.* 1996; Groman and Pellmyr 1999; Martinell *et al.* 2010). Some nocturnal insects are also observed in day censuses (Knop *et al.* 2017), as in our study. Indeed *Chrysoperla carnea*, *Guanchia* sp. and *Phyllodromica brullei* were also recorded in diurnal censuses (Jaca *et al.* 2019), as these animals can have diurnal activity or rest/hide within the flowers.

The most common nocturnal visitors in *E. simplex* were moths and the beetle *Alloxantha* sp. (Oedemeridae). This contrasts with other studies that report beetle visits as merely anecdotal (Stephenson and Thomas 1977; Groman and Pellmyr 1999; Martinell *et al.* 2010, but see Knop *et al.* 2017). When moths land on the inflorescence of *E. simplex* they sometimes walk over the flowers while probing them, and may remain on them for a short period. All body parts can contact the exerted anthers and pistils, and thus they are potentially effective pollinators (Ghazoul 1997). The moth diversity we found on *E. simplex* is much lower than that reported in other studies in both paleartic and neartic realms, such as those on *Manfreda virginica* or *Silene otitis* and *S. sennenii* (Brantjes and Leemans 1976; Groman and Pellmyr 1999; Martinell *et al.* 2010), but is similar to *Catalpa speciosa* or *S. vulgaris* and others (Stephenson and Thomas 1977; Jürgens *et al.* 1996). Beetles feed on pollen and move within the flowers but are probably irrelevant pollinators. In fact, their presence may indeed be deleterious, reducing final reproductive success by removing pollen from the stigmas (Kevan and Baker 1983; Jaca *et al.* 2019). As for other flower visitors, these nocturnal beetles were seen only anecdotally in other studies on night pollination, without being considered as pollinators (Crumb *et al.* 1941; Brantjes and Leemans 1976; Thien 1980; Schneemilch *et al.* 2011; Knop *et al.* 2017).

Regarding visitation frequency, nocturnal visitors were less frequent than diurnal ones (Jaca *et al.* 2019). This pattern is consistent with that found in most nocturnal pollination studies, despite the target species having a nocturnal syndrome (Stephenson and Thomas 1977; Ghazoul 1997; Young 2002 for a comparative table among studies; Martinell *et al.* 2010). It has been suggested that nocturnal visitors are scarcer because of their energetics, as they might require a larger nectar reward because of the cooler night temperatures (Morse and Fritz 1983); it has also been suggested that they could increase their length of visit during the night compared to diurnal pollinators (McMullen 2009).

Reproductive effectiveness of night and day pollination in the inflorescence sections—

In our previous studies on *E. simplex* we found that diurnal flying hymenopterans are the main pollinators responsible for its reproductive success (Jaca *et al.* 2019). In general, control plants set more fruits than diurnally or nocturnally pollinated, and than selfed plants, while diurnally pollinated plants set more fruits than nocturnally pollinated and selfed plants. This result is consistent with other studies (Bertin and Willson 1980; Morse and Fritz 1983; Jennersten and Morse 1991; Guitian *et al.* 1993; Navarro 1999), but not with others in which no differences have been found (McMullen 2009) or where a higher fruit set in nocturnally pollinated plants compared to diurnally pollinated plants has been reported (Martinell *et al.* 2010).

There was no difference in fruit set among plant sections in either control or selfed plants, suggesting absence of competition among sections or maternal constraints, and uniform pollination in *E. simplex*, unlike most studies of reproductive success patterns in inflorescences (Diggle 1995 for a review; Tremblay 2006; Torices and Méndez 2010). It is generally thought that perennial monocarpic species use stored reserves for fruit development rather than resources obtained during the flowering season, even more so than annually fruiting species (Stephenson 1981; Udovic and Aker 1981). However, day- and night-pollinated plants showed a fruit production pattern indicating non-uniform pollination (Karoly 1992; Kudo *et al.* 2001; Tremblay 2006). Some studies (Lee 1988; Tremblay 2006) have reported higher reproductive success in bottom positions due to the behaviour of pollinators; these move distally upward on inflorescences, may become satiated with the resources and thus may leave the plant before visiting the upper flowers; alternatively, the bottom of the inflorescence may be more likely than the distal parts to receive cross pollen. We found that diurnally pollinated plants set more fruits in upper inflorescence sections. One explanation might be that if diurnal insects (mostly bees) do indeed move upwards, upper positions may avoid stigma clogging to some extent (Brown and Mitchell 2001) if *E. simplex* competes with other co-flowering plants for pollinators. By contrast, other studies found higher pollen deposition in the upper flowers of inflorescences, with no relation to directional pollinator foraging and bee preference for higher flowers (Roubik *et al.* 1982; Lortie and Aarssen 1999). The deposition of large amounts of self-pollen, however, may also clog up the stigma and prevent effective pollination (Kikuzawa 1989; Thomson 1989b).

Nocturnally pollinated plants were found to set less fruits in intermediate compared to bottom and upper parts. The presence of *Alloxantha* sp. consuming the pollen in intermediate sections might reduce final reproductive success;

previous studies have documented beetles reducing plant fitness due to pollen consumption (Kevan and Baker 1983).

Diurnally pollinated plants set more seeds per fruit than control plants. This finding in *E. simplex* is consistent with studies by Jennersten (1988) and Martinell *et al.* (2010) who found higher or equal seed set in controls and day-pollinated plants. However, the reduced seed set in control plants may be compensated by the greater fruit production. Although some studies also found higher seed set in diurnally compared with nocturnally pollinated flowers (Jennersten 1988), most studies actually found the opposite (Jürgens *et al.* 1996; Groman and Pellmyr 1999; Young 2002; Martinell *et al.* 2010) or no effect (Jennersten and Morse 1991). In addition, no differences were detected between seed set of nocturnal and selfed fruits, indicating a low effectiveness of nocturnal pollinators, as documented by Jennersten (1988) for *Viscaria vulgaris*.

Seeds of *E. simplex* coming from selfed flowers were heavier than those resulting from cross-pollination. The reason is that the former have a thicker coat, whilst embryo size is similar between the two treatments (Jaca *et al.* 2019). Comparing seed weight between inflorescence sections, bottom seeds were lighter than upper and intermediate ones. This contrast with other studies that have found basal seeds to be heavier (Byrne and Mazer 1990; Navarro 1996; Vallius 2000).

In accordance with findings from other germination studies (Schemske 1983; Navarro and Guitián 2002), heavy seeds showed higher germinability and germinated faster than light ones in all treatments, except those from the bottom sections of inflorescences. The thicker seed coat produced by selfed flowers is probably what slows germination (Crocker 1906; Miyoshi and Mii 1988). Indeed, this was previously reported in at least one species, *Sinapis arvensis* (Paolini *et al.* 2001).

Concluding remarks—

Despite the relatively abundant literature on nocturnal vs. diurnal pollination, and on fruiting patterns along the inflorescences, this is the first study that examined both effects simultaneously. We found that *E. simplex* was visited at night -mainly by moths and beetles-, but at lower rates than during the day. The exclusion experiment indicated that fruiting patterns along the inflorescences in open-pollinated plants show no variation, but nocturnally and diurnally exposed plants do. This indicates different visiting behaviour between nocturnal and diurnal pollinators generating complementarity effects in *E. simplex* pollination services. Both germinability and germination rate were influenced by seed weight, inflorescence section and pollination treatment.

2.2.5 Funding Information

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2.3 Chapter 3

Impact of alien rats and honeybees on the reproductive success of an ornithophilous endemic plant in Canarian thermosclerophyllous woodland relicts

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Abstract

Islands harbour a considerable portion of global biodiversity and endemic biota, and also the recipients of the largest proportional numbers of alien invaders. Such invaders may jeopardize the performance of native species, through either their direct or indirect effects. In this study, we investigated the reproductive ecology of the endemic scrambling perennial herb *Canarina canariensis* in remnants of the former thermosclerophyllous woodland of Tenerife (Canary Islands), assessing how two widespread alien invasive species, the honeybee (*Apis mellifera*) and the black rat (*Rattus rattus*), affect its reproductive success. *Apis mellifera* visits its flowers whereas the black rat consumes both its flowers and fruits. Here, we compared the pollination effectiveness of different animal guilds (vertebrates vs. insects) by means of selective exclosures and determined the level of floral herbivory. Three bird species (*Phylloscopus canariensis*, *Cyanistes teneriffae* and *Sylvia melanocephala*), a lizard (*Gallotia galloti*) and two insects (*A. mellifera* and the butterfly *Gonepteryx cleobule*) were the main flower visitors. *Phylloscopus canariensis* was the most frequent visitor in the early flowering season whereas

A. mellifera predominated in the flowers during mid and late flowering periods. Birds increased fruit set, whilst lizards and insects had a negligible effect. Rats consumed about 10% of the flowers and reduced fruit set to one third. Besides contributing little to plant reproduction, *A. mellifera* might interfere with bird pollination by depleting flowers of nectar. We conclude that both alien species can threaten *C. canariensis* reproduction and hence population sustainability in the thermosclerophyllous vegetation. *Api*s *mellifera*, in particular, may become especially detrimental if apiculture keeps expanding, or if this bee becomes active earlier in the season due to global warming.

2.3.1 Introduction

Biological invasions, together with habitat loss and fragmentation, may represent serious threats to biodiversity (Wilcove *et al.* 1998; Bellard *et al.* 2017). They may imperil native species by direct (e.g., aggression, predation, infection, competition) or indirect effects (altering their mutualistic interactions or ecosystem processes, hybridization, introgression, vectoring diseases) (Vitousek *et al.*, 1996; Mack *et al.*, 2000; Vanbergen *et al.*, 2018). Islands, in particular, which harbor a considerable portion of global biodiversity and endemic biota (Kier *et al.* 2009), are the recipients of the largest proportional numbers of invaders (Vitousek *et al.* 1996; Mooney and Cleland 2001). This fact, together with their intrinsically naïve communities, less rewiring opportunities and no scape routes make them especially vulnerable to invasions (Traveset and Richardson 2006). Some islands already have a greater proportion of alien than native plants (Worthington and Lowe-McConnell 1994; Sax *et al.* 2002; Hofman and Rick 2018), even reaching 100%, as was found on Lehua Island in the Hawaiian archipelago (Pyšek *et al.* 2017).

Rats (*Rattus* spp.) have reached about 90% of the world's islands and are among the most successful invasive mammals (see Martin *et al.* 2000; Donlan *et al.* 2003; Towns *et al.* 2006, and references therein). Introduced rats threaten native plants and animals (Hernández *et al.* 1999; Martin *et al.* 2000; Towns *et al.* 2006), ecosystem functioning (Towns *et al.* 2006) and plant–animal mutualistic interactions (Traveset and Richardson 2006, 2014). They prey directly upon native fauna and also consume plant matter, affecting reproductive, photosynthetic and supporting parts (Cuddihy and Stone 1990; Sugihara 1997; Harper and Bunbury 2015). Indeed, plant material constitutes a large percentage of rat diet (Shiels *et al.* 2014), making up 73-99% of stomach contents in Mediterranean islands (Traveset *et al.* 2009 and references therein).

The honeybee (*Apis mellifera* L.) is another alien invasive species in many areas of the world, including islands (Moritz *et al.* 2005; Whitfield *et al.* 2006; Han *et al.* 2012). Traditionally, *A. mellifera* has been considered an effective flower pollinator and thus beneficial for plants in particular, and for the ecosystem in general. However, an increasing number of studies have noted both positive and negative effects in natural habitats, depending on the ecological context (Agüero *et al.* 2018). This bee is an efficient pollen collector and, at high densities, it can behave as an antagonist more than as a mutualist (Agüero *et al.* 2018; Vanbergen *et al.* 2018).

The Canary archipelago is considered a hotspot of both biodiversity and alien species. Of the 2,091 vascular plant species present there, 539 are endemic (25.78%) whereas 688 are introduced (32.90%) (Arechavaleta *et al.* 2010). Of the 8,283 animal species, 3,165 are endemic (38.21%) and 797 introduced (9.62%), with 183 species (1.69%) considered invasive (Arechavaleta *et al.* 2010). The rate of endemicity in this archipelago is one of the highest per unit area in Europe, with levels similar to the Galapagos Islands, but lower than in Hawaii, New Caledonia or New Zealand. However, its invasion rates are also the highest in Spanish territory (Sanz-Elorza *et al.* 2001, 2005). The thermosclerophyllous woodland is the most threatened habitat in both the Canary Islands and the entire Macaronesian region, being located between the coastal shrubland and the laurel forest that usually occupies higher altitudes (Fernández-Palacios *et al.* 2008).

In the Canaries, rats have been interacting with native plants probably since the arrival of Europeans in the 15th century and are known to prey on several species of land- and seabirds, lizards and gastropod species (Nogales *et al.* 2006; Traveset *et al.* 2009 and references therein). Their distribution is not homogeneous but patchy. Thus, for instance, on Tenerife, rats are more abundant in the east than in the western laurel forests (Hernández *et al.* 1999; Rodríguez-Rodríguez and Valido 2011), and in laurel forests more than in pine forests (Delgado *et al.* 2001). On the other hand, *A. mellifera* is present on all islands except Lanzarote and Fuerteventura. Since it has been domesticated for millennia, its native range and taxonomic status (native vs. introduced) is still controversial (Hohmann *et al.* 1993 claiming introduced status, Arechavaleta *et al.* 2010, native), but the most plausible explanation is that it was introduced by humans as on most other oceanic islands worldwide (Michener 1979; Moritz *et al.* 2005). Previous studies in El Teide National Park (Tenerife) that have examined the impact of this invasive animal insect species on plant reproductive success have found that it significantly reduces the reproductive performance of highly visited native plant species (Valido *et al.* 2014, Valido *et al.* 2019).

In this study we focus on an endemic scrambling perennial herb, the Canary bellflower, *Canarina canariensis* (L.) Vatke (F. Campanulaceae), which is found inhabiting the remnant thermosclerophyllous habitats and laurel forests (Fernández-Palacios *et al.* 2008) and is categorized as a vulnerable species (Barreno *et al.* 1984). Our goal is to assess how the two alien species, rats and honeybees, threaten its reproductive success in the thermosclerophyllous remnants. In laurel forest, the species is already known to be pollinated by *A. mellifera* as well as by birds (Ollerton *et al.* 2009b; Valido and Olesen 2010) and that both flowers and fruits are consumed by rats and semi-slugs *Plutonia* spp. (Rodríguez-Rodríguez and Valido 2011; Figure 14A). We first study the floral traits (reward, morphology, and floral phenology) of *C. canariensis*, and assess the composition and visitation rate of its floral visitors (both diurnal and nocturnal). Our specific questions are: (1) what is the relative contribution of floral visitors, including *A. mellifera*, to plant reproductive success, measured as fruit and seed production? (2) what are the levels of floral damage by rats, in terms of organs affected and extent of damage, and how does this influence fruit production? (3) is floral damage related to the local relative rat abundance? We then compare our finding in the thermosclerophyllous zone with those obtained in previous studies (Ollerton *et al.* 2009b; Valido and Olesen, 2010).

Figure 14 Floral visitors of Canarina canariensis, A) Plutonia sp.; B) Ommatoiulus moreletii; C) Formicidae; D) **Meloe tuccius** ***; E) and F) Lepidoptera larvae; G) Apis mellifera; H) Phylloscopus canariensis; and I) Gallotia galloti. Photo credits: A-F, N. Rodríguez; G-I, J. Curbelo.***

2.3.2 Materials and Methods

Study area and species—

The present study was conducted in one of the best-preserved thermosclerophyllous vegetation remnants, located in the Teno Rural Park, northwest Tenerife (see supplementary material Figure 23), during the 2015-2016 flowering periods. The population of *C. canariensis* is found on a steep slope facing north at an altitude of 220 m a.s.l. and occupies an area of ca. 4 ha. The vegetation is a heterogeneous formation with shrubland and woodland areas, and its flora is closely related to that of the Mediterranean Basin but in an oceanic context. The climate is Mediterranean, with a mean annual rainfall of 250-450 mm and mean temperature of 15-19°C (Fernández-Palacios *et al.* 2008).

The study site is dominated by *Heberdenia excelsa* and *Jasminum odoratissimum*; other species are *Olea cerasiformis, Juniperus turbinata, Pistacia* *atlantica, Pistacia lentiscus, Hypericum canariensis, Convolvulus foetidus, Rhamnus crenulata, Rubia fruticosa, Kleinia neriifolia, Euphorbia lamarckii, Periploca laevigata, Lavatera acerifolia*. *Opuntia maxima* and *Prunus dulcis* are also present in the lower anthropized areas.

We selected nine plots (see supplementary material Figure 23) within the area with high density of *C. canariensis*. Each plot was determined by topography, orientation, sun exposure, vegetation and land use. Vegetation and land use included closed and open thermosclerophyllous woodland and/or shrubland, and orchards. *Canarina canariensis* (L.) Vatke (Campanulaceae), locally known as 'bicacarera' or 'bicácaro', is a scrambling perennial herb endemic to the islands of Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro. It belongs to the Macaronesian bird-flower element (Vogel *et al.* 1984; Olesen 1985; Valido *et al.* 2004; Valido and Olesen 2010), which includes endemic plant species in the genera *Isoplexis, Teucrium, Scrophularia, Musschia, Anagyris, Lavatera, Lotus, Echium* and *Justicia*. These plants exhibit characteristics associated with the ornithophilous pollination syndrome (Fenster *et al.* 2004; Ollerton *et al.* 2009a), and are visited by opportunistic nectar-feeding passerine birds (*Phylloscopus, Cyanistes, Serinus, Sylvia* and *Fringilla*) and the endemic lizard (*Gallotia galloti*) (Valido *et al.* 2004; Valido and Olesen 2010). The bell-shaped reddish-orange flowers (darkening when dried) develop at the end of the stems in a three-flower dichasium. In laurel forests, flowers are reported to last an average of 18.1 (SD: 4.4) days (Ollerton *et al.* 2009b) and contain large quantities (53-109.8 µl) of dilute hexose-dominant nectar (12.2-16.4% sugar; Dupont *et al.* 2004b; Valido *et al.* 2004; Ollerton *et al.* 2009b; Rodríguez-Rodríguez and Valido 2011). The flowers are protandrous and have secondary pollen presentation via pollen-collection hairs. Self-fertilization is possible, but depresses its reproductive success (Rodríguez-Rodríguez and Valido 2011; and present study). Mature fruits are reddish-orange ovoid-shaped berries, containing many seeds, and are consumed mainly by native birds and lizards (Valido 1999; González-Castro *et al.* 2015), and also by introduced rats (Valido *et al.* 2011; this study).

