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**Ciências
ULisboa**

**The pollinator community of the Madeiran endemic
Echium candicans: individual-based network metrics, relation
with plant traits, and pollinator behaviour**

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Mestrado em Biologia da Conservação

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2019

Acknowledgements

A big thank you to both of my advisors for all their help throughout my thesis.

A special thanks to Mário Boeiro for being a great teacher, colleague and friend. For all the guidance during the fieldwork in Madeira and all of the advice during the identifications and writing of this work I will be forever grateful.

I would also like to express my gratitude towards Rui Rebelo for all his patience, guidance and commitment in teaching us how to become excellent professionals.

A kind thanks to Miguel Andrade, Elisa Teixeira and Fabiana Esposito for their assistance during the sampling of the pollinators.

I would like to give my warm gratitude to Carla Rego and Andreia Penado for the time they spent guiding me to properly identify some of the pollinator groups.

A special big thank you to Ana Hasse for her help with the statistical analysis, and for clarifying my doubts when using R software.

I would like to give honourable mentions to my fellow colleagues and the PhD students Maria João Verdasca and Fernando Madeira, for their suggestions in our weekly group meetings.

I am very grateful to 2gether project (PTDC/BIA-BIC/1013/2014) for giving me the chance to do this work, and Fundação para a Ciência e a Tecnologia for funding this project.

A big thank you to the Instituto das Florestas e da Conservação da Natureza for allowing our study of the *Echium candicans* populations in the area of the Central Mountain Massif.

Massive warm and fuzzy feelings to my family who have always supported my path, and have helped me in any way they could. To my friends, for all the support they have given me when I was doubting my capacity to properly finish this thesis. And to my girlfriend, Patrícia, whom I owe so many hugs for all of the patience and the help during these long months of work.

A generous thank you to any and all other persons that have helped to make this study possible.

Abstract

The study of plant-pollinator interactions is particularly important in insular environments, not only because these are rich in unique plant species, but also due to the threats that affect these communities and their interactions. The Macaronesia is a biogeographic region well known for its rich diversity of endemic plants that radiated in these archipelagos. A good example is the genus *Echium*, which has a total of 29 species. Despite their diversity, studies focused on the pollinator communities of these plants are restricted to some species in the Canary Islands and mostly isolated records on the other archipelagos.

The focus of this work was the community of diurnal pollinators that visit the Madeiran endemic *Echium candicans*. We intended to assess the relations between individual plant traits and the community of pollinators, following an individual-based design. We also aimed to characterize the behaviour of some of the most commonly observed pollinator species.

Overall we sampled 24 individuals distributed between two sites (one at 1500m and the other at 1800m), near Pico do Arieiro (Madeira). For each plant, we determined its largest diameter and height, the average size of its inflorescences, estimated the number of flowers as well as plant isolation. The pollinator community of each plant was sampled during observation sessions. We also used pan-traps and net trapping during timed-transects to sample the local community of potential pollinators. We recorded the time, the number of inflorescences and the number of flowers visited for a maximum of twenty different pollinators of each selected species.

As expected, we observed a diverse community of pollinators visiting *E. candicans*, with 50 different insect and one lizard species observed. The bumblebee *Bombus ruderatus* was the most common species at both sites, while the endemic butterfly *Hipparchia maderensis* and the bee *Amegilla quadrifasciata* were the second most common at each site. The honeybee *Apis mellifera* and *H. maderensis* were more common at the site at a lower altitude, while *A. quadrifasciata*, *Scaeva pyrastris* and the Madeiran lizard (*Teira dugesii*) were more common higher altitude. The diversity of bees sampled on *E. candicans* was similar to the one seen in Canary *Echium*, but the Madeiran species was visited by a much higher diversity of hoverflies and butterflies.

The plant traits were related to the network metrics by obtaining standardized regression coefficients. The number of flowers per plant and the average size of the inflorescences were the traits that presented a positive relation with most network metrics. Nested rank showed that the average size of the inflorescences was related to an increase in plant generalization. The other metrics showed that plants with more flowers and larger inflorescences were more central in the network, had a higher number of pollinators and seemed to be more important for their pollinators. Most species and groups of pollinators also showed a positive relation with the previously mentioned plant traits. Only three species (*A. quadrifasciata*, *Eristalis tenax* and *T. dugesii*) showed a negative relation with isolation, which might be due to the short distances involved. Two of them seemed to prefer denser patches of resources, having also the strongest relations with flower number.

Large bees had the highest visitation rates. Only the two studied hoverflies had a similar value to that of *B. terrestris*. Butterflies, the small bee *Lasioglossum wollastoni* and the Madeiran lizard had much lower visitation rates. However, butterflies spent more time on the visited flowers, possibly being equally important pollinators. Larger bees are probably responsible for the highest amount of pollination while species like *H. maderensis* might be more effective pollinators.

This study showed that *E. candicans* is visited by a great diversity of pollinators, which included a reptile. The amount and diversity of pollinators seemed to be mostly related to the amount of available floral resources at each plant. Overall, the different pollinators might complement each other due to their different foraging behaviours.

Keywords: Pollination, Macaronesia, Endemic plant, Pollinator behaviour, Individual-based networks

Resumo

As ilhas são locais prioritários para o estudo das interações planta-polinizador, não só por serem ricas em espécies únicas, mas também devido às ameaças que afetam as suas comunidades nativas. Os arquipélagos que compõem a Macaronésia são um exemplo de como os ambientes insulares podem ser ricos em biodiversidade, com vários exemplos de géneros de plantas que radiaram em muitas espécies endémicas. Tipicamente esta diversidade de plantas em ilhas oceânicas não é acompanhada por igual diversidade de insetos polinizadores. Contudo, outros grupos como répteis e aves surgem como potenciais polinizadores ao passarem a incluir recursos como néctar na sua dieta.