In the laurel forest (or laurisilva), *C. canariensis* flowers are visited primarily by opportunistic, native passerine birds, mainly *Phylloscopus canariensis*, but also *Cyanistes caeruleus*, and three species of warblers, *Sylvia atricapilla, S. conspicillata* and *S. melanocephala* (Valido *et al.* 2004). In open areas, the endemic Canarian lizard *Gallotia galloti* also visits *C. canariensis* flowers, acting as a potential pollinator (Siverio and Rodríguez-Rodríguez 2012). The flowers are visited by several species of insects including *A. mellifera* (Valido *et al.*, 2004; Rodríguez-Rodríguez and Valido, 2011). Other animals visit the flowers for shelter, like the isopod *Armadillidium vulgare*, whereas endemic semi-slugs and introduced black rats *Rattus rattus* consume floral tissue (Rodríguez-Rodríguez and Valido, 2011). During this study, an important leaf and flower herbivory attack by the native beetle *Meloe tuccius* was noted (Figure 14D), in which some of the plants were even completely defoliated by this insect.

Floral traits and longevity—

A total of 51 flowers from 37 plants were sampled for floral rewards and morphometry. We scored each flower according to its phenological phase (male or female) and measured corolla length, corolla bell diameter at opening and nectary level, counting the number of petals, stamens and stigmatic lobes. Moreover, we estimated floral rewards (nectar volume and sugar concentration) as nectar standing crop, extracting the nectar with 5µl micropipettes using 0-50% (Bellingham and Stanley LTD, Tunbridge Wells) and 45-82% (Bellingham and Stanley, Eclipse 020310) handheld sugar refractometers.

A total of 60 flowers were sampled for floral phenology from two plots (‘Plot 6’ and ‘Plot 1’ (see supplementary material Figure 23). ‘Plot 6’ was located in a shady humid ravine completely covered with dense native vegetation, whereas ‘Plot 1’ was below the mountain with sparse open anthropized vegetation. Floral buds close to anthesis were labeled and checked daily in order to record the days of sexual phase change and flower wilting.

Identification and visitation rate of main floral visitors—

Diurnal censuses

To identify flower visitors and quantify their visitation frequency, a total of 2,039 haphazardly chosen flowers were observed during focal censuses, by means of binoculars from a site located 5-10 m away from the plant. These direct observations were supplemented by video recordings and photographs. Animals were considered as flower visitors whenever they contacted the sexual organs inside the corolla. For each flower visitor, we recorded: species identity, number of flowers visited, and number of probes per flower. We performed 124.48 h of observations (*n* = 144 periods of 15-60 min) from December 2015 until mid-March 2016. Based on the dates of the flowering season, censuses were classified as early (December - mid-January), mid (late January - mid-February) and late (late February - mid-March), with the goal of assessing if different pollinators were available during the flowering season. Censuses were distinguished according to time of day as morning (8:00-11:30h), midday (11:30-15:00h) and afternoon (15:00-18:30h) censuses.

Nocturnal censuses

A total 21.12 h of observations were performed on a total of three nights in January, February and March 2016 starting at 8:00 pm and lasting up to 7:00 am. We censused a total of 103 flowers divided into three patches separated from each other ca. 30 and 50 m. Observed flowers varied in each census, 40, 47 and 16 flowers in January, February and March, respectively. We censused every half hour, for periods of 7 min, separated by 23 min intervals, at a distance of 0.5 m from the plant. Infrared light was used to avoid disturbing nocturnal visitors to flowers.

Pollinator effectiveness—

Based on the observations of flower visitors, and using flowers not involved in floral surveys, we conducted experiments to study the contribution by the different guilds of pollinators (passerine birds, lizards and insects) to pollination success. In each plot, five randomly assigned exclusion treatments were performed on each of 9-13 plants: (1) total exclusion: flowers were bagged (with a muslin bag) to exclude any type of flower visitor (Figure 15A); (2) bird-exclusion: flowers were enclosed in a 5 x 2 cm plastic mesh to avoid birds accessing the flowers, but near the ground or with perches for lizard access (i.e. access to lizards and insects; Figure 15B); (3) lizard-exclusion: a 4 cm acetate funnel was placed at the base of pendent flowers to avoid lizards climbing them (i.e., access to birds and insects; Figure 15C); and (4) vertebrate (bird + lizard) exclusion: pendent flowers were covered with a suitable plastic net (i.e., access to insects; Figure 15D). Another set of flowers was left open to all flower visitors, as controls. All exclusions were imposed at the flower bud stage, before flower anthesis. A total of 570 flowers from 96 plants were used for the experiment. Flowers from all treatments were inspected daily to assess that the exclosures were functioning correctly.

Fruits from all treatments were covered with chicken wire on beginning to ripen, to avoid fruits being consumed by animals and were collected on March and April 2016. For each treatment, fruit were weighed, and the fruit set was calculated as the proportion of flowers that became fruit. Seed set was the amount of viable seeds produced per fruit. Non-viable (aborted) seeds were identified according to their characteristic grayness and smaller size.

In another set of female flowers, we further tested whether the frequency of bird visits influenced plant reproductive success, by looking for evidence of corolla piercing and scratches resulting from hanging bird visits. Flowers were classified as ‘frequently visited’ or ‘infrequently visited’, based on whether they had less or more than 20 marks, respectively (this threshold was established after estimating that in each visit, birds usually leave ca. 10 marks on the flower). The total of 76 flowers marked were monitored, and fruit and seed set was assessed for each.

Figure 15 Experimental treatments applied, A) Total exclusion; B) Bird-exclusion; C) Lizard-exclusion, and D) Vertebrate exclusion.

Herbivory assessment—

We determined the level of floral herbivory (fraction of damaged flowers) and the effects on plant reproductive success (reduction in fruit and seed set), conducting four monthly floral surveys, once per month (from December to April, n_{total}= 1,680 flowers). In each survey we checked for the level of herbivory on all flowers from all nine monitored plots. For each flower, we recorded the floral position (on the ground or hanging at low, medium or high levels) and herbivory using the following criteria: (1) presence or absence of floral herbivory; (2) herbivore (rat, semi-slug, or both); (3) structures affected: corolla, sexual organs (anthers, style), or a combination of both; and (4) fraction affected. Damaged flowers were tagged and monitored until they withered or produced fruit. Fruits were protected, collected and evaluated using the procedure described above. Semi-slug and rat damage can easily be identified, as semi-slugs leave slimy trails and/or feces inside the flower, and may consume any part of the flower, but mainly the androecium, whereas rats usually tear the corolla and consume the style to reach the nectar (Rodríguez-Rodríguez and Valido 2011).

To assess the relative abundance of rats, we used bait units composed of ten toasted-honey corn rings hung up by a wire 20 cm above the ground. Once per month, on three occasions from January to March, we left ten bait units overnight (at 5 m intervals) at all plots. Baits were collected early the next morning and the remaining corn rings were counted.

Statistical analyses—

Generalized linear mixed models (GLMM) and generalized linear models (GLM) in R software version 3.3.3 (R Core Team 2018) were used, followed by a Tukey test for the required multiple comparisons of regression coefficients. Differences in flower biometry and nectar were estimated using a Gaussian or gamma error distribution. To test for differences in flower visitation rate, our response variable was the number of visits to each flower per unit time, whereas the predictor variables included in the model were: animal group, day (grouped into early, mid, and late flowering, as previously indicated), and time of day (early, mid and afternoon), with a Gamma error distribution. We further included flower standing crop as a covariate, as this may be an important factor determining the visits of a given guild of pollinators.

To test for differences in plant reproductive success after exclusion experiments and after flower herbivory, we analyzed two variables related to female fitness (percentage of flowers that set fruit and number of viable seeds per fruit). Data were adjusted to binomial or Poisson distributions and using plot as a random factor. The standard errors were corrected using a quasi-GLM model where the variance is given by f x μ, where μ is the mean and f the dispersion parameter when dispersion was detected. Consumption rates of baits and flowers, location and date were analyzed with a binomial error distribution. To test for differences in fruit set in preyed on flowers we used five predictor variables: date, plot, type of damage, percentage of damage, and flower location. We evaluated colinearity by means of the variance inflation factor (VIF), and variables with VIF value > 3 were removed (Zuur *et al.* 2009). The best model was selected with the dredge function in the package *MuMIn* (multimodel inference), adjusting data to a gamma error distribution. Again, the individual plant observed was used as a random effect. The VIF analysis showed that all predictors had low colinearity (< 3) and thus were included in the models. Throughout the text, all means are accompanied with their standard errors unless otherwise indicated.

2.3.3 Results

Floral traits—

Morphological traits did not differ significantly between the male and female phase, except for corolla bell diameter at the flower opening, which is smaller during the male phase (Table 5). Nectar standing crop and sugar concentration did not differ between sexual phases either ($\chi^2_1 = 0.73$, $P = 0.39$), and were also similar between areas in the shade of the mountain *vs* fully exposed to sun; $\chi^2_1 = 0.31$, $P = 0.58$, Table 5).

Individual floral longevity varied among sites, ranging from 10.71 ± 0.51 days, N = 35 flowers) in 'Plot 1' to 14.70 ± 1.33 days, N = 20 flowers days in 'Plot 6' ($\chi^2_1 = 16.21$, $P < 0.001$). Greater longevity was caused by a significantly longer duration of the male phase in 'Plot 6' (10.35 ± 1.58 days) compared with 'Plot 1'

(6.09 ± 0.33) (χ^2_1 = 29.19, P < 0.001), whereas there were no significant differences in the duration of the female phase (4.53 ± 0.27 days; χ^2_1 = 0.22, P = 0.64).

Table 5 Quantitative characterization of male and female stages of floral traits of Canarina canariensis.

Flower trait	Df	χ^2	P	X ± SE	Range	*n*
Corolla length (mm)	1	0.020	0.889	38.525 ± 0.764	27.15-51.85	51
Corolla bell diameter at nectary level (mm)	1	0.005	0.941	16.779 ± 0.365	11.72-21.66	51
Corolla bell diameter at opening (mm)	1	9.151	**0.002**	Male: 49.596 ± 1.389	36.20-62.63	24
				Female:53.097 ± 1.580	39.65-69.40	27
Number of petals	1	0.334	0.563	6.137 ± 0.079	5-8	51
Number of stamens	1	0.008	0.929	6.176 ± 0.072	5-8	51
Number of stigmatic lobes	1	-11.326	1	6.071 ± 0.050	6-7	28
Nectar volume (µl)	1	1.557	0.212	35.4343 ± 5.235	0-150.50	51
Sugar concentration (%)	1	1.421	0.233	42.738 ± 2.882	13-74	46

Identification and visitation rate of main floral visitors—

During the diurnal censuses, we detected four species of vertebrates visiting the flowers of *C. canariensis*: three species of birds, the Canary Islands chiffchaff *Phylloscopus canariensis* being the most frequent visitor (Figure 14H), *Cyanistes teneriffae* and *Sylvia melanocephala*, observed only once, and the endemic lizard *Gallotia galloti* (Figure 14I). Among insects, we observed *Apis mellifera* (Hymenoptera: Apidae; Figure 14G), *Gonepteryx cleobule* (Lepidoptera: Pieridae), and unidentified ants (Hymenoptera: Formicidae; Figure 14C) visiting the flowers. However, the latter feed on the nectar without contacting the sexual organs and are thus not legitimate pollinators of *C. canariensis*. The millipede *Ommatoiulus moreletii* (Julida: Julidae; Figure 14B) and two caterpillars of unidentified species (Figure 14E and Figure 14F) were also found inside some flowers, apparently searching for shelter. These three species and the ants were excluded from the analysis of visitation rates.

Animal visits were detected in 100 out of 144 diurnal censuses. Flower visitation rate was affected by flowering phenology and time of the day (χ^2_2 = 7.59, P = 0.02; and χ^2_2 = 17.87, P < 0.001, respectively). It increased during the flowering season (Figure 16A) and was consistently higher at midday (Figure 16B).

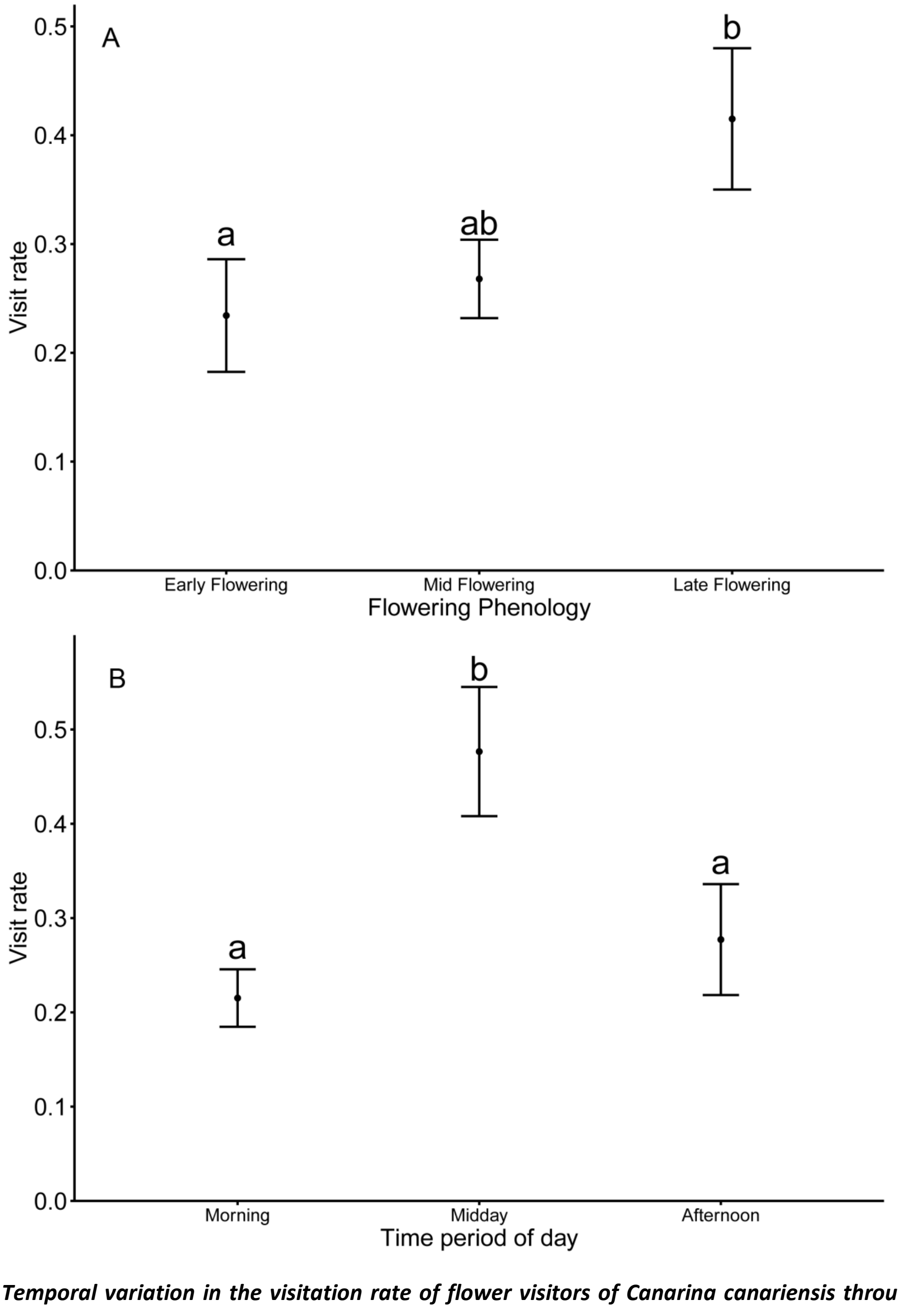

***Figure 16** Temporal variation in the visitation rate of flower visitors of Canarina canariensis throughout the flowering season (a) and the day (b). Visitation rate is arrivals per flower per hour, represented by the number of visits per census · minutes of census^{-1} · flowers observed^{-1}· 60. Letters indicate significant differences during flowering phenology and time period of the day using Tukey´s test after GLM (α = 0.05).*

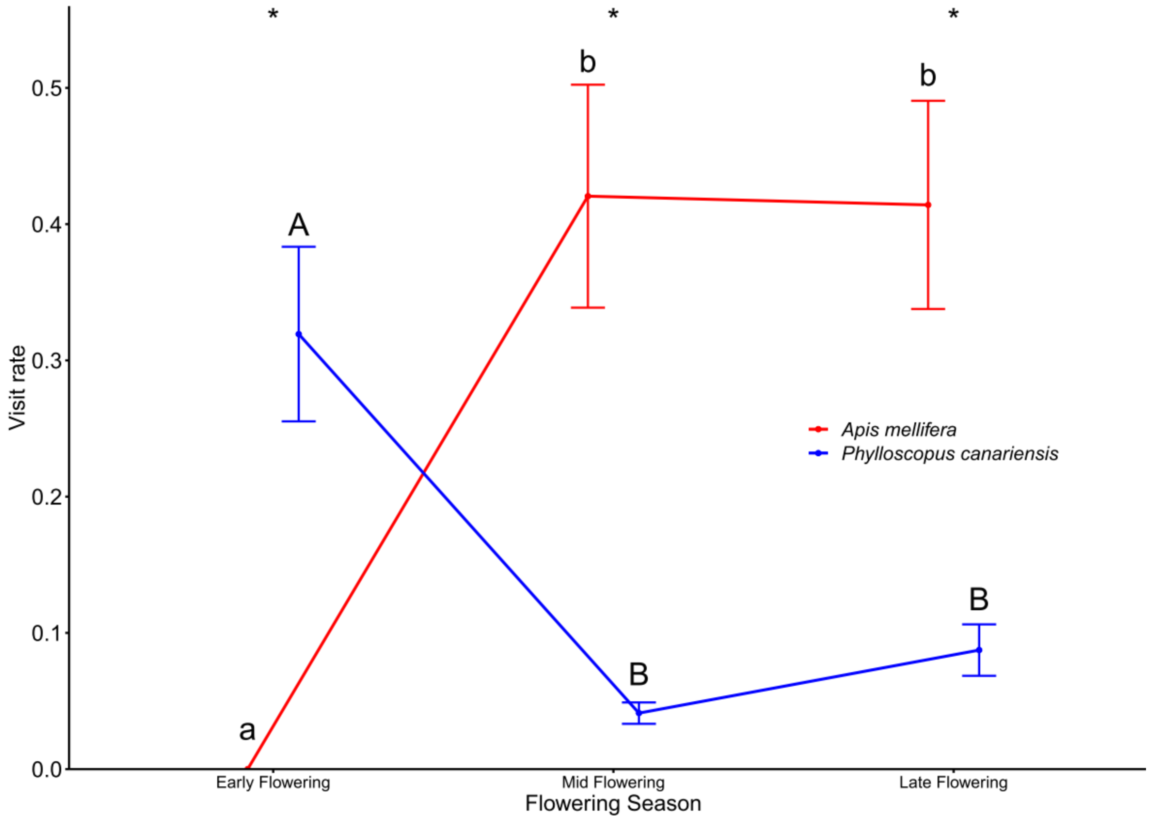

Figure 17 Temporal variation in the visitation rate of flower visitors Apis mellifera and Phylloscopus canariensis to Canarina canariensis throughout the flowering season. Visitation rate is arrivals per flower per hour, represented by the number of visits per census · minutes of census^{-1} · flowers observed^{-1}· 60. Early flowering lasts from December to January, mid from January to February and late from February to March. Data are means ±SE. Letters indicate significant differences in visitation rate during the flowering seasons within each animal group, and asterisks indicate significant differences between animal groups within each flowering season using Tukey´s test after GLM (α = 0.05)..

Of the 407 observed pollination visits, *A. mellifera* was the most frequent visitor (82.06% of the floral visits), followed by the bird *P. canariensis* (15.97%). The butterfly *G. cleobule* and the lizard *G. galloti* were observed in only 0.98% and 0.49% of the visits, respectively. The bird probed each flower more frequently than *A. mellifera* (2.15 ± 0.15 probes/flower, *n* = 91 flowers, vs. 1.17 ± 0.03 probes/flower, *n* = 557 flowers) (χ^2_1 = 49.74, P < 0.001). However, both species probed a similar number of flowers per visit (1.63 ± 0.06 flowers/visit, *n* = 399 visits; χ^2_1 = 2.71, P = 0.10). The time of day had no effect on flower visitation rate in either of the two species (see supplementary material Figure 24, χ^2_2 = 3.71, P = 0.16, and χ^2_2 = 4.70, P = 0.10, respectively). However, the effect of the animal group on flower visitation rate did vary with flowering phenology (χ^2_2 = 35.73, P < 0.001). While P. *canariensis* was the most common visitor in the early flowering period when *A. mellifera* was absent, the latter became the most frequent visitor as the flowering season progressed (Figure 17).

No visitors were recorded during any of the nocturnal censuses, indicating that *C. canariensis* is pollinated mostly (or only) by diurnal animals.

Pollination effectiveness of the different flower-visitor groups—

Fruit set varied significantly among treatments ($\chi^2_4 = 26.53$, $P < 0.001$). It was higher in the open-pollination (control) and in the lizard-exclusion treatment than in either the autogamy treatment (all animals excluded) or the bird and vertebrate exclusions (Figure 18). That is, the plant appears to be mainly pollinated by birds. This is supported by the strong decrease in fruit set when they were excluded from visiting flowers. Insects did not appear to contribute much to pollination success, given that the effects of vertebrate exclusions and autogamy treatment were not significantly different (Figure 18).

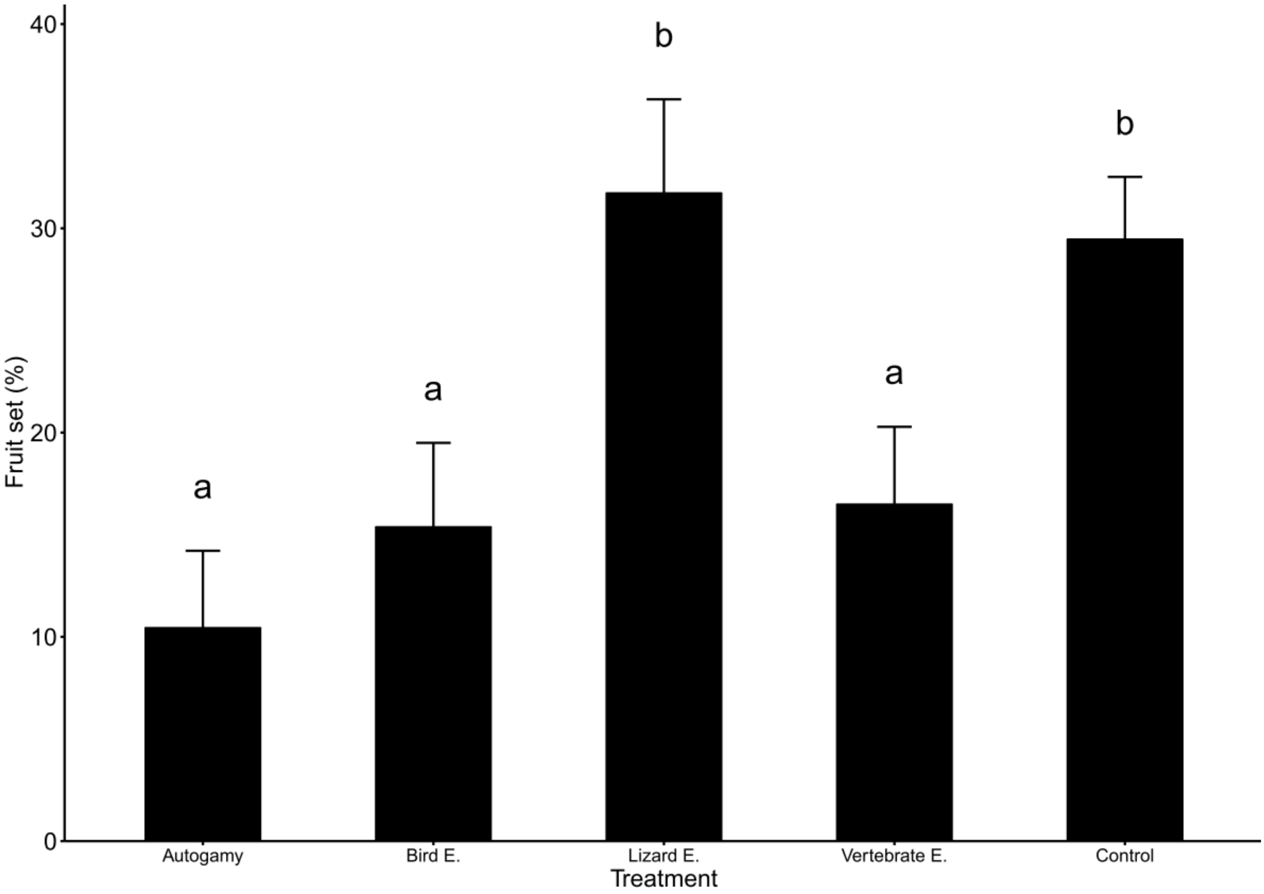

Figure 18 Mean (±SE) percentage of flowers that set fruit per plant. Sample sizes are Autogamy, n = 67 flowers; Birds Exclusion, n = 78 flowers; Lizard Exclusion, n = 104 flowers, Vertebrate Exclusion, n = 97 flowers; Control, n = 224 flowers. Letters indicate significant differences between treatments using Tukey´s test after GLM (α = 0.05).

Seed set (number of viable seeds per fruit) was on average 272.98 ± 30.24 seeds/fruit, not differing significantly across treatments ($\chi^2_3 = 4.46$, $P = 0.22$). This variable could be estimated only from 54 fruits out of the 134 total fruits produced (see supplementary material Table 10), owing to high fruit predation by rats despite the chicken-wire cages. No fruits from the autogamy treatment were available and this treatment was therefore not included in the analysis.

Regarding reproductive success based on intensity of bird visitation, no differences in either fruit or seed set were found between frequently *vs* infrequently visited flowers (fruit set: $\chi^2_1 = 0.46$ $P = 0.50$; 75 ± 0.05%, *n* = 76 flowers; and seed set: $\chi^2_1 = 0.01$, $P = 0.92$; 402.96 ± 60.84 seeds per fruit, *n* = 23 fruits, see supplementary material Table 10).

Floral herbivory by rats and semi-slugs and consequences on plant fitness—

Pooling data from the four months of monitoring, a total of 1,680 flowers were inspected for herbivory. Of these, 10.83% (182 flowers) were damaged mainly by rats (97.80%, *n* = 178 flowers) and only a small fraction by the semi-slugs *Plutonia* (2.20%, *n* = 4 flowers). All flowers consumed by semi-slugs had the male sexual organs missing to some extent (occasionally the corolla had also been pierced, leaving a slimy trail inside the flower). Such consumed flowers were located in a low or intermediate position within the plant, and none of them produced fruit. By contrast, rats usually tore the corolla and consumed the style to reach the nectar. They usually damaged both reproductive organs and corolla (62.92%; *n* = 112 flowers). In 29.78% of the cases (*n* = 53 flowers) they tore only the corolla, whereas less frequently (7.30% of the cases; *n* = 13 flowers) they damaged only the reproductive organs ($\chi^2_2 = 125.41$, $P < 0.001$; Figure 19). Moreover, rats consumed flowers preferentially at ground level or on low hanging flowers (65.17%; *n* = 116), then on flowers at medium level (26.97%; *n* = 48 flowers), and finally on flowers at high level (7.87%, *n* = 14 flowers) ($\chi^2_2 = 136.38$, $P < 0.001$).

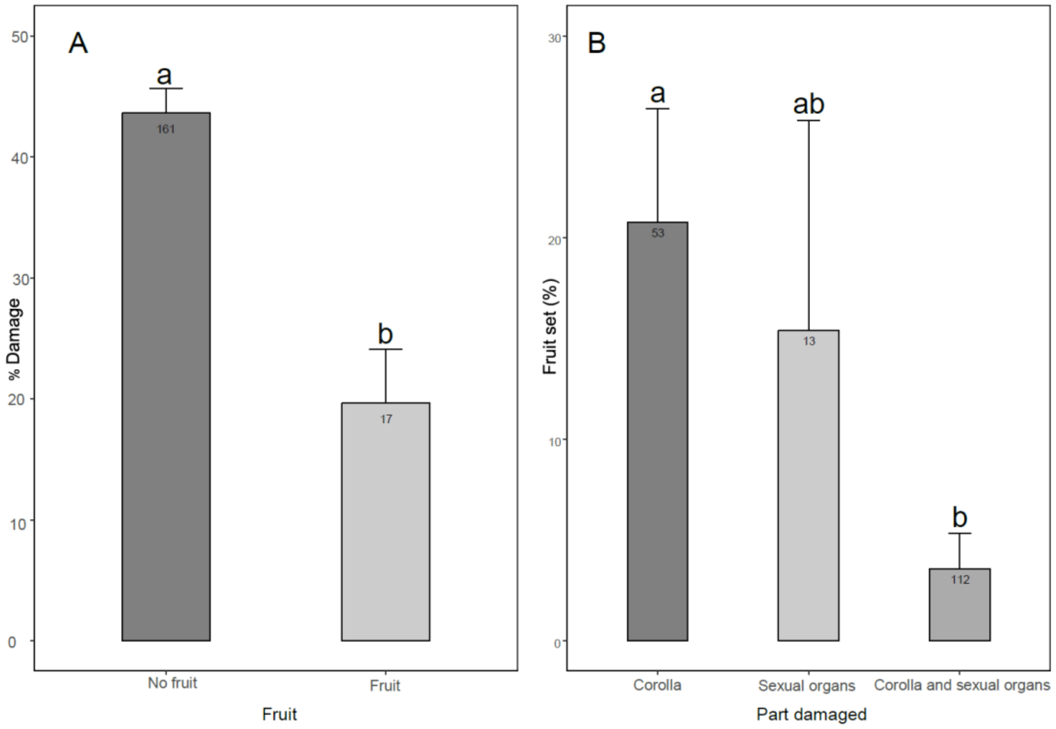

Figure 19 Mean (+SE) percentage of damage to preyed upon flowers that set fruit or not (a); and percentage of fruit produced by flowers preyed upon in different parts (b). Numbers below bars are sample sizes. Letters indicate significant differences between classes using Tukey´s test after GLM (α = 0.05).

Of the total 2,568 baits set to assess rat abundance, 863 (34.19%) were consumed in three monthly surveys. Consumption rates were not consistent between food types (flowers or baits), date, and location ($\chi^2_{12} = 62.67$, $P < 0.001$, see supplementary material Figure 25); therefore, flower and bait consumption were analyzed separately.

Floral herbivory varied in time and site ($\chi^2_{12} = 142.87$, $P < 0.001$), ranging from 0% to 100% (Supplemental Figure 3). Likewise, bait consumption was not consistent in time and space ($\chi^2_{16} = 386.72$, $P < 0.001$) and ranged from 10 to c. 95% (see supplementary material Figure 25).

A higher percentage of baits were consumed compared to flowers (34% *vs* 11%) because baits may be more palatable and accessible to rats, as found in Hernández *et al.* (1999) with artificial nests of endemic pigeons.

The extent of flower damage by rats determined fruit set ($\chi^2_{1} = 16.11$, $P < 0.001$); undamaged flowers set about three times more fruits than damaged flowers (29.46% vs. 10.06%) (χ^2_1 = 11.93, P < 0.001; Table 6 and Figure 19A). Fruit set also depended on the damaged floral organ (χ^2_2 = 16.11, P < 0.001). As expected, when both corolla and sexual organs were damaged, fruit set was lower than when only the corolla was consumed (Figure 19B).

Table 6 Percentage of flowers that set fruit (fruit set), number of total seeds/fruit (seed set) and fruit biometry obtained from flowers damaged and undamaged by rats. Data are means ± SE.

	Treatment		GLMM	
Variables	Damaged flowers *(n)*	Undamaged flowers *(n)*	χ^2_1	P
Fruit set	10.06% (178)	29.46% (224)	11.933	<0.001
Seed set	304.667±143.260 (3)	297.375±43.692(32)	0.0025	0.9599
Fruit weight (mg)	2.429±0.5232 (3)	4.893±0.646 (34)	1.428	0.232
Fruit length (mm)	26.200±2.020 (2)	25.441±1.234 (30)	1.295	0.255
Fruit width (mm)	18.170±2.344 (3)	19.193±1.033 (29)	0.101	0.751

2.3.4 Discussion

Our results show that *C. canariensis* in this thermosclerophyllous woodland remnant on Tenerife relies mostly on passerine birds for reproductive success, mainly *P. canariensis*. This is consistent with findings by Rodríguez-Rodríguez and Valido (2011) from the laurel forest. Although *A. mellifera* is the most common flower visitor, it seems to play a very minor role as pollinator of this plant, not enhancing either fruit or seed set. However, this common alien bee might interfere with bird pollination by depleting nectar from flowers and also by removing substantial amounts of pollen. Alien rats, on the other hand, were found to reduce fruit set significantly, which in the long run could threaten the populations in this habitat.

Floral traits of C. canariensis in the thermosclerophyllous relict vs the laurel forest—

Except for nectar characteristics, flower morphology was similar to that in the laurel forest (Rodríguez-Rodríguez and Valido 2011). Flower morphology did not vary between the male and female phase either, and the wider corolla bell at the opening during the female phase may simply be a consequence of the delayed sequential expansion of the corolla end during anthesis. Interestingly, flower nectar volume in the thermosclerophyllous habitat was about threefold lower but sugar concentration threefold higher than values reported in the laurel forest (Ollerton *et al.* 2009b; Rodríguez-Rodríguez and Valido 2011). Lower air humidity and water availability, and higher temperature compared to laurel forest are probably the causes of such differences. Both temperature and humidity are known environmental factors that influence nectar secretion and sugar concentration (Bertsch 1983; Wyatt *et al.* 1992; Pacini *et al.* 2003 and references therein). Laurel forests are by far the wettest habitats of the Canary Islands, with an average annual precipitation of 600-900 mm, but through mist condensation (so-called 'horizontal precipitation') it can reach up to 1,200 mm, about three times higher than in our study area. Mean annual temperature in the laurel forest is ca. 15 °C (Wildpret and Martín 1997), a few degrees lower than in thermosclerophyllous woodlands, where the mean temperature can reach 19 °C.

Flower longevity (10 - 15 days) is also lower than in the laurel forest, where flowers can last on average 18.1 ± 4.4 days (Ollerton *et al.* 2009b). Flower longevity reflects a balance between the benefit of increased reproductive success and the cost of flower maintenance (Primack 1985). In general, lower pollinator activity (Ashman and Schoen 1994; Giblin 2005; Trunschke and Stöcklin 2017), together with higher environmental stresses, reduced water availability (Jorgensen and Arathi 2013) and high temperature (Yasaka *et al.* 1998; Teixido and Valladares 2015), result in shorter flower longevities (reviewed in Primack 1985). The milder environmental conditions (higher water availability and mild temperatures) (Wildpret and Martín 1997), together with the reduced pollination rate (see next section on flower visitors) in the laurel forest might thus be the cause of a longer flower lifespan there, compared to thermosclerophyllous habitats.