Uma das grandes radiações de plantas na Macaronésia ocorreu no género *Echium*, com 29 espécies endémicas. Estas ocorrem nos arquipélagos de Canárias, Madeira e Cabo Verde, ocupando uma grande diversidade de habitats, desde a costa até zonas de alta montanha. Apesar da sua diversidade, as interações entre estas plantas e os seus polinizadores encontram-se restritas a estudos focados em algumas espécies das ilhas Canárias com algumas observações isoladas nos restantes arquipélagos. Segundo estes estudos, os *Echium* da Macaronésia podem ser visitados por diferentes grupos de polinizadores, de insetos a vertebrados, sendo as abelhas os seus principais polinizadores.

Neste trabalho estudámos a comunidade de polinizadores diurnos que visita *Echium candicans*, uma espécie endémica da ilha da Madeira que ocorre em habitats de montanha. Esta espécie está classificada como *Data Deficient* segundo a IUCN, estando o seu habitat ameaçado por incêndios, tais como os que ocorreram nos últimos anos, e pela expansão de espécies exóticas, na zona do Maciço Montanhoso Central. Averiguámos também a relação das comunidades em indivíduos de *E. candicans* com as suas características e isolamento em relação a conspécíficos seguindo um modelo com base no indivíduo. Por último, caracterizámos o comportamento de alguns dos polinizadores mais comuns quando visitam as flores de *E. candicans*.

Ao todo foram seleccionados 24 indivíduos de *Echium candicans* distribuídos por dois locais (um a 1500m e outro a 1800m) nas proximidades do Pico do Arieiro. Estes indivíduos foram caracterizados: diâmetro, altura, tamanho médio das suas inflorescências, número de flores e isolamento (distância média aos três conspécíficos mais próximos). A comunidade de polinizadores de cada planta foi amostrada através de seis observações de 10 minutos distribuídas entre as 10h e as 16h. Foram também realizadas duas amostragens complementares, recorrendo a pratos coloridos e capturas com rede em transetos de 10 minutos com o objetivo de caracterizar a comunidade de potenciais polinizadores que ocorrem na proximidade dos indivíduos de *Echium candicans* estudados. As espécies de polinizadores mais observadas de cada ordem foram seleccionadas para o estudo do comportamento. Neste segundo estudo, foram cronometradas até 10 minutos cerca de vinte visitas de cada espécie, registando-se o número de inflorescências e flores visitadas pelo polinizador.

Com os resultados obtidos construímos três redes de polinizadores e de indivíduos de *Echium candicans* visitados, uma para cada local amostrado e uma com todas as observações registadas. Tal como era esperado, os *E. candicans* estudados revelaram ter uma comunidade diversa de polinizadores, com 50 espécies e grupos de insetos e um réptil. Os dois locais estudados demonstraram alguma variabilidade nos seus polinizadores principais, sendo o abelhão *Bombus ruderatus* a espécie mais observada em ambos, e a borboleta endémica *Hipparchia maderensis* e a abelha *Amegilla quadrifasciata* as segundas espécies mais abundantes, respetivamente nos dois locais. A abelha-do-mel *Apis mellifera* e a borboleta *H. maderensis* foram observadas em maior número no local de menor altitude, em contraste com a abelha *A. quadrifasciata*, o sírfideo *Scaeva pyrastris* e a Lagartixa da Madeira (*Teira dugesii*), em maior número no local de maior altitude.

Apesar de ter sido observada uma diversidade de abelhas semelhante à reportada para as espécies de *Echium* mais estudadas das Ilhas Canárias (*E. wildpretii* e *E. simplex*), a nossa espécie revelou uma diversidade muito superior de sirfídeos e borboletas.

As características das plantas foram relacionadas com parâmetros da rede, através de coeficientes de regressão estandardizados obtidos a partir de modelos lineares generalizados (GLMs). O número de flores por planta e o tamanho médio das suas inflorescências foram as características das plantas que apresentaram uma relação positiva com a maioria das métricas utilizadas, com exceção do Nested rank. Esta métrica demonstrou um aumento na generalização das plantas com o aumento do tamanho das suas inflorescências. As restantes métricas demonstraram que os *E. candicans* com maior número de flores e maiores inflorescências são os indivíduos mais centrais na rede, tendo um maior número de polinizadores e sendo de maior importância para estes. A maioria das espécies e grupos de polinizadores estudados apresentaram relações positivas com o número de flores e/ou com o tamanho das inflorescências. Os módulos obtidos demonstraram que as comunidades de polinizadores amostrados apresentam um padrão de ocorrência semelhante à distribuição dos indivíduos de *E. candicans* pelos dois locais, assim como dentro de cada local. Ao contrário do esperado apenas três espécies de polinizadores tiveram uma relação negativa com o isolamento das plantas, podendo isto ser devido às distâncias envolvidas, entre as plantas e os seus conspecíficos, serem relativamente pequenas. As três espécies de polinizadores que responderam a esta variável poderão preferir as manchas com maior número de plantas próximas, uma vez que apresentaram as relações mais fortes com o número de flores das plantas que visitaram.

No que toca ao comportamento das espécies selecionadas, as abelhas de maior tamanho (*A. quadrifasciata*, *Apis mellifera*, *Bombus ruderatus* e *B. terrestris*) apresentaram as maiores taxas de visitação (número médio de flores visitadas pelo tempo de visita). Somente as duas espécies de sirfídeos (*S. pyrastris* e *E. tenax*), apresentaram um valor semelhante ao abelhão *B. terrestris*. As borboletas (*C. croceus* e *H. maderensis*), a abelha *L. wollastoni* e a lagartixa apresentaram taxas muito inferiores às das abelhas de maior tamanho. Contudo, as borboletas, por passarem mais tempo nas flores que visitam e por contribuírem com maior ocorrência de polinização cruzada, poderão ser igualmente de grande importância para *E. candicans*. Globalmente estes polinizadores poderão ser complementares entre si em termos da polinização de *Echium candicans*, devido aos seus diferentes comportamentos quando visitam as flores. Espécies mais abundantes e com altas taxas de visitação polinizam mais flores, mas poderão levar a um aumento de autopolinização por geitonogamia. Em contrapartida, outras espécies mais raras, mas que favoreçam a ocorrência de polinização cruzada poderão ser responsáveis por uma polinização mais eficaz das flores.

Este estudo demonstrou que, tal como os seus parentes das Ilhas Canárias, *Echium candicans* é visitado por uma grande variedade de polinizadores, entre os quais se incluem vertebrados. Contudo, aparenta ter uma elevada importância para uma maior diversidade de polinizadores de muitos outros grupos para além das abelhas, tais como sirfídeos e borboletas. A diversidade e as abundâncias registadas parecem estar relacionadas principalmente com a abundância de recursos em cada planta e um pouco com a densidade de indivíduos de *E. candicans* presentes nos locais, pelo menos para algumas espécies. As maiores taxas de visitação foram registadas para as abelhas de maior tamanho, o que aliado às suas abundâncias pode fazer destes os principais responsáveis por grande parte da polinização das populações estudadas. Em contraste, outras espécies como a borboleta *H. maderensis* poderão ser mais eficazes na polinização devido à diferença no seu comportamento durante as visitas.

Palavras-chave: Polinização, Macaronésia, Planta endémica, Comportamento do polinizador, Redes com base no indivíduo

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1. Introduction

1.1. The importance of studying plant-pollinator interactions

According to the International Union for Conservation of Nature (IUCN), 21% of the plant species are threatened with extinction (Willis, 2016). Among the reasons for the decline is the loss of pollinators (Biesmeijer et al., 2006), as some studies have shown worldwide decreases of abundance and diversity of different groups of pollinators and their consequences for plant conservation (Potts et al., 2010, Anderson et al., 2011, Carvalheiro et al., 2013, Nieto et al., 2014). Plant-pollinator interactions might disappear even when both interacting species are present (Valiente-Banuet et al., 2015). This can happen if one of the mutualist populations is reduced to a point where it is considered “ecologically extinct”, meaning the interaction has been lost (Aslan et al., 2013). The loss of pollination interactions can lead to the decline of plant populations, with species associated to or specialised in a particular type of pollinator being the most affected (Biesmeijer et al., 2006, Anderson et al., 2011, Pauw and Hawkins, 2011). Some examples of these threatened interactions occur on insular environments (Cox and Elmqvist, 2000, Magnacca, 2007, Anderson et al., 2011), where many plants are severely threatened by the introduction of exotic species and the loss and fragmentation of their habitats (Kaiser-Bunbury et al., 2010, Valido and Olesen, 2010, Lobo et al., 2017a).

1.2. Pollination networks on islands

Pollination networks have been widely used to study plant-pollinator interactions on different islands and archipelagos (e.g. Olesen et al., 2002, Dupont et al., 2003, Traveset et al., 2013). Networks are constructions of interlinked nodes used by ecologists to study interactions, to understand the patterns observed in nature (Heleno et al., 2014). In the case of pollination interactions, the links between species represent exchanges of ecological services for both parts (Olesen et al., 2012a), and are normally estimated using a proxy of pollinator effectiveness (e.g. flower visitation rates) (Heleno et al., 2014). It has been shown that island pollination networks, especially in oceanic islands, are poorer in species and interaction links, and show a wider plant niche overlap compared to the mainland ones (Traveset et al., 2016). This is a consequence of filtering effects that drive island colonization by plants and especially by pollinators, leading to communities poorer in species when compared to the mainland (Whittaker and Fernández-Palacios, 2006). Also the low dispersal ability of several animal groups, such as large flower-visiting insects, contributes to a low pollinator to plant species ratio on islands (Olesen and Jordano, 2002, Valido and Olesen, 2010), which seems to favour generalism on plants (Armbruster, 1998, Traveset et al., 2016). This niche widening is not restricted to plants, as certain reptiles and birds often include flower resources such as nectar in their diet, which might be due to low interspecific competition and food availability on island ecosystems (Olesen and Valido, 2003, 2004), giving rise to novel pollination interactions (Valido and Olesen, 2010).

One of the new avenues of network studies is the focus on individual-based studies (Olesen et al., 2012a). Studying communities using an individual-based approach is vital to understand if and how the network patterns differ from those obtained from species-based studies (Heleno et al., 2014), as well as what is the functional position of individuals in populations and communities (Kuppler et al., 2016). However, despite some focus on the relation between individual plant traits and the observed pollinator community (e.g. Valido et al., 2002), studies using an individual-based network approach on insular plants are still lacking.