Composition and visitation rate of the main floral visitors to C. canariensis—

The high diversity of flower visitors found in this study contrasts with previous findings in the laurel forest (laurisilva) (Vogel *et al.* 1984; Olesen 1985; Ollerton *et al.* 2009b; but see Rodríguez-Rodríguez and Valido 2011). Moreover, most flower visitors reported in the latter formation were also found in thermosclerophyllous habitats (Vogel *et al.* 1984; Ollerton *et al.* 2009b; Siverio and Rodríguez-Rodríguez 2011) except for *Sylvia conspicillata* among the vertebrate visitors (Olesen 1985), and *Lasioglossum viridae, Paravespula germanica* and *Armadillidium vulgare* among the invertebrates (Valido and Olesen 2010; Rodríguez-Rodríguez and Valido 2011). The endemic lizard, *G. galloti,* is considered an anecdotal flower visitor –in thermosclerophyllous relicts- as it is mainly active at the end of the flowering season, when the temperature is warmer, and in cleared sites where flowers often lie on the ground (Siverio and Rodríguez-Rodríguez 2012).

One main difference in the flower visitors of *C. canarina* between the two types of vegetation is the absence of *A. mellifera* in the laurel forest (Rodríguez-Rodríguez and Valido, 2011). Contrastingly, this species was the most frequent floral visitor at our study site. Moreover, flower visitation by *A. mellifera* accounted for the variation in the flower visitation peaks during midday and at the end of the flowering season. Despite the high number of *A. mellifera* visits, however, bird visitation -and presumably pollination- was strikingly higher than in the laurel forest (Ollerton *et al.* 2009b; Rodríguez-Rodríguez and Valido 2011). This might also account for the reduced flower longevity compared with pollinator-scarce environments (Primack 1985; Ashman and Schoen 1994).

According to previous pollination studies from the Canary Islands, bees visit more flowers at midday whereas vertebrate flower visits are more frequent in the morning or evening (Kraemer and Schmitt, 1997; Dupont *et al.*, 2004a; Fernández de Castro *et al.*, 2017). Our findings are partly concordant with such a pattern; we detected higher insect activity at midday and more bird visits in the mornings, but birds were also seen frequently on flowers at midday. During the flowering season, birds were frequent visitors early in the season, whereas at mid and late season, birds stopped feeding on flowers, coinciding with an increase in *A. mellifera* activity. Indeed, the number of beehives managed in the Canary Islands has dramatically increased in the last 20 years, from ca. 20,000 in 1997 to ca. 39,000 in 2017 (Dirección General de Ganadería 2006; Subdirección General de Productos Ganaderos 2017). This activity has been supported by European, national and local funds, and about half of the hives are on Tenerife (Dirección General de Ganadería 2006). One possible explanation for our observed pattern, thus, is that nectar traits may change during the season (e.g., less but more concentrated nectar later in the season), which is known to influence visitors' foraging patterns (Torres and Galetto 1998; Pacini *et al.* 2003). More dilute nectar at the beginning of the season may be preferred by birds (Faegri and van der Pijl 1966; Johnson and Nicolson 2008), whereas more concentrated nectar in the middle and the end of the season may promote insect visitation (Olesen 1985). Another explanation could be that the birds are consuming other resources during the middle and end of flowering season of *C. canariensis*. However, *P. canariensis* is mainly insectivorous and insect resources are scarce during all the winter (Wolda 1988; Kishimoto-Yamada and Itioka 2015). Alternatively, *A. mellifera* could be interfering with the interaction between birds and *C. canariensis*. Since *A. mellifera* can deplete or lower the standing crop of nectar, we also hypothesize that the change in the flower visitation pattern could be due to exploitative competition between birds and bees, as claimed for other plant species (Kraemer and Schmitt 1997; Hansen *et al.* 2002; Valido *et al.* 2002; Dupont *et al.* 2004a).

Pollination effectiveness of the different flower-visitor groups—

Our findings indicate that birds act as the only legitimate pollinators of *C. canariensis*. Spontaneous selfing produced as many fruits as flowers accessed only by bees and lizards, which suggests that visits by either of these two taxonomic groups do not contribute to pollination success. At least two factors might explain the low efficiency of *A. mellifera* as a pollinator of this plant. First, when collecting nectar, *A. mellifera* might not contact pollen or stigmas due to their small size, and second, when collecting pollen they ensure maximal amounts of pollen are removed, thus reducing the pollen subsequently available for pollination (Vaughton 1996).

Apis mellifera is well integrated in the Tenerife pollination networks (Dupont *et al.* 2003; Padrón *et al.* 2009), although the impact of this alien on native pollination interactions has not been thoroughly evaluated in all invaded ecosystems. However, the only two studies in the sub-alpine desert of Teide indicate that the presence of *A. mellifera* reduces the diversity of native flower visitors and the reproductive performance of *Echium wildpretii* and *Spartocytisus supranubius* (Dupont *et al.* 2004a, Valido *et al.* 2014, Valido *et al.* 2019). In other oceanic islands (e.g. Japan, Tasmania, Mascarene Islands, Seychelles or Mauritius), *A. mellifera* is also well integrated and affects plant fitness (Kaiser-Bunbury *et al.* 2010 and references therein). Despite all the negative consequences of *Apis mellifera* on islands, studies on mainland show mixed effects, the ecological context (resource availability, wild pollinator community, etc.) as well as the *A. mellifera* density (see the reviews of Agüero *et al.* 2018, and Vanbergen et el 2018) playing an important role.

Lizards were not effective pollinators due to their merely anecdotal visits, as mentioned before. Both, in this study and in Rodriguez-Rodriguez and Valido (2011), flower visitation rate was associated with fruit production. However, Ollerton *et al.* (2009b) recorded fewer bird visits to *C. canariensis* in the laurel forest but higher natural fruit set (62 ± 14%) in the absence of *A. mellifera*. Thus, we might expect fruit sets even higher than those in Ollerton *et al.* (2009b). This cannot however be tested since exclusion of only *A. mellifera* is logistically difficult.

Although the levels of autogamy were low (10.45 ± 3.77%), we found that *C. canariensis* can self-pollinate in the thermosclerophyllous woodland. This was unexpected given the results of previous studies in the laurel forest, which did not detect selfing in this plant (Ollerton *et al.* 2009b; Rodríguez-Rodríguez and Valido 2011). Self-fertility levels may vary from population to population due to a number of operational factors (Antonovics 1968), including environmental conditions and different pollinator species (Lloyd and Schoen 1992). In general, unfavorable pollination conditions are likely to increase the amount of autogamy

(Kalisz *et al.* 2004; Busch and Delph 2012). However, given the higher frequency of pollinators in the thermosclerophyllous relict, a lower level of selfing would actually be expected there compared to the laurel forest. In contrast, different environmental factors between the two vegetation zones, such as temperature, light and humidity (Lloyd and Schoen 1992) may affect the levels of self-fertilization.

Floral herbivory and consequences on plant fitness—

Only about 11% of all surveyed flowers suffered from herbivory, mainly by rats. Such a figure contrasts with the 46% reported from the nearby laurel forest, also in Teno (Rodríguez-Rodríguez and Valido 2011). Moreover, as these authors point out, most flower herbivory in the latter was caused by semi-slugs rather than by rats. These differences may be explained by differences in species abundances in the two habitats. *Plutonia* sp. is clearly more abundant in the laurel than the thermosclerophyllous habitat because of the difference in humidity, but their respective rat abundance is not so clear. Rats are usually abundant in the laurel forest (Hernández *et al.* 1999). We attribute the higher abundance of rats at our study site to the short distance from agricultural areas. Further studies are needed to quantify differences in rat abundance and flower herbivory rates between the two types of vegetation.

Interestingly, the behavior of rats consuming flowers also differed between the two formations. In the laurel forest, rats seem to prefer the gynoecium (Rodríguez-Rodríguez and Valido 2011) whilst we found that they consumed both reproductive organs and corolla at our study site, thus increasing floral damage. The floral damage reduced potential fruit set in both habitats but it was slightly more severe in the thermosclerophyllous remnant (Rodríguez-Rodríguez and Valido 2011). Likewise, rats preferentially consumed flowers on the ground or pendent at a low height, whereas they showed no such preference in the laurel forest (Rodríguez-Rodríguez and Valido 2011).

The impacts of *Rattus rattus* on the reproductive success of *C. canariensis* might be underestimated, as rat pollination and rat fruit consumption/seed destruction were not evaluated. Rodents are the main postdispersal seed predators in many ecosystems (Jensen 1985, Price and Jenkins 1986, Traveset *et al.* 2009). The negative consequences of fruit consumption and seed destruction by rats recorded in the Canaries (Delgado *et al.* 2000, Traveset *et al.* 2009) and other islands (Lord 1991, Shiels 2011, Shiels and Drake 2011, Shiels *et al.* 2014) outweigh the benefits of a possible rat pollination (Ecroyd 1996, Vitousek 1987, Pattermore *et al.* 2012) or fruit dispersal by caching for further consumption (Delgado *et al.* 2000, Shiels and Drake 2010). However, the probability of predation depends on the ecological context (e.g. plant accessibility, actual rat abundances, flower, fruit and seed densities, resource phenological predictability, etc.) (Price and Jenkins 1986, Herrera 1985).

***Concluding remarks, conservation and future avenues of research*—**

Compared with laurel forest, *C. canariensis* received more visits from *P. canariensis*, but also more from the alien *A. mellifera*. Besides being the most common flower visitor, *P. canariensis* was the only effective pollinator of this endemic plant in the early flowering season, but it was replaced by *A. mellifera* in the mid and late season. If *A. mellifera* does disrupt the *Phylloscopus-Canarina* interaction, hindering effective pollination, the increasing trend in the number of beehives on Tenerife might threaten the maintenance of *C. canariensis* in the thermosclerophyllous habitat. This would be exacerbated by the effect of climate change, enhancing *A. mellifera* activity earlier in winter. In order to maintain the presence of *C. canariensis* in such relicts, we thus recommend reducing the extensive use of *A. mellifera* near *C. canariensis* populations close to places of human activity. Moreover, as *C. canariensis* fruits are also consumed and dispersed by birds, it would be also be interesting to determine to what extent *P. canariensis* contributes to each stage of the life cycle (i.e., pollination, fruit consumption and seed deposition) of this endemic plant, in both laurel forests and thermosclerophyllous woodlands.

2.3.5 Funding Information

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2.4 Chapter 4

Pollination and seed dispersal effectiveness of birds and lizards in a double mutualism system

Abstract

Mutualistic plant-animal interactions are one of the most important ecological processes to generate and maintain global biodiversity. In the cases of ‘double mutualisms’, the same plant species may be pollinated and dispersed by the same animal species. In this study, we investigated the relative effectiveness of different vertebrate groups (birds and lizards) at two different stages (i.e., pollination and seed dispersal) of the reproductive cycle of the endemic scrambling perennial herb *Canarina canariensis* in remnants of the former thermosclerophyllous woodland of Tenerife (Canary Islands). From field data on pollination and seed dispersal, we built up three stochastic models (namely ‘pollination’, ‘dispersal’ and ‘pollination+dispersal’ models) to simulate seedling recruitment. The dispersal stage contributed more than the pollination stage through the recruitment process. Moreover, birds and lizards exhibited a functional complementarity, with birds contributing more in the pollination model whilst lizards in the dispersal model. We argue that the use of stochastic simulation models constitutes a novel approach to assess the relative importance of two mutualist processes different from that based in effectiveness landscapes implemented by Schupp *et al.* (2010, 2017).

2.4.1 Introduction

Mutualistic plant-animal interactions are widely spread across many different ecosystems worldwide and have been proposed as one of the most important ecological processes to generate and maintain the global biodiversity (Thompson 2005). Both pollination and seed dispersal of plants by animals are among the most studied mutualisms, not only for single pairs of species but at the community level, leading to the emergence of different questions of growing interest for the study of mutualistic interactions.

One of such questions deals with the effectiveness of mutualisms and its ecological and evolutionary consequences for interacting species (Schupp *et al.* 2017). The reproductive success of many plant species depends on how effective animals are both in the concatenated pollination and seed dispersal processes. Another important aspect within the framework of mutualistic interactions is the so-called 'double mutualism' phenomenon (Hansen and Müller 2009), which occurs when the same animal species utilizes the same plant species in two different reproductive stages of the plant (i.e., harvesting its floral resources and feeding upon its fruits), acting both as pollinator and seed disperser of the same plant species. Therefore, a double mutualism may lead to a double positive feedback loop (Olesen *et al.* 2018) whereby an animal species that collects nectar and pollen as reward when visiting flowers originates a viable fruit crop, which in turn will lure the same animal species to feed on the fruits leading to its seed dispersal. Double mutualisms have shown to be an island phenomenon, where paired with the threat status of island species suggest that the loss of a double mutualist and its cascading consequences may have severe consequences on community composition and functioning of fragile island ecosystem (Fuster *et al.* 2019).

However, although an increasing number of studies worldwide have aimed at elucidating the pollination and the seed dispersal effectiveness provided by animals (Schupp *et al.* 2017), we still lack much information on the relative importance of pollination and seed dispersal effectiveness as determinants of plant reproductive success (but see Fontúrbel *et al.* 2017).

Frequently, there are systems in which pollination and/or seed dispersal rely on different animal groups. In such cases, the effectiveness of mutualists usually vary much among animal groups (e.g. Traveset and Sáez 1997; Rodríguez-Rodríguez *et al.* 2013; González-Castro *et al.* 2015; Jaca *et al.* 2018), often leading to complementarity between pollinators or between dispersers. For instance, González-Castro *et al.* (2015) reported complementarity between birds and lizards as seed dispersers for a plant community, some plants depending more on birds while others on lizards for their recruitment, which might have important consequences for plant species coexistence.

A given plant species can be both pollinated and dispersed by more than one species or species groups. For instance, in island ecosystems, generalist birds and lizards have been reported to fed on both nectar and fruits from several species (Olesen and Valido 2004; Valido and Olesen 2019). Consequently, there may be cases of 'two double mutualisms', where the same plant species may be pollinated and dispersed by both birds and lizards (see Fuster *et al.* 2018 for a review). In such cases, knowing the relative effectiveness of different vertebrate groups at the different stages (i.e., pollination and seed dispersal) of the reproductive cycle of plant species could help us to understand their natural regeneration and further persistence in the community, especially if we consider that highly-dependent mutualistic relationships in a community reduce the resilience of the system (Allesina and Tang 2012).

In this study, we focus on an endemic scrambling perennial herb, the Canary bellflower, *Canarina canariensis* (L.) Vatke (F. Campanulaceae), which is found in the thermosclerophyllous shrublands and laurel forests from the central and Western Canary Islands (Fernández-Palacios *et al.* 2008). In the thermosclerophyllous shrubland, the species is already known to be pollinated and dispersed by both birds and lizards (González-Castro *et al.* 2015; Jaca *et al.* 2019). From field data on pollination and seed dispersal, we built up three stochastic models (namely 'pollination', 'dispersal' and 'pollination + dispersal' models) to simulate seedling recruitment. Our specific aims were: 1) to assess to what extent our models predict seedling recruitment in the field, 2) to evaluate the relative importance of each mutualistic process (pollination and seed dispersal) as driver of the natural regeneration of *C. canariensis*, and 3) to know the relative contribution of birds and lizards to both pollination and seed dispersal processes. Answering this latter question is key in a changing world, where many plant species are rapidly losing services provided by both pollinators and dispersers, mainly vertebrate animals (Kearns *et al.* 1998; Mokany *et al.* 2014; Harter *et al.* 2015).

2.4.2 Materials and Methods

Study area—

The present study was conducted in one of the best-preserved thermosclerophyllous vegetation remnants, located at the Teno Rural Park, northwest Tenerife, during the 2015-2016 flowering and 2016 fructification periods. Additionally, we used some data obtained from a previous study carried out in the same study site (González-Castro *et al.* 2015). This site is found on a steep slope facing north, at an altitude of 220 m a.s.l., and occupies an area of ca. 4 ha. The Canary thermosclerophyllous habitat is a heterogeneous formation with shrubland and woodland areas, and its flora is closely related to that of the Mediterranean Basin but in an oceanic context. The climate is Mediterranean, with mean annual rainfall of 250-450 mm and mean temperature of 15-19°C (Fernández-Palacios *et al.* 2008).

The study site is dominated by *Hypericum canariense*, *Jasminum odoratissimum* and *Rhamnus crenulata,* and other species present are *Heberdenia excelsa, Pistacia atlantica, Convolvulus floridus, Rubia fruticosa, Kleinia neriifolia, Euphorbia lamarckii, Periploca laevigata, Lavatera acerifolia*. *Opuntia maxima* and *Prunus dulcis* are also present in the lower anthropogenic areas.

Study species—

Canarina canariensis (L.) Vatke (Campanulaceae), locally known as 'bicacarera' or 'bicácaro', is a scrambling perennial herb endemic to the islands of Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro. It is protected by the Canarian Governement (Gobierno de Canarias, 1991). This plant is a geophyte that passes the dry season underground and sprouts and blooms from early winter (November) to late spring (May). It grows prostate and creeping with hollow and laticiferous stems up to 3 m long making up expanded mats with several overlapping individuals (Bramwell and Bramwell 1990). Propagation is both by seeds and by shoots from the base of the tuber (Bramwell and Bramwell 1990). The flowers are protandrous and have second pollen presentation via pollen-collection hairs. Self-fertilization is possible, but selfing depresses its reproductive success (Rodríguez-Rodríguez and Valido 2011; Jaca *et al.* 2019). It belongs to the Macaronesian bird-flower element (Vogel *et al.* 1984; Olesen 1985; Valido *et al.* 2004; Valido and Olesen 2010), exhibiting characteristics associated with the ornitophilous pollination syndrome (Fenster *et al.* 2004; Ollerton *et al.* 2009a). In the laurel forest, *C. canariensis* flowers are visited primarily by opportunistic, native passerine birds, mainly *P. canariensis*, but also *C. teneriffae*, and the three species of warblers inhabiting the Canary Islands: *Sylvia atricapilla, S. conspicillata* and *S. melanocephala* (Valido *et al.* 2004). In open areas, the endemic Canarian lizard *Gallotia galloti* also visits *C. canariensis* flowers, acting as a potential pollinator (Siverio and Rodríguez-Rodríguez 2012, Jaca *et al.* 2019). Mature fruits are reddish-orange ovoid-shaped berries, containing many seeds, and are consumed mainly by native birds and lizards (Valido 1999; González-Castro *et al.* 2015). *Canarina canariensis* is hence an example of a species involved in a double mutualism in the Canary Islands.