1.3. Plant-pollinator interactions in Macaronesia

Insular environments are known to be rich in endemic plant species (Kier et al., 2009), and one good example is the Macaronesia. This biogeographical region includes the archipelagos of Madeira, Canaries, Azores, Selvagens and Cape Verde (Fernández-Palacios, 2010). Worldwide known for its endemic biodiversity (Arechavaleta et al., 2005, 2010, Borges et al., 2005, 2008), the unique floral elements of Macaronesia span from very old groups of ferns and trees, like the ones found on the Laurel forests (Fernández-Palacios, 2010, Fernández-Palacios et al., 2011), to more recent radiations that resulted from few colonization events (Cronk, 1997, Fernández-Palacios, 2010). Some large radiations can be found in various plant groups, such as in *Sonchus*, *Argyranthemum*, *Aeonium* and *Echium* (Böhle et al., 1996, Francisco-Ortega et al., 1996, Kim et al., 1996, Mort et al., 2002). These radiations are not restricted to plants, with many studies showing high radiation in multiple invertebrate groups after arriving at these archipelagos (e.g. Oromí et al., 1991, Percy, 2010), which in the case of insect pollinators is observed at a much lower level (e.g. Brunton and Hurst, 1998).

Although several studies on pollination have already been conducted in the Azores and the Canary Islands, they are still lacking on the other archipelagos (Valido and Olesen, 2010). In the case of the Madeira archipelago, there were no specific studies about pollination until very recently (Olesen et al., 2012b, Kratochwil et al., 2019a), being mostly restricted to isolated records of some plant-pollinator interactions (Fernandes et al., 2007, Valido and Olesen, 2010).

Many Madeiran endemic plants have their habitats threatened by the spread of exotic plant species, which were favoured by human activities during the last centuries (Silva et al., 2008, Lobo et al., 2017a). Nevertheless, these impacts of introduced species in Macaronesia are not restricted to invasive plants, with some studies showing competition for resources, disruption of interactions and reduced plant reproductive success caused by the presence of the non-native honeybee *Apis mellifera* (e.g. Dupont et al., 2004, Jaca et al., 2019a, Valido et al., 2019). Since the survival of many endemic plants of these archipelagos is at risk, it is especially urgent to evaluate the state of the interactions between them and both native and introduced pollinators.

1.4. The Macaronesian *Echium*

One of the best examples of radiation in the Macaronesia archipelagos is the plant genus *Echium* (Boraginaceae) (García-Maroto et al., 2009, Givnish, 2010, Valido and Olesen, 2010). With a total of 29 endemic species in these archipelagos (García-Maroto et al., 2009, Carvalho et al., 2010), it constitutes a very diverse group of plants occupying various habitats, from coastal to mountain areas (García-Maroto et al., 2009). This plant genus seems to have initially colonised and diversified on the Canary Islands archipelago (Böhle et al., 1996) between 20 and 15 Ma ago, in the Miocene (Böhle et al., 1996, García-Maroto et al., 2009). After the initial radiation in the Canary Islands, it colonized Cape Verde and then Madeira, and this last colonization could have resulted from multiple colonization events (García-Maroto et al., 2009).

Animal pollinators are important for proper cross-pollination and fruit production (Sedlacek et al., 2012, Jaca et al., 2019b) for the *Echium* species of Macaronesia. Despite this, the pollination studies done so far have been focused only on Canary Islands species, mostly *E. wildpretii* (Olesen, 1988, Valido et al., 2002, Dupont et al., 2003, 2004, Dupont and Skov, 2004, Sedlacek et al., 2012, Valido et al., 2019) and more recently on *E. simplex* (Jaca et al., 2019b). Some studies have also addressed the diversity of bees observed visiting *Echium* on the Canary Islands and Madeira (Dupont and Skov, 2004, Kratochwil et al., 2019a), as well as the pollination effectiveness of insects and vertebrate pollinators (Sedlacek et al., 2012, Jaca et al., 2019b).

However, information about pollinator groups other than bees and vertebrates, such as flies and butterflies, is still missing for most species (Valido and Olesen, 2010).

1.5. Importance of pollinator behaviour

A particular pollinator's importance cannot be measured only by the number of times it visits a plant species, but it is also necessary to evaluate its effectiveness (Herrera, 1987). Bees for example, which are known to minimize flight distances, visit many flowers in the same plant before moving to another individual (Herrera, 1987, Montaner et al., 2001). This can promote selfing by geitonogamy (transfer of pollen between flowers in the same individual plant), which can lead to inbreeding depression, sometimes only detected in the long term (Melser et al., 1999, Dupont et al., 2004). Because of their high abundance on certain sites, species like the honeybee can be the main responsible for the occurrence of geitonogamy (Montaner et al., 2001, Dupont et al., 2004), which can reduce the reproductive success of plants (Herrera, 1987).

In contrast, less common pollinators, such as butterflies and hoverflies, can be more effective in pollination if they deposit pollen from other conspecific plants, contributing to cross-pollination (xenogamy) (Herrera, 1987, Herrera, 1990, Fontaine et al., 2006). To evaluate the effectiveness of particular pollinator species, the studies normally focus on assessing plant reproductive output by determining seed set and/or fruit set (e.g. Herrera, 1987, Sedlacek et al., 2012). However, this task is time-consuming and involves hard work at recording which pollinator or pollinators visit specific flowers, as well as the assessment of pollen grains deposited on stigmas and their viability (Herrera, 1987). Consequently, low sample size, particularly for less abundant pollinator groups, such as Diptera and Lepidoptera (e.g. Jaca et al., 2019b), may turn difficult to interpret their role as pollinators (Herrera, 1987, Fontaine, 2006). A possible solution to overcome this problem is to use the visitation rate of a pollinator species as a proxy of pollinator importance (e.g. Herrera, 1989) jointly with the record of pollinator foraging behaviour when visiting the plant (Herrera, 1987). Recording the foraging behaviour of a pollinator is especially valuable when studying insular plants, which tend to hold a lower genetic diversity than continental species (Barret et al., 1996), as it helps understand the pollinator's movements in the plant and the probability of occurring geitonogamy (Herrera, 1987, Montaner et al., 2001).