Description of simulation models to estimate the mutualistic services provided by birds and lizards—

Pollination model

The pollination service was estimated as the density of seeds of *C. canariensis* contributed by birds and lizards, as well as by autogamy, in the study area. We used a stochastic simulation model (Figure 20, blue boxes and ellipses) that follows the seed production processes and it was parameterized with quantity and quality components of pollination effectiveness measured in the field (see below: Simulation model parameterization).

The model consists of a series of stages connected by processes, each with its own empirically measured set of transition probabilities. It starts with the number of flowers per unit area, according to their density (per m^2) sampled in the field. To avoid very small and decimal numbers of flowers, we extrapolated it to flowers per 100 m^2 and numbers were rounded to the unit. Then, it applies the transition probabilities corresponding to: (1) flower visits made by either birds, lizards or non-visited (i.e., percentage of flowers that were visited by each animal at each individual census), (2) probability of fruit production (i.e., the fruit set as the percentage of flowers exposed to each pollination treatment that produced fruits) and, lastly, (3) the number of seeds per fruits. The original sample of transition probabilities for each process, measured empirically, was resampled 100,000 times by random selection with replacement (bootstrapping; Manly 1998). The final output of each simulation iteration (the seeds produced per 100 m^2) is the result of the product of the randomly selected transition probabilities at each seed production stage.

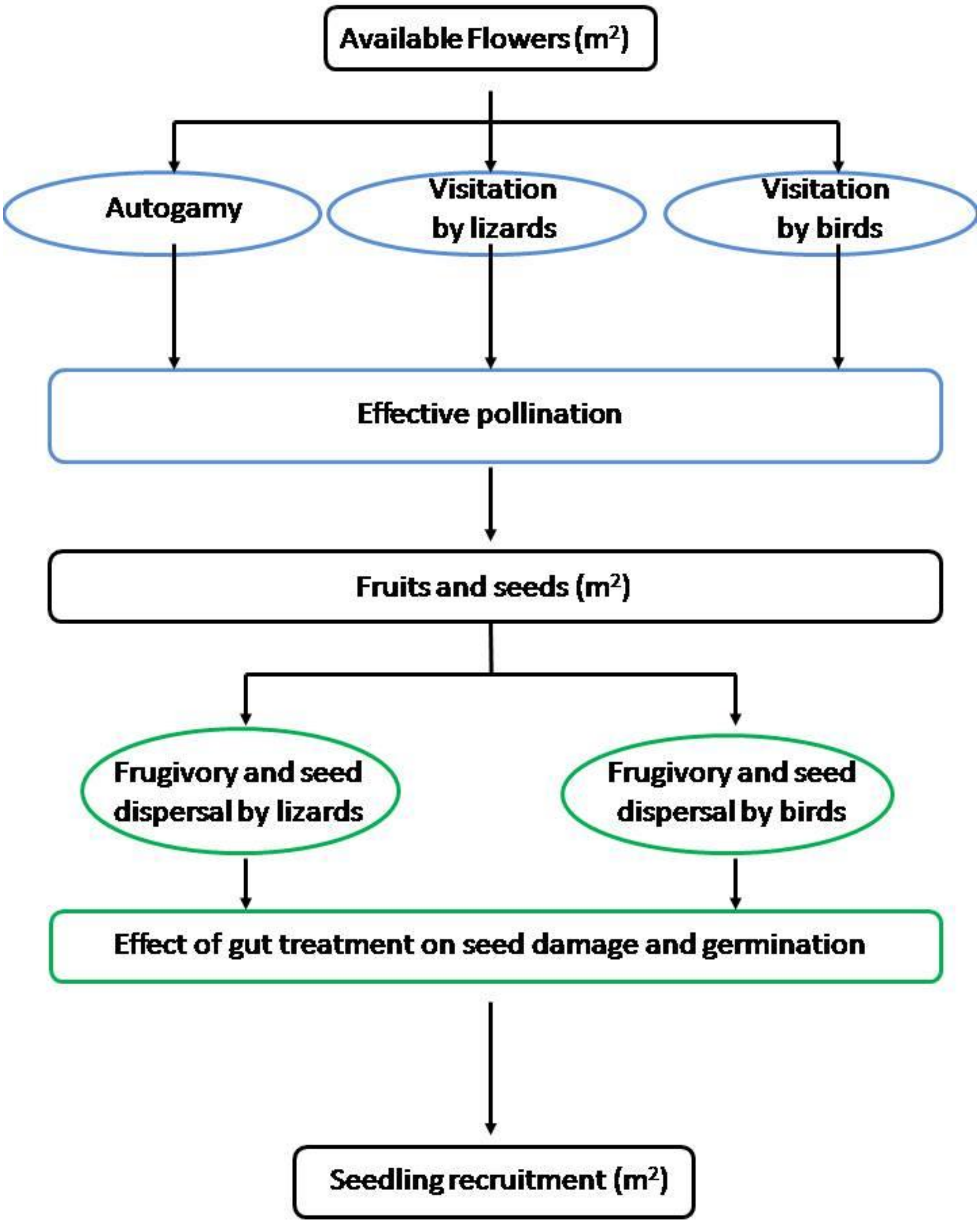

Figure 20 Diagram of the recruitment process showing the different stages considered in the model

Seed dispersal model

The dispersal service provided by birds and lizards was estimated as the density of seedlings of *C. canariensis* contributed by both dispersers in the study area. For this, we used again the stochastic simulation model (Figure 20, green boxes and ellipses) that follows the seedling recruitment processes and it was parameterized with quantity and quality components of the seed dispersal (*sensu* Schupp *et al.* 2010) measured in the field and greenhouse (see below: Simulation model parameterization).

In this case, the model starts with the number of seeds per unit area, according to their density sampled in the field (again, extrapolated to seeds per 100 m^2 and rounded to the unit to avoid small and decimal numbers). Then, it applies the transition probabilities corresponding to: (1) probability of dispersal (as the relation of seeds found in seed traps relative to the available seeds), (2) the probability of seeds being dispersed by either birds or lizards (percentage of seeds in bird or lizard defecations/regurgitations in the seed traps), (3) seed damage caused through ingestion by animals (percentage of damaged seeds in bird and lizard feces/regurgitations) and, lastly, (4) seedling emergence, considering both the effect of the site (percentage of control seeds, taken from the mother plants, that germinate in the field) and the effect of seed ingestion by dispersers (ratio between the probability of seedling emergence obtained from seeds defecated/regurgitated by birds or lizards and that of control seeds, as measured in a greenhouse experiment). Like for the pollination model, the original sample of transition probabilities for each process, measured empirically, was resampled 100,000 times by bootstrapping. The final output of each simulation iteration (the seedlings emerged) was the result of the product of the randomly selected transition probabilities at each seedling recruitment stage.

Pollination + seed dispersal model

This combined model was a combination of the two previously described models. In this case, each iteration at the dispersal phase starts with the number of seeds produced during the pollination phase (i.e., the pollination model outputs at each of its 100,000 iterations) in order to concatenate both phases (i.e., the flower pollination followed by the seed dispersal). These stochastic models allow us to estimate the fate of individuals (flowers or seeds) throughout the process of pollination, dispersal and both together.

Models' parameterization—

The parameters used in each model are shown in the supplementary material Table 11, and were obtained using the following methodology.

(a) Amount of available flowers

Three different transects were established parallel to three different paths covering the study area (see supplementary material Table 12). Each one of these paths was subdivided in different 50 × 2 m sub-transects as follows: 12 sub-transects for 'Path 1', five for 'Path 2'and 14 for 'Path 3'. The nature of the study area constrained the length of paths, which thus determined the number of sub-transects used within each path. At each sub-transect, we counted the number of flowers, so we could estimate the density of flowers per unit area, extrapolated to 100 m^2 to avoid small and decimal numbers (see section for model description above).

(b) Probability of flower visitation by vertebrate pollinators

Throughout the study area, we selected nine plots, separated by at least 11 m from each other, within the area with high density of *C. canariensis*. In eight of them, we performed a total of 141 censuses for flower visitors (20.3 censuses per population on average and ranging between 1 and 72). The number of censuses was constrained by the length of flowering period at each population, so the larger the flowering period, the higher the number of censuses. They lasted 52.97 ± 13.38 minutes (mean ± SD; ranging between 10 and 60 minutes and encompassing a total of 124.5 hours of observation). During each census, we recorded the number of flowers and those that were visited by either lizards or birds. We estimated the visitation probability by each vertebrate type as the ratio between the number of flowers visited by each type of vertebrate and the number of flowers observed during each census. The probability of a given flower to receive only its own pollen was set as 1 minus the probability of visit by either lizards or birds.

Censuses were performed with binoculars to a prudential distance from the plants to avoid interference with the activity of vertebrates around the plant. Although some insect visits were recorded, we excluded them from analyses because their role as pollinators is not significant when compared to that of birds or lizards (Rodríguez-Rodríguez and Valido 2011; Jaca *et al.* 2019).

(c) Probability of fruit production and number of seeds per fruit

At each of the nine plots above mentioned, we performed a flower-exclusion experiment to assess fruit set in flowers only visited by insects and birds (lizards excluded), flowers visited by insects and lizards (birds excluded), flowers visited by insects (vertebrates excluded) and flowers only exposed to its own pollen (all visitors excluded). At the end of the experiment we counted the number of flowers developing into fruits within each treatment. With this experiment we could estimate the probability of fruit production depending on whether the flower was visited (either by bird or lizard) or not (autogamy). We discounted the effect of insects in both bird-exposed and lizard-exposed flowers by using the average fruit set produced by flowers only visited by insects, following the method proposed by Reynolds and Fenster (2008).

We randomly selected 323 flowers for the experiment. Due to the scrambling nature of *C. canariensis*, it was difficult to have a precise number of independent individual plants within each plot, i.e. we could not distinguish among individual plants within the nine plots. For this reason, we could not account for the mother plant effect on fruit set across exclusion treatments.

To assign an amount of seeds to each fruit produced at each exclusion treatment, we collected 57 fruits and counted their seeds. We could not use the number of seeds contained within fruits of each exclusion treatment separately because the number of fruits was very low for some of the exclusions (especially for the 'autogamy' treatment). Therefore, we had to assume that differences between birds and lizards as effective pollinators affect fruit set but not seed set.

(d) Seed availability

Within the same 31 sub-transects used to estimate the flower density, we counted the number of fruits produced (Table 12). Then we multiplied it by the mean number of seeds per fruit to have an estimation of seed density as seed per 100 m^2. These 31 values of seed density were bootstrapped 100,000 times to set the starting point for the model only based on seed dispersal.

(e) Probability of seed dispersal by each vertebrate group

To estimate the probability of seed dispersal for *C. canariensis* we monitored 102 seed traps during the fruiting period (between May 2016 and June 2016). Seed traps consisted of 0.12 m^2 square trays 2 cm deep and made of grey plastic mesh (1 mm aperture), to retain feces and provide good drainage. Traps were placed parallel to the above-mentioned paths running across the study area. The distance from the starting point of the path as well as between seed traps was established by generating random numbers between 5 and 12 by using two dices (i.e. 5 to 12 m distances). Feces of passerine birds and lizards and regurgitations of the former were easily distinguishable in the traps.

Seeds of *C. canariensis* contained in feces and regurgitations were identified, counted and extrapolated to seeds dispersed per unit area. For each seed trap, we estimated the probability of dispersal as the ratio between the number of seeds found in the trap (scaled to the unit area) and the number of seeds available per unit area. Once the seed is dispersed, to estimate the probability of being dispersed either by lizard or by bird, for each seed trap containing seeds of *C. canariensis* we calculated the proportion of seeds that were dispersed by lizards and birds, respectively.

(f) Seed damage caused by passage through the digestive tract of animals

We used a dissecting stereomicroscope (16×) to visually determine the percentage of damaged seeds (Figure 20) in feces or regurgitations of birds and lizards (*n* = 12 and 70, respectively) collected at the study site from 2006 to 2009 in a previous study (González-Castro *et al.* 2015) (total: 1,827 seeds). Bird feces and regurgitations were collected during mist-netting sessions and at water feeders. Lizard feces were collected along a 500 × 2 m transect (the same used to estimate lizard and bird densities).

(g) Seedling emergence

We estimated both the effect of environmental conditions at the study site and ingestion by dispersers on seedling emergence. This part of the study was made with previously published data (González-Castro *et al.* 2015). To test the effect of environmental conditions, 440 control seeds of *C. canariensis* were sown in 20 square plots throughout the study site (22 seeds per plot). Plots were distributed in the study area as described for seed traps. Control seeds were obtained from depulped ripe fruits picked from those available on randomly selected plants (*n* = 15 mother plants). We installed a metal mesh covering the plots to avoid potential disturbances. Seeds were sown in October 2010 and seedling emergence was surveyed every 15 days until the end of March 2011, thus comprising the rainy season in the Canaries, when seedling emergence mainly occurs (Rodríguez *et al.* 2007).

The effect of ingestion by dispersers was computed as the ratio between the probability of seedling emergence from control seeds and the probability of seedling emergence from undamaged seeds defecated/regurgitated by birds or lizards. This ratio equals 1 when there was no effect (emergence of control seeds equals that of defecated seeds), is higher than 1 when seed ingestion has a detrimental effect and lower than 1 when positive. Control and ingested seeds were obtained as described previously. Seeds collected in each fruiting season (2006-2007, 2007-2008 and 2008-2009) were sown the following October. Seeds from each treatment (230 for control, 244 for bird and 230 for lizard) were individually sown in 4 cm^2 pots filled with a standard substrate (50% peat and 50% agricultural soil), and were distributed randomly in the greenhouse. Pots were watered every two days and seedling emergence was monitored every five days for six months, until the end of March.

The probability of seedling emergence for seeds defecated by birds and lizards in each site type was estimated as the product of the probability for control seeds in each site and the effect of seed ingestion, computed as previously explained.

Models´validation and statistical analysis—

For each simulation model (i.e., ‘Pollination’, ‘Dispersal’ and ‘Pollination + Dispersal’), we used their outputs (i.e., seeds produced, and seedling emergence) to plot the density function of simulated seedlings per area. To validate each model, we performed three visits to the study area from October to November 2016 to record all naturally emerging seedlings of *C. canariensis*. Seedling surveys were made at the same three paths and 31 sub-transects used to estimate flowers and fruit abundance. We validated the probability of the 95% of seedling recruitment under the density curves generated by each of the models by integrating their density function between the q005 and q095 quantiles of the empirical data on recruiting.

Relative importance of pollination and dispersal on the overall recruiting process

To assess the relative importance of pollination vs. seed dispersal as drivers of seedling recruitment in *C. canariensis,* we calculated for each model the percentage of overlap between the density function of each single model and that of the combined model (i.e., that considering both the pollination and seed dispersal processes).

Relative importance of birds and lizards for each mutualistic process

The output for the pollination model was the number of seeds/100 m^2 produced, whereas that of the combined model was the number of seedlings/100 m^2. As both models allowed estimating the seeds and seedlings contributed either by birds or lizards, for each model iteration, we could estimate the contribution by each vertebrate type to the total seed production and seedling recruitment at the pollination and the combined models, respectively. To compare the relative contribution to the total seeds and seedlings between vertebrate groups we used a Generalizad Linear Model (GzLM) with a negative binomial error distribution.

Both the model validation and the statistical analyses were performed with R software version 3.3.3 (R Core Team 2019). Model validation and the relative importance of pollination and dispersal as drivers of natural recruitment were analysed with the ‘sfsmisc’ package (Maechler 2018), whereas the GzLM to assess the relative importance of birds and lizards were analysed with the ‘MASS’ package (Venables and Ripley 2002).

2.4.3 Results

Models and their validation—

The pollination model had the highest values of median, mean and quantiles 0-95%, and the lowest range, whereas the opposite occurred in the pollination+dispersal model (Table 7, Figure 21A).

Table 7 Model outputs (seeds or seedlings produced · 100 m^{-2})

Model	$\tilde{x}$	μ	Quantiles 0-95%	Range
Pollination	7	298.47	0-1805	0-3488
Dispersal	0	280.87	0-758	0-26999
Pollination +Dispersal	0	64.40	0-19	0-66331

Quantiles 0.05 and 0.95 of our validation data were 0 and 29 plants, respectively. ‘Path 1’ transect accounted for the highest recruitment, with ca. 16 new plants/100 m^2, whereas ‘Path 3’ transect had the lowest recruitment with ca. 5 new plants/100 m^2 (Table 8). Given these values, their probability under the predictions of the pollination, dispersal and mixed models were 10.04%, 12.37% and 24.32%, respectively (Figure 21B).

Table 8 Median, mean and range of new recruited seedlings · 100 m^{-2} in each transect

	New seedlings 100 m^{-2}		
Transect	$\tilde{x}$	μ	Range
Path 1	18	15.92	0-34
Path 2	20	14.6	0-27
Path 3	0	4.86	0-21

Stagewise analysis and overall probability of recruitment—

Overall, birds had the most important role in pollination compared to lizards, both in pollination/visitation and fruit set, whereas lizard dominated the dispersal, especially in frugivory/seed dispersal and germination stages (see supplementary material Table 11). Seed set and damage to seeds was similar for both vertebrates, and autogamy did not produce any seeds despite its high probability of pollination (see supplementary material Table 11).