1.6. Study species

Considering the variety of flower-visitors of the Macaronesia *Echium* (Valido et al., 2002, Jaca et al., 2019b, Valido et al., 2019), these plants could be considered generalists, as well as key species in their habitats (Dupont et al., 2003). However, so far, the visitors reported from Madeiran *Echium* have been mostly bees (Valido and Olesen, 2010, Kratochwil and Schwabe, 2018, Kratochwil et al., 2019a) and the Madeiran lizard (*Teira dugesii*) (Elders, 1977). In this work, we decided to study the community of flower-visitors, hereafter designated pollinators, of the Madeiran endemic *Echium candicans* (Linnaeus, 1781). Like most other Macaronesia *Echium*, it is a shrub, which can grow up to 2 meters on forest-cliff habitats and terraces above 800 meters, on the cloud zone of the Madeira Island (Bramwell, 1972, Carvalho, 2011). Typically, *E. candicans* is found in open forest border areas, in the *Vaccinio padifolii-Ericetum maderinicolae* community, and rocky cliffs of the *Argyranthemo montani-Ericetum maderensis* and *Bystropogono punctati-Telinetum maderensis* communities (Costa et al., 2004, Lobo et al., 2017a). *Echium candicans* is currently protected by the Habitats Directive (Annex II and IV) and classified as *Data Deficient* by the IUCN (Carvalho, 2011), the main threats identified

being the loss of habitat due to human activities and wildfires, as well as the spread of introduced species (Carvalho, 2011, Lobo et al., 2017a). Macaronesian *Echium* are traditionally organized in different sections, although with no phylogenetic support (Bramwell, 1972). Like its two Madeiran congeners (*E. nervosum* and *E. portosanctensis*), *Echium candicans* is included in the *Virescentia* group, which is the largest and more widespread Macaronesian group (Bramwell, 1972, Carvalho et al., 2010). Plants of this group are characterized by cylindrical inflorescences, non-laterally compressed flowers (funnel-shaped in the case of *E. candicans*) and blue or pink corolla with more or less equal lobes (Carvalho et al., 2010). Its flowers are protandrous, meaning that the male phase precedes the female, which might be an adaptation to reduce autogamy (Bramwell, 1972, Bramwell, 1973). The flowering period lasts from April to August (Press and Short, 1994), depending on the altitude where the population is located.

1.7. Objectives

Our study has three major goals: 1) to identify the diurnal community of pollinators of *Echium candicans*, from the local pool of pollinators; 2) to evaluate which plant traits influence the diversity and abundance of pollinators as well as pollination network metrics; 3) to assess differences in the behaviour of the most common visitor species that may influence pollination effectiveness. We expect that the plants with a higher number of flowers and larger inflorescences will be visited by a more diverse and abundant pollinator community, acting as hubs, having a more central and generalist position in the network. Given their overall importance in mainland ecosystems, we also expect that bee species show higher visitation rates compared to other groups, such as butterflies and hoverflies.

2. Methods

2.1. Study area

The two studied populations of *Echium candicans* were located near Pico do Arieiro in Madeira Island, one at 1500 meters (S1) and the other at 1800 meters (S2) above sea level (Figure 2.1). Fog and precipitation are common in the study area during the study period (Lobo et al., 2017a), but following the standard protocols for sampling pollinators, our sampling was restricted to days with sunny weather with some occasional fog. The study area is part of a Special Conservation Area (Habitats Directive; PTMAD0002 – Central Mountainous Massif), enclosed in the Madeira Natural Park (Lobo et al., 2017a). *Echium candicans* was common in both study sites, with less than 100 individuals in both S1 and S2. However, because these communities are fragmented above 1650 meters (Lobo et al., 2017a), and the studied plants were very close to man-made paths and roads, we suspect that the studied populations are likely mostly composed of planted individuals. In the past, the area was severely affected by grazing, which was only completely banned from the mountain regions in 2003 (Lobo et al., 2017a). In recent years, a high number of wildfires has affected large areas of the island and several were severe, like the one that happened in the summer of 2010 affecting the Central Mountain Massif (Carvalho, 2011). This fire affected the vegetation that was still recovering from grazing and contributed to the spread of some exotic species, such as the common broom (*Cytisus scoparius*) (Lobo et al., 2017a) which is one of the top 25 worst invasive species in the Macaronesian archipelagos (Silva et al., 2008). This species is a severe threat to Madeira mountain biodiversity in many areas of the Paul da Serra plateau and the Central Mountainous Massif, including the site S2.

Recent work of the LIFE project Maciço Montanhoso in Madeira, revealed that *E. candicans* populations seem to be recovering spontaneously in the areas affected by the wildfires, occurring sporadically across the Central Mountainous Massif, with their highest densities near Pico Ruivo and Pico do Gato (Lobo et al., 2017b). Apart from *E. candicans*, the native plant community of the study sites was composed of several shrub species, such as the endemics *Erica maderensis*, *Vaccinium padifolium*, *Melanoselinum decipiens*, *Teline maderensis* and *Argyranthemum pinnatifidum*, and herbs, like *Armeria maderensis*, *Andryala* spp., *Erysimum bicolor*, *Hypochoeris* sp., *Vicia* spp, *Plantago* spp. and *Anthyllis lemanniana*, (Costa et al., 2004, Lobo et al., 2017b). The study sites were sampled during the flowering peak at these altitudes (personal observation), from early July to the beginning of August 2018, with additional fieldwork in June 2019.