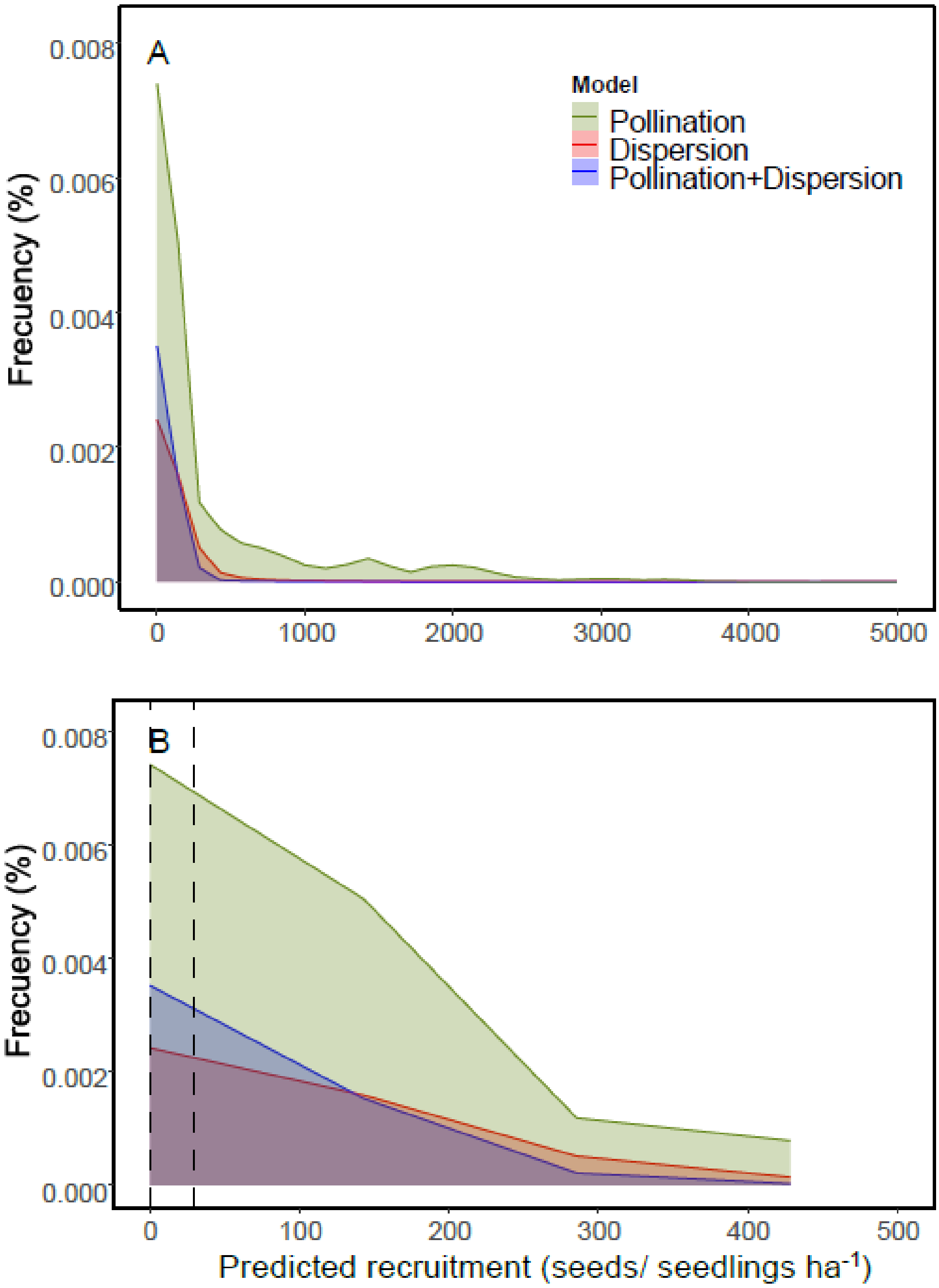

Figure 21 Density plots of the models in seeds or seedlings · ha^{-1}. Vertical lines indicate the quantiles q005 and q095 or our validation data.

Relative importance of pollination and seed dispersal by birds and lizards for seedling recruitment—

The curves of the pollination model and the mixed model shared a 17.86% of area, whereas the curves of the dispersal model and the mixed model shared a 36.12% of area. Overall, the dispersal model overestimates the intermediate densities while the pollination model the high densities (Figure 21).

Birds contributed more than lizards in the pollination model (χ^2 = 292.44, df = 1, P < 0.001), whereas the opposite occurred in the dispersal models (χ^2 = 655.02, df = 1, P < 0.001) (Figure 21).

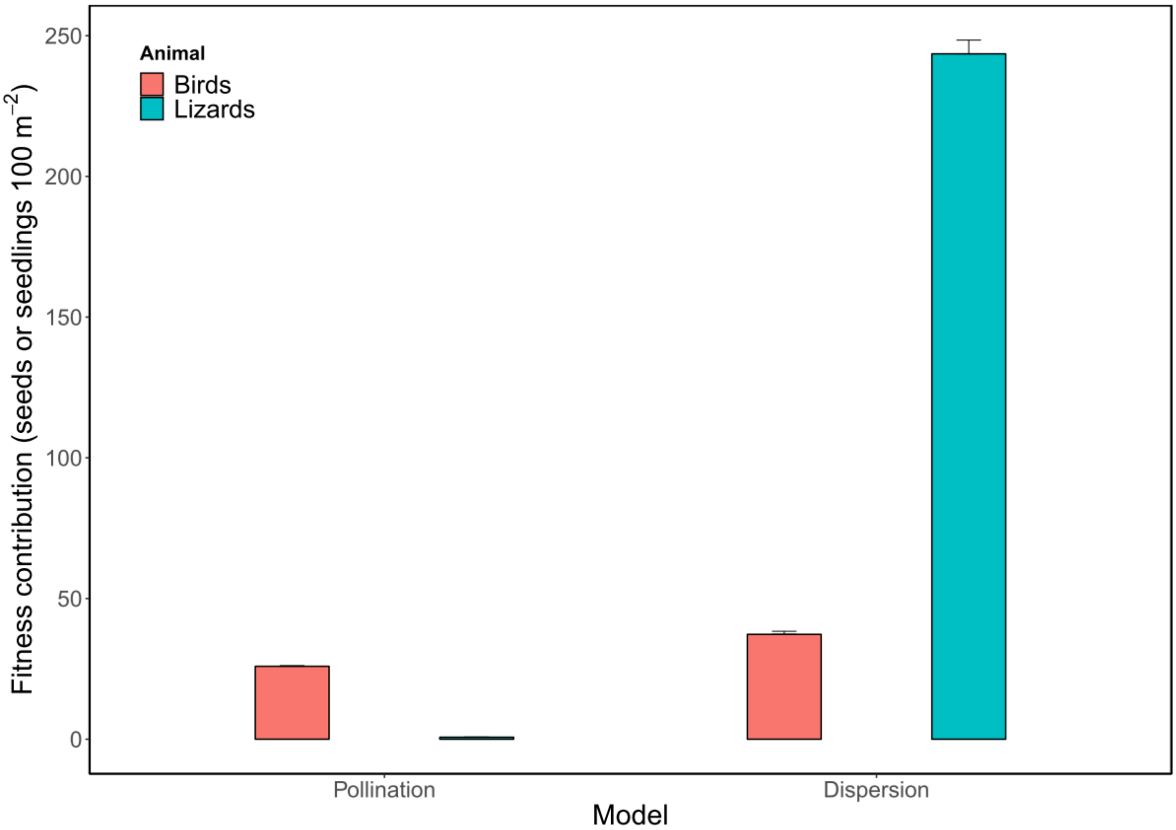

Figure 21 Mean fitness contribution (+SE) to C. canariensis of each vertebrate (birds or lizards) in pollination and dispersal models.

2.4.4 Discussion

As plant recruitment depends on pollination and subsequent seed dispersal, the sequential coupling of these stages is of paramount importance to assess plant recruitment. In spite of this, most studies focus on the effects of animal-mediated pollination and seed dispersal separately, and the joint outcome of both mutualisms is rarely assessed in an integrated way (e.g. Gomes *et al.* 2014; Fontúrbel *et al.* 2017).

The use of stochastic simulation models constitutes a novel approach to assess the relative importance of two mutualist processes different from that based in effectiveness landscapes implemented by Schupp *et al.* (2010, 2017).

The dispersal stage was more relevant, i.e. contributed more, than the pollination stage through the whole recruitment process. This contrasts with the results found by Fontúrbel *et al.* (2017), who reported the opposite in the mistletoe *Tristerix corymbosus*, with pollination effectiveness being ca. three times higher than seed dispersal effectiveness.

Bird and lizard complementarity—

Our results show the functional complementarity between lizards and birds in the reproduction success of *C. canariensis* in the thermoesclerophyllous vegetation of the Canary Islands. Both birds and lizards participated in the pollination and dispersal stages of recruitment. This phenomenon of having more than one double mutualist has been recorded in at least other 36 plant species around the world, many of them including both birds and lizards (see supplemetal material in Fuster *et al.* 2018). However, birds delivered most of the pollination service whereas lizards accomplished most of the seed dispersal service. This is the first study evaluating the relative importance of each mutualist in the double mutualism.

This functional complementarity between birds and lizards has also been observed in the dispersal community of the same area (González-Castro *et al.* 2015), in which lizards dispersed more seeds of certain plant species in open sites and shrublands, and birds dispersed more seeds of other plant species in woodlands and beneath canopies.

The role of birds as pollinators and seed dispersers is well known. The figures of bird species involved in nectar-drinking reaches up to 30% (Westerkamp 1990 citing Schuchmann 1987). Moreover, few thousands of plant genera (Olesen and Valido 2004 citing Porsch 1931) and up to 100% of woody plants in some locations exhibit fleshy fruits adapted for bird dispersal (Howe and Smallwood 1982). Regarding lizards, their role as pollinators and seed dispersers has been regarded as negligible. Indeed, lizards are the least common pollinators and seed dispersers among vertebrates; however, new studies show that their mutualistic role has been underrated, especially on islands. About two hundred lizard species are known as fruit consumers and 39 as flower visitors (Valido and Olesen 2019).

Both *Gallotia galloti* and *Phylloscopus canariensis* represent a clear example of density compensation on this archipelago (Valido and Delgado 1996; Olesen and Valido 2003b), with the highest lizard and bird population densities, and both might be equally important in both mutualistic processes. However, birds were more important in pollination and lizards in dispersal and this may be related to the resource phenology and location within the vegetation canopy. The bird *P. canariensis* is, however, mainly insectivorous and the flowering period of *C. canariensis* (i.e. winter) coincides with the minimum availability of arthropods within the thermosclerophyllous remnant (Wolda 1988; Kishimoto-Yamada and Itioka 2015). The lizard *G. galloti* is mainly active from the end of the flowering season (and starting of fruiting season) onwards when the temperature is warmer and insects begin their activities, and in cleared sites where flowers often lie on the ground (Siverio and Rodríguez-Rodríguez 2012).

Additionally, despite *P. canariensis*is the most common flower visitor among birds, seed dispersal is carried out by birds like the warblers (*Sylvia* spp.), that also visit flowers, but not as frequently as *P. canariensis* (Jaca *et al.* 2019). This may be, among others, one of the factors that explain why birds are so important for pollination but not for dispersal. Moreover, the pendant flowers usually located few meters above the ground are easily accesed by birds, while ripen fruit may fall to the ground and be consumed by lizards. In spite of this, all ecological processes are context-dependent, and an important disperser or pollinator in one context may be less important in another, and viceversa (Schupp 2007; Calviño-Cancela and Martín-Herrero 2009; Chamberlain *et al.* 2014).

Plant vulnerability to the loss of mutualists—

Canarina canariensis is extremely vulnerable to the loss of any of their mutualist partners; if birds were lost, the plant would reduce much its capacity to produce seeds, whereas if lizards disappeared, it would lose its dispersal capacity. This high dependence of plants on a single or a few pollinators and dispersers is indeed quite common in islands (Olesen and Jordano 2002; Dupont *et al.* 2003; Philipp *et al.* 2006; González-Castro *et al.* 2015), largely as a result of their depauperated faunas of mutualists (Carlquist 1974; Barrett 1998; Traveset and Richardson 2014). Such higher dependence results into higher vulnerability to disturbances, which may lead to mutualism disruptions when local extinctions of pollinators and dispersers occur. Pollination limitation results in reproductive impairment and generally plant population declines (Aguilar *et al.* 2006; Potts *et al.* 2010 and references therein).

Losses of animal dispersers may lead to a genetic lower heterozygosity, higher relatedness among individuals (Calviño-Cancela *et al.* 2012) and have also ecological consequences, such as reduction of survival probability (Rodríguez-Pérez and Traveset 2010). Extinction of the mutualist animal can result into the plant extinction, or at least makes it vulnerable to extinction (Aslan *et al.* 2013), even though compensatory effects may reduce such risk (Bond 1994), which varies greatly among taxa and regions.

Concluding remarks—

Finally, our findings highlight the necessity in future studies to investigate the relative importance of each process involved in a double mutualism system as well as the relative importance of different animal mutualist species for plant fitness.

3. EPILOGUE

3.1 General Discussion

From this thesis on the pollination and seed dispersal effectiveness of opportunistic nectarivorous vertebrates (birds and lizards) in two plant species (*Echium simplex* and *Canarina canariensis*) in the Canary Islands, we can highlight two key results: (1) there is a functional complementarity between birds and lizards in the effectiveness of the mutualism; and (2) non-native animal invaders (goats in the case of *E. simplex,* and rats and honeybees in the case of *C. canariensis*) threaten native mutualistic interactions and long-term population sustainability.

Flower visitors and visitation rates

During the study, the generalist floral morphology of *E. simplex* attracted ca. 100 different animal species (mainly insects), whereas the specialist floral syndrome of *C. canariensis* was only visited by six different species, mostly birds. Indeed, the insect diversity found in *E. simplex* is not frequent on oceanic islands, where insect fauna is commonly depauperate (Carlquist 1974; Whitaker 1987; Olesen and Valido 2004). Floral morphologies and phenology, together with pollinator activity, may explain the differences in number of flower visitors between *E. simplex* and *C. canariensis.*

Floral morphologies of the Macaronesian bird flower element are diverse and could be a useful predictive tool for vertebrate pollination effectiveness. *Canarina canariensis* was effectively pollinated by birds and its floral traits match with the ornithophily syndrome (large nectar standing crop, long floral duration and orange-reddish corollas), indicating that it has 'adapted' to a pure strategy of opportunistic bird pollination (Olesen 1985; Dupont *et al.* 2004a; Ollerton *et al.* 2009a; chapter 3). In the case of *E. simplex*, it is frequently visited by birds, but has a floral morphology that better matches insect generalized pollination (low nectar standing crop, pale corollas, short floral duration) and is in fact mainly pollinated by insects, with birds being rather inefficient (chapter 1). A third pollination system, mixed bird-insect pollination, has also been described in the

Canarian endemic *Scrophularia calliantha* (Ortega-Olivencia *et al.* 2012); however, this does not seem to be the case in our two studied species.

Apart from the floral features exhibited for *E. simplex* and *C. canariensis*, floral phenology might also influence the number and type of visitors. *Echium simplex* flowers in springtime, when insects start to be active and abundant and vertebrates can prey on them, thus reducing their dependency on nectar. In contrast, *C. canariensis* flowers in winter, when few insects are available for pollination (Wolda 1988; Kishimoto-Yamada and Itioka 2015), and thus vertebrates may have a higher dependence on nectar.

Pollinator activity is also conditioned by weather. Birds can forage under inclement conditions when insects remain inactive, and are therefore more likely to feed on nectar (if available) during cold and/or rainy conditions (Stiles 1978). We might thus expect that *E. simplex* to receive more frequent flower visits by insects, whereas *C. canariensis* would be mainly pollinated by birds.

Opportunistic nectar-feeding vertebrate visitors

Not all visits to the flowers result in effective pollen transfer to stigmas (King *et al.* 2013). Some visitors may be efficient at removing pollen from anthers but not at depositing pollen on stigmas (Wilson and Thomson 1991; Irwin and Adler 2006; Bartomeus *et al.* 2008), while others may actually not carry pollen at all (Alarcón 2010; Popic *et al.* 2013). In addition, some may act as nectar or pollen thieves or robbers (Irwin *et al.* 2010; Rojas-Nossa *et al.* 2016).

Our results indicate that the main pollinators of *E. simplex* are flying insects (mainly hymenopterans), and the main pollinator of *C. canariensis* in thermosclerophyllous habitats is the passerine Canarian chiffchaff, *Phylloscopus canariensis*. Despite being self-compatible, both plant species require pollinators to increase their female reproductive success. Flowers visited by their pollinators sired higher proportions of fruits compared to the absence of floral visitors. In both species, the most common visitor group was also the most effective pollinator (but see discussion about honeybee invaders in *C. canariensis*).

The effectiveness of opportunistic vertebrate pollination in plants from the Macaronesian bird-flower element was previously studied for *Isoplexis* *canariensis*, *Navaea phoenicea* and *Canarina canariensis* in the laurel forest (Rodríguez-Rodríguez and Valido 2008, 2011; Fernández de Castro *et al.* 2017). Valido *et al.* (2004) suggested that the origin and evolution of ornithophilous traits in plants from Macaronesian bird-flowers took place mostly in mainland areas prior to island colonization (relict ornithophily). *Echium* species are an exception, their flowers evolved from mainland insect-pollinated ancestors after island colonization (*de novo* ornithophily). The directional changes in floral traits support both the relict and *de novo* ornithophily hypotheses. For example, the sugar composition of nectar evolved in response to the main pollinator group of a plant, from high-concentrated sucrose-rich, typical of specialist nectar-feeders, to a low concentrated hexose-rich composition (Dupont *et al.* 2004b) typical of opportunistic bird-pollinated systems (Johnson and Nicolson 2008).

Echium simplex is included in the Macaronesian bird-element (Vogel *et al.* 1984), and it is truly visited by birds. However, the results of this thesis indicate that birds cannot drive the evolution of this plant species, as they are not effective pollinators, possibly due to morphological constraints, i.e. mismatch between flower and bird's bill shapes.

Despite not being effective pollinators of *E. simplex*, the high visitation rates of birds to its flowers tell us that nectar constitutes an important energy source to them, which we have not evaluated. This would be certainly worth investigating in the future, as Cecere *et al.* (2011) did on Ventotene Island with opportunistic nectar-feeding birds.

The most effective guild of pollinators enhanced fruit set in *E. simplex*, but no differences were detected in seed set, indicating there is no qualitative variation between birds and insects in effectiveness once the flower is pollinated by either of them. Hence, flowers are obtaining sufficient pollen to fertilize all ovules, but the availability of resources for seed and fruit maturation restricts female reproductive success (Bateman´s principle) (Burd 1994).