Figure 2.1. Location of Pico do Arieiro in Madeira and the study sites (S1 at 1500m and S2 at 1800m). Source: <https://earth.google.com>.

2.2 Characterizing individual *Echium candicans*

We randomly selected 24 flowering individuals for this study (14 at S1 and 10 at S2). To assess the role of plant characteristics on pollinator visitation we measured the maximum height and diameter of all plants, the distance of each one to its three closest conspecifics (an indication of plant isolation) and the size of five randomly chosen inflorescences. We also registered the geographic coordinates of every individual. In small plants (up to 10 inflorescences), we counted all the inflorescences, but for larger plants (more than 10 inflorescences) this task proved inaccurate and time-consuming. So, on these plants, we counted all the inflorescences in one-quarter of the plant and multiplied the results by four. To estimate the number of flowers in each measured inflorescence, we counted the number of flowers on half of each selected inflorescence and multiplied the results by two.

2.3 Sampling the pollinator community

2.3.1. Record of pollinators on the selected *E. candicans* plants

We sampled daytime pollinators, six times on each of the 24 selected plants, between 10:00-16:00. The observations on each plant were distributed throughout the day (two per two-hour

period) to consider the differences in foraging activity of pollinators. Sampling consisted of a 10 minute observation period of animal visitation to the flowers of a focal plant. We registered the lowest taxonomic identity possible of each pollinator, as well as the number of visits to flowers they made on each plant. Overall, 24 hours of observations were made on the individual plants to identify the pollinator assemblage and assess differences in pollinator visitation. Each pollinator that we could not identify to the species level was captured and preserved in ethanol (70%) for later identification in the laboratory. Insect sampling was authorized by the legal authorities, in this case, the Instituto das Florestas e Conservação da Natureza (IFCN).

2.3.2. Assessing the local pollinator community

To identify the community of pollinators present at the study sites, we used pan-traps and net sampling during timed transects, as they are two complementary sampling strategies (Popic et al., 2013). Net sampling can better capture the diversity and abundance of pollinators, also enabling the record of behaviour and interactions they undergo with plants, while pan-traps are better at capturing specific species, not commonly captured by net sampling (Roulston et al., 2007, Popic et al., 2013).

For pan-trapping, at each site we used 4 groups of plates set randomly at least 20 meters apart. Each group was composed of a blue, a yellow and a white plate, which are considered the most effective colours at attracting pollinators (Roulston et al., 2007). Within each group, plates were distanced by nearly 2.5m. We monitored the traps twice per day, at 10:00 and 16:00, for four consecutive days, removing the trapped specimens and storing them in ethanol (70%) for later identification in the laboratory.

We also performed eighteen 10-minute transect walks at each site. During these transects, we registered the lowest possible taxonomic identity and number of flower visitors observed on all flowering species, except on *Echium candicans*. All insects captured were stored in ethanol (70%) for posterior identification.

2.3.3. Identification of insect specimens in the lab

We identified all captured insects on the selected *E. candicans*, pan-traps and transects to the lowest taxonomic level possible using an Olympus SZX7 stereomicroscope at the entomology lab of the Faculty of Sciences, University of Lisbon. Insect identification followed a two-stage process: first, we identified all specimens to family-level using general literature (Unwin, 1981, 1988, Barrientos, 1988), then we identified the specimens from the most common pollinator groups to the species level (i.e. bees, butterflies, hoverflies). This was done using specific literature with the help of colleagues more knowledgeable of the Madeiran diversity of bees and hoverflies (Cockerell, 1922, Fumero, 1977, Erlandsson, 1983, Wakeham-Dawson et al., 2001, Ortiz-Sánchez et al., 2003, Smit et al., 2004, Veen, 2010, Ball, 2013, Kratochwil et al., 2014, Falk, 2016, Kratochwil, 2018, Kratochwil et al., 2018). All specimens are deposited in the entomological collection of the Faculty of Sciences, University of Lisbon.

2.4. Differences in the activity of the main pollinators of *E. candicans*

For this study, we selected the most common pollinators of *E. candicans* of each of the main orders. The species selected for this behavioural study were: five bees (*Amegilla quadrifasciata*, *Apis mellifera*, *Bombus ruderatus*, *B. terrestris* and *Lasioglossum wollastoni*), two hoverflies (*Eristalis tenax* and *Scaeva pyrastris*), two butterflies (*Hipparchia maderensis* and *Colias croceus*) and the Madeiran lizard (*T. dugesii*). This study was carried out mostly during 2018, with additional sampling in 2019.

Up to twenty individuals of each selected species were observed visiting the flowers of randomly selected *Echium candicans* plants. During each observation period, we recorded the duration of the visit (up to 10 minutes), and the number of inflorescences and flowers visited by the pollinator. Overall we registered the activity of 198 individual pollinators that lasted a total of 665 minutes of observation.

2.5. Statistical analysis

2.5.1. Visitation networks

We built three individual-based visitation networks, one using all the different pollinators observed on the twenty-four *E. candicans* studied, and two using the information from each site separately. The study of these networks was made by calculating specific network metrics that are described below and in Table 2.1.

Modularity can be seen as the degree to which an ecological network is composed of different sub-communities, with modules being sets of interacting species (Dormann et al., 2009). The modularity metrics consider the existence of these modules (Dormann and Strauss, 2014), which in our case are link-rich clusters of different *Echium candicans*. The information given by each metric is described in Table 2.1. We compared the metrics from the two study sites using multiple Wilcoxon tests. We also tested for negative correlations between the number of visits of particular species and groups, to check for avoidance between pollinators.

Table 2.1. Network metrics and the information they provide about the individual *E. candicans* plants and their pollinators (Hill, 1973, Olesen et al., 2007, Alarcon et al., 2008, Dormann, 2011).

Metrics		Definition
Species richness (SR)		Number of species recorded.
Exponential of the Shannon diversity index (EShan)		Mean proportional abundance obtained from an intermediate diversity-number, in what concerns rare species.
Total number of visits		Total number of pollinator records
Number of visits by specific groups		Number of records of selected groups (Bees, Hoverflies and Butterflies).
Number of visits by specific species		Number of records of selected species.
Species-level metrics	Normalized degree	The number of visitor species per individual <i>E. candicans</i> in relation to the total number of visitor species, ranging between 0 and 1.
	Species strength	Individual <i>E. candicans</i> relevance across all its pollinators, by comparing the proportion of visits done by each pollinator to the other <i>E. candicans</i> .
	Nested rank	Ranks individual <i>E. candicans</i> according to their generality, measured as the position in a maximum nestedness matrix. Generalist plants are those interacting with more pollinator species and thus those with a rank closer to 0, while on specialists ranks have higher values.
	Weighted closeness	Describes the centrality of an <i>E. candicans</i> in the network: it is the inverse of the average distance from the focal node to all other nodes.
Modularity metrics	c (Among-module connectivity)	The level to which the <i>E. candicans</i> in a module is linked to its conspecifics in other modules.

	z (Within-module degree)	The standardized number of links to other <i>E.candicans</i> in the same module.
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2.5.2. Association between plant characteristics and pollinator visitation

The plant traits we used were diameter, height, isolation, which was the average distance of the plant to its three closest conspecifics, the average size of the inflorescences, obtained from the five measured inflorescences at each plant, and the number of flowers per plant. This last variable was estimated by multiplying the total number of inflorescences on the plant (an estimate in the case of larger plants) by the average number of flowers counted on the five measured inflorescences, multiplied by two. The different network metrics obtained for the individual *E. candicans* were used as response variables (Table 2.1).

We analysed the relation between the plant characteristics and: i) pollinator diversity; ii) the number of observations of particular species and pollinator groups, which consisted on the previously mentioned species, plus *Eupeodes* sp. and *Sphaerophoria scripta* in the case of hoverflies, as they were common and could help to better reflect hoverflies relations with the variables; iii) species level and modularity metrics, by fitting Generalized Linear Models (GLMs) (Crawley, 2007, Zuur et al., 2009). The plant traits (diameter, height, isolation, inflorescence size and flower number) were used as predictors in the analysis and, when necessary, transformations were applied to the data to comply with the assumptions of the GLMs analysis (Table 2.2). The model used for each response variable varied according to the type of data and variable (Crawley, 2007, Zuur et al., 2009, R Core Team 2018).

Initially, we tested for correlations among the different plant traits. Diameter and height were highly correlated with each other ($r > 0.9$, $p < 0.001$) and with number of flowers per plant ($r > 0.8$, $p < 0.001$; $r > 0.6$, $p < 0.001$), so these variables were discarded. Afterwards, we created a model for each response variable with all remaining plant traits. The explanatory variables were selected in each model using Akaike's information criterion (AIC). Only the variables significant at a 0.05 level and whose removal would result in a worse model fit were kept in the final model (Burnham and Anderson, 2002, Crawley, 2007). We computed standardized regression coefficients to show the relative importance of significant variables (Gelman and Hill, 2006), following Gelman (2008).

Table 2.2. Models used for each response variable. The model type was chosen for z, EShan and Species strength using Akaike's information criterion (AIC).

Response Variables	Transformation and Distribution	Link Function
z and the exponential of the Shannon diversity index	Gaussian distribution	Identity
c, Nested rank, Normalized degree and Weighted closeness	Arcsine transformed and Gaussian distribution	Identity
Species strength	Gamma distribution	Log
Species richness and total Number of visits, as well as the number of visits of particular species and groups	Negative binomial	Log
Number of visits of the Madeiran lizard	Zero-inflated negative binomial	Logit

2.5.3. Behaviour analysis

First, we calculated the visitation rate of each species by dividing the number of flowers visited by the total time spent by each pollinator on the plant and then averaging the different observations from conspecific pollinators (Herrera, 1989). We then tested if the variables could be analysed using a parametric variance test, but these did not meet the requirements. For this reason, we used the Kruskal-Wallis non-parametric test to check for differences between species

in any of the four variables registered (duration of the visit, number of inflorescences visited, number of flowers visited and the visitation rate). The statistically significant results ($p < 0.05$) were followed by post-hoc Dunn's tests to determine which pairs of species diverged for the specific behaviour analysed.

2.5.4. *Statistics software*

For all statistical analyses, we used R software version v.3.6.0 (R Core Team 2018). The *E. candicans* networks and their metrics were obtained with the R package "bipartite" (Dormann et al., 2009, Dormann, 2011, Dormann and Strauss, 2014, R Core Team 2018). The Wilcoxon test, Kruskal-Wallis test and the Akaike's information criterion (AIC) were implemented from the "stats" package (Crawley, 2007, R Core Team, 2018). Species richness and EShan results were obtained with the package "vegan" (Oksanen et al., 2019). Count variables were analysed with the package "MASS" (Venables and Ripley, 2002). The zero-inflated negative binomial model was applied from the "pscl" package (Zeileis et al., 2008). Standardized regression coefficients were computed using the "arm" package. (Gelman and Su, 2018). The post-hoc Dunn's tests were computed using with the "PMCMR" package (Pohlert, 2014).