It must be noted, however, that we have only evaluated the female function as a proxy of reproductive success, disregarding the male function and thus misrepresenting whole-plant reproduction. Assessing only fruit and seed production is much easier in the field than following the fate of pollen grains. The research oriented to male fitness evaluates ‘pollen transfer efficiency’ measuring pollen removal and pollen loss or pollen wastage in pollinator performance by means of complex and expensive laboratory techniques (Ne’Eman *et al.* 2010).

Birds vs. lizards as pollinators

Among opportunistic vertebrates, birds are seen to play a more important role than lizards as effective pollinators in *C. canariensis* and as fequent visitors in both *E. simplex* and *C. canariensis*. In previous studies in the Macaronesian region in which both birds and lizards were reported as flower visitors, passerines were quantitatively more effective in their mutualistic service, i.e. had higher visitation rates (Ollerton *et al.* 2009b; Ortega-Olivencia *et al.* 2012), but see Rodríguez-Rodríguez and Valido (2011), or higher pollination effectiveness (Rodríguez-Rodríguez and Valido 2008). Outside the Macaronesian region, the inclusion of opportunistic lizards and birds in pollinator assemblages is relatively frequent in insular floras around the world (Olesen and Valido 2004), where again birds are quantitatively more effective than lizards (Mortensen *et al.* 2008; Sazima *et al.* 2009; Traveset *et al.* 2013; Hopkins *et al.* 2015).

Birds present high pollination effectiveness in some Macaronesian plants. This might indicate that their floral traits have been under strong selection by these taxa on the mainland prior to their arrival on the islands. The origin of the vertebrate-visited flora was thought to be the relict Tertiary humid forest from (2.5-66 mya), and originally pollinated by sunbirds. So, according to this hypothesis, islands acted as natural refuges of this ancient flora (Vogel *et al.* 1984; Olesen 1985) and new vertebrate species started to visit them. However, recent molecular analyses have started to weaken the hypothesis of the past existence of specialist nectarivores to explain the origin and/or persistence of this flora in the Canary Islands. For example, *C. canariensis* evolved from bird-pollinated species that colonized this archipelago ca 0.8 mya (Mairal *et al.* 2015a; Mairal *et al.* 2015b), or *Lotus* sect. *Rhyncholotus* evolved ornithophilous traits after island colonization ca. 1.2-1.7 mya (Ojeda *et al.* 2012) when opportunistic birds were already present in the Canaries between 0.01 and 2.6 mya (Illera *et al.* 2012).

Higher bird pollination effectiveness might be also related to lizard biological constraints. On the one hand, foraging for nectar requires that lizards spend long periods at high predation-risk sites, such as the periphery of canopies or on open inflorescences (Olesen and Valido 2004). On the other hand, lower lizard metabolic activity reduces their nectar consumption and disproportionately limits their movement distances and floral visitation frequency compared to birds. Moreover, lizards are forced to spend time thermoregulating (Nagy *et al.* 1999), which for instance limits their quantitative effectiveness via higher floral visitation rates. Finally, the flowering phenologies of our plant species, especially that of *C. canariensis*, do not match with the highest annual activity of lizards in terms of hours needed for thermoregulation.

However, despite their low impact, local lizard activity might supply a complementary service to the long-distance pollen dispersal provided by birds. While birds can transport pollen over long distances, between floral patches or distant populations, lizards can be important dispersal vectors over short distances. Within the patches, they may be considered highly important for population dynamics (Schupp *et al.* 2010). The importance of lizards as mutualists may be even higher than birds in plant populations where birds are scarce but lizards reach high densities, such as arid habitats with reduced vegetation cover (Traveset and Sáez 1997; Olsson *et al.* 2000; Hansen *et al.* 2007; Rodríguez-Rodríguez and Valido 2011; Siverio and Rodríguez-Rodríguez 2012; González-Castro *et al.* 2015).

Birds vs. lizards as seed dispersers

Seed dispersal is one of the most important processes in the life cycle of many plant species (Howe and Smallwood 1982). This is so because, after the fertilization produced by pollination, the seed dispersal process confers a series of advantages on the new generations that increase their chances of survival. Examples of such benefits are: avoiding competition with already established parents and seedlings, reducing the risk of exposure to pathogens and predators, and increasing the chances of reaching a suitable site to become established (Connell 1971; Janzen 1975; Clark and Clark 1981; Howe and Smallwood 1982;

Willson and Traveset 2000). In addition, the movement of seeds favours the increase in the range of distribution of plant species, their metapopulational dynamics and their gene flow (Hanski 1997; Jordano and Godoy 2002; Bohrer *et al.* 2005; Nathan 2006; García *et al.* 2009).

In our system, lizards played a minor role as pollinators in both plant species but were fundamental in the dispersal of the fleshy fruits and seeds of *C. canariensis,* with birds playing a minor role. However, both animal guilds benefit from nutrients in the fruits.

Hence, the importance of a given mutualistic interaction is context-dependent. A pollinator or a disperser important in one plant population or habitat may be irrelevant in another (chapter 3, Rodríguez-Rodríguez and Valido 2011). It means that pairwise interactions will yield conditional reproductive outcomes with a magnitude and/or sign dependent on the ecological context in plant-pollinator and plant-dispersal mutualisms (Thompson 1988; Bronstein 1994; Chamberlain *et al.* 2014; Fuster 2019).

The spatial variation in mutualistic assemblages certainly alters the landscapes of pollination and dispersal effectiveness, with their subsequent evolutionary implications. Indeed, this local variation in both the abiotic and biotic environment constitutes the raw material for coevolution (i.e. cold-hot spots of interaction; Thompson 1994, 2005).

The higher context-dependence would also imply that opportunistic plant-mutualistic relationships are less stable in terms of evolution than specialized systems, not being strong enough to drive floral or fruit evolution. However, some studies have shown that opportunistic nectar feeders react to changes in floral features (Johnson *et al.* 2006; Brown *et al.* 2010; Odendaal *et al.* 2010), and probably act as selective agents behind floral traits (Dupont *et al.* 2004b; Johnson and Nicolson 2008; Hervías-Parejo and Traveset 2018).

Threats to mutualisms and consequences

Plants that rely on vertebrate services for pollination and seed dispersal face many threats due to the extinction, decline or disappearance of their mutualists. This especially occurs in today’s changing world, where animal populations can decline at an alarming rate (Sekercioglu *et al.* 2004; Memmott *et al.* 2007; Tylianakis *et al.* 2008; McConkey and Drake 2015). These threats include poisoning by pesticides, habitat alteration, invasions by animal species, and fragmentation/insularization of habitats (Bond 1994). Plants that have lost their mutualists are likely to undergo severe reproductive declines (40 - 58%) (Aslan *et al.* 2013). After habitat destruction, alien invasive species seem to represent the second most common threat associated with species extinction since 1,500 CE (Wilcove *et al.* 1998; Bellard *et al.* 2016).

In our system, apart from opportunistic vertebrate pollinators, alien species acting as antagonists constitute an important fraction of the partners of the studied plant species. Introduced goats consumed the vegetative parts and flowers of *E. simplex.* In 2016, ca. 99% of the surveyed plants were damaged and at least 30% of the inflorescences were totally consumed and thus produced no fruits or seeds. *Canarina canariensis* interacted with two alien species: honeybees and rats. Honeybees were the most common visitor from March onwards, displacing birds as single legitimate pollinators of this species. In turn, rats consumed about 10% of the flowers and reduced fruit set to one third. All these antagonistic interactions disrupt effective pollination, seed dispersal, and finally plant reproduction.

Regarding pollination disruption, if pollen grains and ovules are consumed, plants could shift their mating systems towards increased selfing in order to compensate the effects of plant damage or associated inadequate pollination, as demonstrated in other systems (Ivey and Carr 2005; Penet *et al.* 2009; Soper Gorden 2013). Additionally, antagonistic pollinator interactions can have a differential impact on female and male fitness (Irwin and Brody 1999; Krupnick *et al.* 1999). This suggests that measuring only female function may misrepresent whole-plant reproduction depending on the antagonistic context of pollination.

Seed dispersal disruption, as a consequence of seed disperser loss, entails lower seedling recruitment, with recruitment occurring almost exclusively under the parent plants. In the long term, this process may lead to species regression

(Traveset and Riera 2005; Rodríguez-Cabal *et al.* 2012; Traveset *et al.* 2012) and even local extinction (Farwig and Berens 2012 and references therein).

Another threat to mutualisms is climate change. The Earth's climate has warmed by approximately 0.6 °C over the past 100 years. The consequences of recent global warming include responses in the phenology and physiology of organisms, distribution ranges of species, composition of communities and interactions within them, and the structure and dynamics of ecosystems (Walther *et al.* 2002; Walther 2010; Hegland *et al.* 2012; Scaven and Rafferty 2013). These responses can be detrimental for pollinator species. Some include a decreased distribution range and the shift (mostly advancing) of their life history events, all this resulting in mismatches between a population and its food resources. Most of this research, however has been conducted on invertebrates, including pollinators such as butterflies (Warren *et al.* 2001; Kingsolver and Buckley 2014; Kingsolver and B. 2018), the honeybee (Bartomeus *et al.* 2011) or the bumblebees (Kerr *et al.* 2015; Miller-Struttmann *et al.* 2015).

3.2 General Conclusions

I. The reproductive success of *Echium simplex* is enhanced by animal pollinators. It is a self-compatible plant, but selfing decreases fruit set compared to cross-pollination, which might be due to a partial temporal mismatch in the sexual phases, more specifically to protandry. Contrary to expectations, seeds coming from selfed flowers were heavier than those resulting from cross-pollination. The reason is that the former have a thicker coat whilst embryo size is similar between the two treatments. This thick seed coat reduced both the germinability and the germination rate

II. The flowers of *E. simplex* were visited by a high diversity of species, including more than 100 insect species, five bird species and a lizard species. Hymenopterans and coleopterans were the most common insect visitors, whereas the African blue tit and the Canarian chiffchaff were the most prevalent vertebrates visiting flowers.

III. Different animal guilds showed different pollination effectiveness in *E. simplex*. Contrary to expectations, flower visitation by vertebrates did not contribute to increased reproductive success and actually was found to play only a minor role in the reproduction of this plant. Flying insects, especially bees, were those contributing the most to seed production of *E. simplex*. On the contrary, small beetles decreased fruit set suggesting that they mostly consume pollen rather than depositing it on the stigmas.

IV. Naturalized goats showed to be important herbivores of *E. simplex* adult plants, in this way impeding or at least reducing their recruitment probabilities. Up to 99% of the plants were consumed to some extent and at least 30% of the inflorescences were totally preyed on. Although further studies are needed to quantify the long-term effects of this herbivory and its threat level to the maintenance of the population, we recommend goat control campaigns be considered in this area where *E. simplex* lives.

V. *Echium simplex* is also visited by a higher number of nocturnal animals than usually reported in nocturnal pollination studies. Those nocturnal visitors include moths, beetles, neuropterans, dictyopterans, dermapterans and julidans. Moths were the most frequent insect group visiting the higher (distal) section within the inflorescence, followed by coleopterans at intermediate and low positions, and other species mainly in the low sections. In any case, the attractiveness of this plant to insect visitors remained greater during the daytime, with both higher insect diversity and visitation rate.

VI. As expected, control plants yielded more fruits than excluded ones, and diurnal pollination also led to higher fruit set than nocturnal pollination. Among plant sections, there was no difference in fruit set between control and selfed plants, suggesting absence of competition among sections or maternal constraints, and uniform pollination. However, nocturnal pollination led to more fruits in both upper and bottom inflorescence sections, while it was higher in only the upper section of diurnally-pollinated plants, probably due to the different behaviour of nocturnal and diurnal animal visitors.

VII. Besides the laurel forest, the endemic *Canarina canariensis* is also present in remnants of the former thermosclerophyllous woodland of Tenerife, where its assembly of interacting species is different. This plant is only diurnally visited by three species of birds, two insect species and one lizard species. Flower visitation rate increased along the flowering season and peaked at midday, with the alien *Apis mellifera* being by far the most frequent visitor, followed by the Canarian chiffchaff. Compared to laurel forest, where honeybees are absent, *C. canariensis* was visited more frequently and by more animal species in the thermophilous forest.

VIII. Only birds acted as legitimate pollinators of *C. canariensis*, consistent with what was reported for the laurel forest. Spontaneous selfing produced as many fruits as flowers visited only by bees and lizards, which suggests that these two taxonomic groups contribute little to its pollination success. Bees might not contact anthers or stigmas due their small size, and lizard visits were merely anecdotal. Although autogamy levels were low, we found that *C. canariensis* can self-pollinate in the thermosclerophyllous woodland. This was unexpected, given previous studies in the laurel forest.

IX. *Apis mellifera* acted as an antagonist of *C. canariensis*, decreasing plant fitness. Two factors may explain this. First, when collecting pollen, honeybees ensure maximal removal of pollen, thus reducing male fitness. Second, by depleting flowers of nectar, they discourage legitimate bird pollinators from flower visitation. A second alien species, rats (*Rattus* spp.), absent in the laurel forest, interacted as an antagonist with *C. canariensis* in the thermosclerophyllous forest; rats consumed 10% of the flowers and reduced fruit set to one third.

X. The use of stochastic simulation models constitutes a novel approach to assess the relative importance of two mutualist processes. We built three models to predict seedling recruitment in *C. canariensis*. Their probability under the predictions of the 'pollination', 'dispersal' and 'pollination + dispersal' models were ca. 10%, 12%, and 24%. The dispersal stage contributed more than the pollination stage throughout the whole recruitment process of *C. canariensis* in thermosclerophyllous habitat.

XI. Both birds and lizards participated in the pollination and dispersal stages of recruitment of *C. canariensis* in the thermoesclerophyllous vegetation. However, there was functional complementarity between lizards and birds in reproduction success. Birds played a more important role compared to lizards, both in pollination/visitation and fruit set, whereas lizards dominated dispersal, especially during the frugivory/seed dispersal and germination stages.

XII. Taken together, our results indicate that it depends on the plant in question whether assemblages of opportunistic vertebrate flower visitors are effective or not for plant sexual reproduction. We need further research on the rest of the plant species from the Canaries, and those from other oceanic archipelagos whose flowers are known to be visited by vertebrates. This will provide better insights on the reproductive effects of opportunistic pollination, compared to more specialized systems. Considering the effect of alien species is also important in assessing the final plant reproductive outcome. These interactions decrease plant fitness and may threaten their long-term survival.

4. SUPPLEMENTARY MATERIAL

4.1 Chapter 1

4.1.1 Supplementary Tables Chapter 1

Table 9 Flower visitors of Echium simplex found during 2015 and 2016.

Order Coleoptera (Family)

Acalles sp (Curculionidae)
Anaspis proteus (Scraptidiidae)
Attalus pellucidus (Malachiidae)
Attalus sp (red) (Malachiidae)
Attalus sp1 (Malachiidae)
Attalus sp2 (Malachiidae)
Attalus sp 2 (dark) (Malachiidae)
Attalus sp3 (Malachiidae)
Calomicrus sp (Chrysomelidae)
Ceutorhinchini sp1 (Curculionidae)
Cryptocephalus sp (Chrysomelidae)
Cryptolestes (Laemophloeidae)
Dasytes sp (Dasytidae)
Longitarsus persimilis (Chrysomelidae)
Longitarsus sp (Chrysomelidae)
Maltinus mutabilis (Cantharidae)
Maltinus sp (Cantharidae)
Melyrosoma hyrtum (Melyridae)
Mesites sp (Curculionidae)
Mordellistena sp (Modellidae)
Nesotes transversus (Tenebrionidae)
Scolitidae sp
Scymnus sp (Coccinellidae)
Tropinota squalida (Scarabeidae)

Order Diptera (Family)

Agromyzidae sp1
Agromyzidae sp2
Calliphora vicina (Calliphoridae)

Order Hymenoptera (Family)

Andrena sp1 (Apidae)
Andrena sp2 (Apidae)
Anthophora alluaudi (Apidae)
Aphelinidae sp (Superfam. Parasitica)
Apis mellifera (Apidae)
Bombus canariensis (Apidae)
Bracon sp (Braconidae)
Braconidae sp 1
Braconidae sp2
Braconidae sp3
Camponotus (subg Mymosericus) sp (Formicidae)
Camponotus (subgTamaemyrmex) sp (Formicidae)
Chelonus sp (Braconidae)
Crematogaster alluaudi (Formicidae)
Euzera gracillipes (Apidae)
Eumenidae sp
Formicidae sp1
Formicidae sp
Hylaeus canariensis (Apidae)
Lasiussp (Formicidae)
Lassioglossum sp (Apidae)
Microgastrinae sp1 (Braconidae)
Osmia canaria (Apidae)
Parasitica sp2
Paravespula germanica (Vespidae)
Pimpla sp (Ichneumonidae)
Torymidae sp (Superfam. Parasitica)

Calliphora sp (Calliphoridae)
Campiglossa sp (Tephritiidae)
Chloropidae sp1
Delia sp (Anthomyiidae)
Dilophis sp (Bibionidae)
Estheritia simonyi (Tachinidae)
Lucilia serrata (Calliphoridae)
Musca domestica (Muscidae)
Myopa sp (Canopidae)
Mythicomyiidae sp
Sarcophagidae sp1
Sarcophagidae sp2
Sarcophagidae sp3
Sphunculina (Chloropidae)
Syrphidae sp1
Tephritiidae sp
Thereva sp (Therevidae)

Orden Lepidoptera (Family)

Thymelicus christi (Nymphalidae)
Pararge xiphiodes (Nymphalidae)
Danaus chrysippus (Nymphalidae)

Order Hemiptera (Family)

Aphidoidea (Sternorrhycha)
Brachycarinatus tigrinus (Rhopalidae)
Lygaeidae sp1
Miridae sp1
Miridae sp2
Miridae sp3
Nysius sp1 (Lygaeidae)
Orius sp (Anthocoridae)
Oxycarenus lavaterae (Lygaeidae)
Sphilostethus pandurus (Lygaeidae)
Tingidae sp1
Tingidae sp2

Other (Order, Family)

Chrysoperla carnea (Plannipedia, Chrysopidae)
Guanchia sp (Dermaptera, Forficulidae)
Linyphiidae sp1 (Araneae)
Phyllodromica brullei (Blattaria, Ectobiidae)
Thysanoptera sp

4.1.2 Germination patterns

In 2015 seed weight did not affect germinability of control seeds but had a positive effect on germination from ‘flower dwellers’, and bird, lizard and total-exclusion treatments, whereas a negative effect on germination of seeds from vertebrate exclusion treatment (see figure below A and B). By contrast, in 2016, heavier control seeds germinated more whereas heavier seeds from the dwellers and total exclusion treatments showed reduced germinability. Regarding germination rate consistently the two years, seeds from the vertebrate exclusion, total exclusion, lizard exclusion and the ‘flower dwellers’ treatments took longer to germinate as seed weight increased, whereas light seeds of the other treatments (i.e. control and bird exclusion) germinated faster than heavy seeds (see figure below C and D).

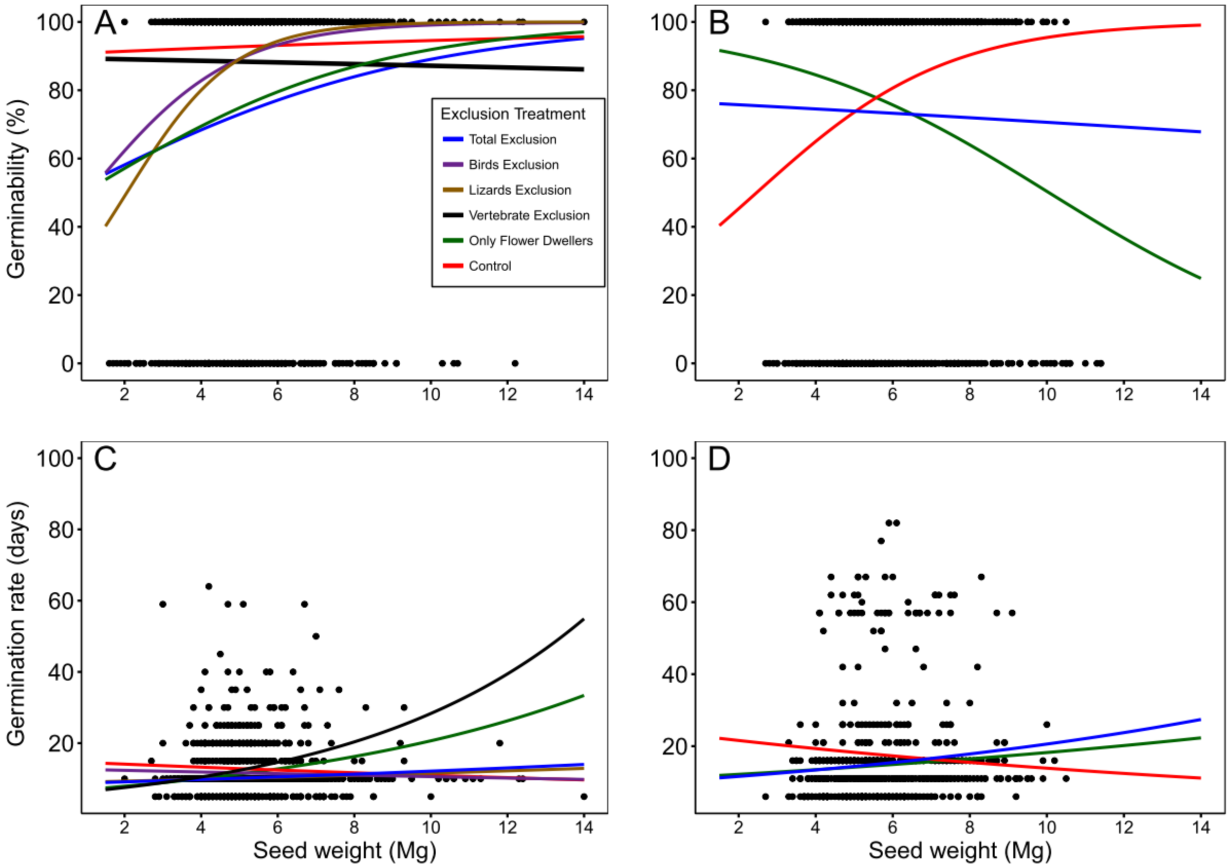

Figure 22 GLMM predicted probabilities of germination (A and B) and days to germination (C and D) along seed weight for each exclusion treatment in 2015 (A and C) and 2016 (B and D). Sample sizes from germinability studies were: Total Exclusion 2015 and 2016, N=198 and N=331 seeds, respectively; Birds Exclusion, N=200 seeds; Lizards Exclusion, N=175 seeds; Vertebrates Exclusion, N=155 seeds; Flower Dwellers 2015 and 2016, N=181 and 385 seeds, respectively; and Control 2015 and 2016, N=264 and N=310 seeds, respectively. Sample sizes for germinated seeds were Total Exclusion 2015 and 2016, N= 140 and N=268 seeds, respectively; Birds Exclusion, N=166 seeds; Lizards Exclusion, N=148 seeds; Vertebrates Exclusion, N= 129 seeds; Flower Dwellers 2015 and 2016, N=140 and 268 seeds, respectively; and Control 2015 and 2016, N=235 and N=223 seeds, respectively.

4.2 Chapter 3

4.2.1 Supplementary Tables Chapter 3

Table 10 Number of fruits collected per treatment and range of seeds per fruit in exclusion and intensity of bird visitation experiments

Treatment	***n***	**Seed set (range)**
Exclusion experiment		
Bird Exclusion	2	275 - 525
Lizard Exclusion	17	0 - 766
Vertebrate Exclusion	7	15 - 397
Control	28	13 - 925
Intensity of visits		
Frequently visited	8	4 - 879
Infrequently visited	15	34 - 945

4.2.2 Supplementary Figures Chapter 3

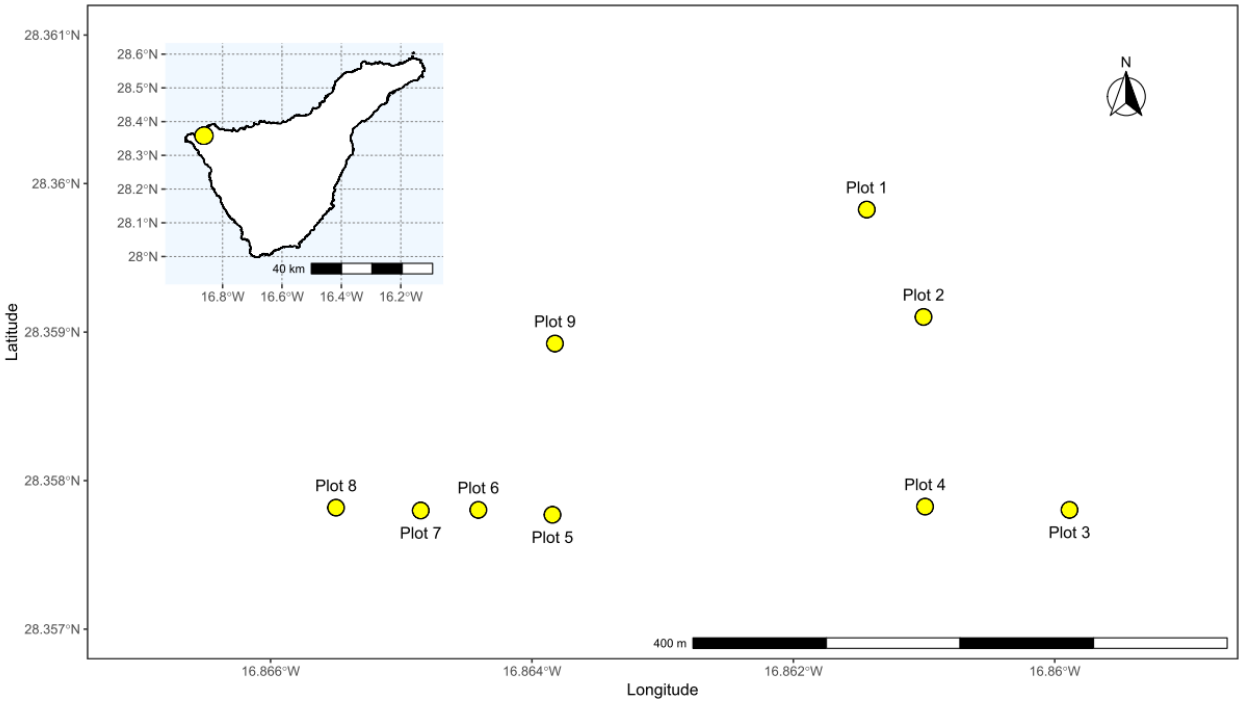

Figure 23 Location of the study area on Tenerife with C. canariensis plots indicated

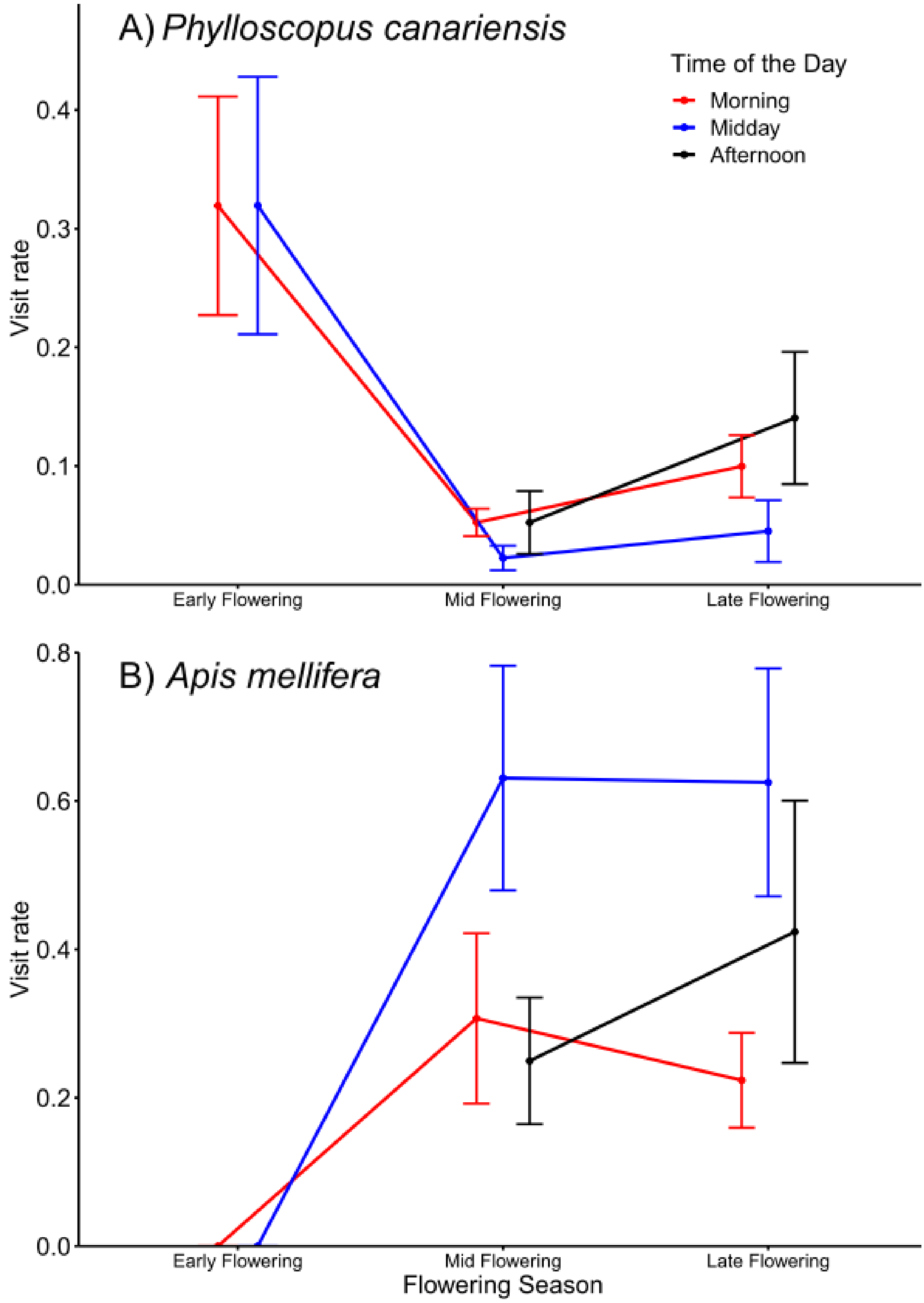

Figure 24 Temporal variation in the visitation rate of flower visitors to Canarina canariensis throughout the flowering season in morning, midday and afternoon hours performed by a) Phylloscopus canariensis and b) Apis mellifera. Data are means ±SE.

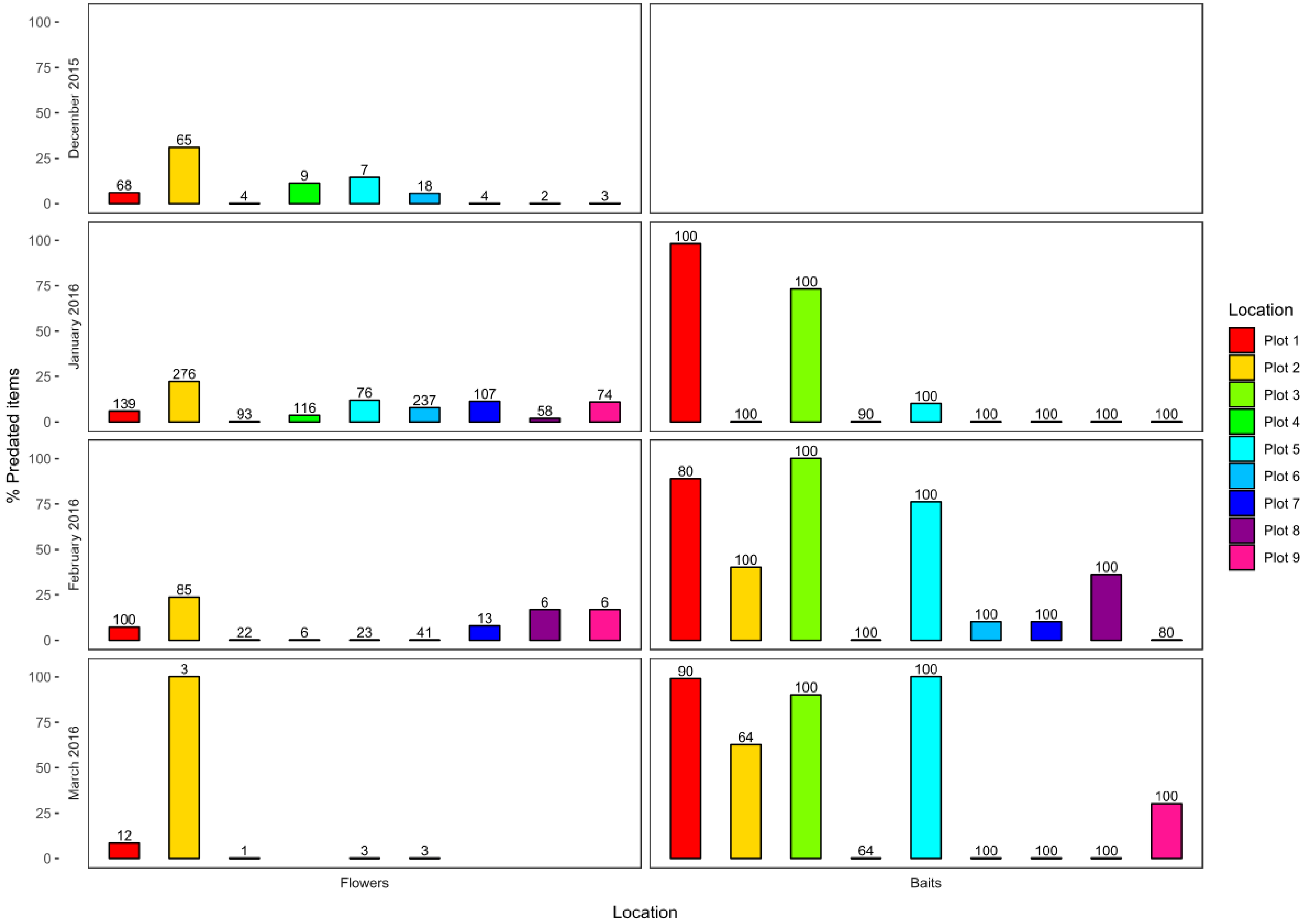

Figure 25 Percentage of flowers and baits preyed on by rats from December 2015 to March 2016 at the studied locations. Numbers on top of the bars are sample sizes

4.3 Chapter 4

4.3.1 Supplementary Tables Chapter 4

Table 11 Empirical values used for parameterization of transition probabilities used in the simulation models. For each stage and treatment, median, mean and range of values are shown.

Flower visits and pollination			
	Autogamy	Lizards	Birds
Pollination/visitation ($\tilde{x}$, μ , range)	0.946 , 1, 0.5 - 1	0, 0.005, 0 - 0.5	0, 0.049, 0 - 0.43
Fruit set ($\tilde{x}$, μ , range)	0, 0.085, 0- 0.33	0, 0.049, 0-0.3035	0.135, 0.160, 0 – 0.349
Seed set ($\tilde{x}$, μ , range)	0	369, 328.182, 80 -543	217.5 , 258.5, 0 -766
Frugivory and seed dispersal			
Probability of seed dispersal ($\tilde{x}$, μ , range)	0	1, 0.7, 0 -1	0, 0.3, 0 -1
Effect of gut treatment on seed damage ($\tilde{x}$, μ , range)			
Undamaged seeds	-	1, 0.993, 0.778-1	1, 0.984, 0.833-1
Damaged seeds	-	0, 0.007, 0-0.222	0, 0.016, 0-0.167
Germination in field ($\tilde{x}$, μ , range)	-	0.295, 0.309, 0-0.727	
Germination in greenhouse (%)	-	0.909	0.333

Table 12 Number of flowers and fruits per transect

Transect	Nº subtransects	Length (m)	Nº flowers	Nº fruits
Path 1	12	594.1	260	99
Path 2	5	255.7	213	17
Path 3	14	697	43	20

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