3. Results

3.1. The flower-visitors of *Echium candicans*

We observed a total of 5612 visitors from 51 different morphospecies (32 identified at the species level; Appendix 1). Of the identified species, 25% are endemic at the species or subspecies level, 66% are native non-endemic and only 9% are non-native (Borges et al., 2008, Kratochwil et al., 2018). For a few genera (for example, *Eupeodes*), it was not possible to properly identify the species in the field, due to their similar morphology and rapid movement. So, despite that we were able to identify the collected specimens in the laboratory, most individuals observed in the field (and not collected) could only be identified at the genus level. Pollinators were mostly insects belonging to five different orders (Hymenoptera, Lepidoptera, Diptera, Coleoptera and Blattodea), with Hymenoptera, Lepidoptera and Diptera being the groups with the higher numbers of observations. In these groups, the most common pollinators were bees (55% of all observations), butterflies (20% of all observations), and hoverflies (9% of all observations). The most common species of each main order were five bees (*Amegilla quadrifasciata*, *Apis mellifera*, *Bombus ruderatus*, *B. terrestris* and *Lasioglossum wollastoni*), two butterflies (*Hipparchia maderensis* and *Colias croceus*) and two hoverflies (*Eristalis tenax* and *Scaeva pyrastris*). In addition, we also observed the Madeiran lizard (*Teira dugesii*), lapping the nectar on some *E. candicans* (Figure 3.1a). Altogether, these ten species accounted for 81% of all the observations registered in the 24 study plants.



Figure 3.1. Pollinators observed on the studied *Echium candicans*: a – *Teira dugesii*, b – *Bombus ruderatus*, c – *Hipparchia maderensis*, d – *Amegilla quadrifasciata*.

3.2. Visitation networks of *Echium candicans* in the study area

The visitation network of the different pollinators to the study plants is presented in the form of an ecological individual-based network for the study area (Figure 3.2), as well as for the two studied sites separately (Figures 3.3 and 3.4). Of all the visitors, *B. ruderatus* was the most commonly observed species (Figure 3.1b) both overall and in the two sites separately (23% of all the observations), the second most common being the endemic butterfly *H. maderensis* (18% of all the observations – Figure 3.1c). Looking at the two sites separately, the second most common pollinator was *H. maderensis* at S1 and the bee *A. quadrifasciata* (5.6% of all the observations) at S2 (Figure 3.1d). The only vertebrate observed (*T. dugesii*), accounted for only 1.6% of all the observations.

Comparing the study sites in terms of the number of observations of the selected groups and species, S2 showed a higher number of hoverflies, in particular, *S. pyrastris*, as well as more observations of the bee *A. quadrifasciata* and the Madeiran lizard *T. dugesii* (Table 3.1). In contrast, S1 had a higher number of butterflies observed, in particular, *H. maderensis*, as well as more observations of *A. mellifera* (Table 3.1). Concerning the network metrics for each study site, only *c* was different ($p < 0.05$), having higher values at S1 (Appendix 2) in comparison with S2

(Appendix 3). No negative correlations of visits between pollinator species and groups to individual *E. candicans* were detected.

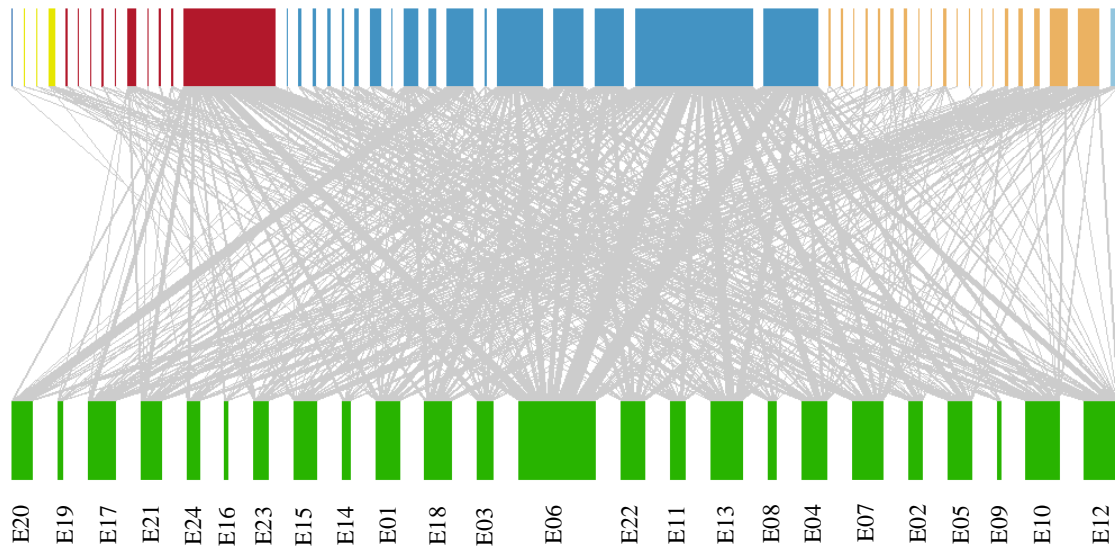


Figure 3.2. Pollination network with the entire pool of pollinators observed (upper side) on all the *Echium candicans* plants (lower side) at the study area. The size of each box on the upper part is proportional to the number of visits performed by each group of pollinators. The different groups are organized from left to right as: Blattodea (Dark Blue), Coleoptera (Yellow), Lepidoptera (Red), Hymenoptera (Blue), Diptera (Orange) and Squamata (Light Blue). In the lower part, bar width is proportional to the number of visits done to each individual *E. candicans*. E01-E14 are *E. candicans* that belong to S1 while E15-E24 belong to S2. The link width shows the number of visits done by a group of pollinators on an individual *E. candicans*.

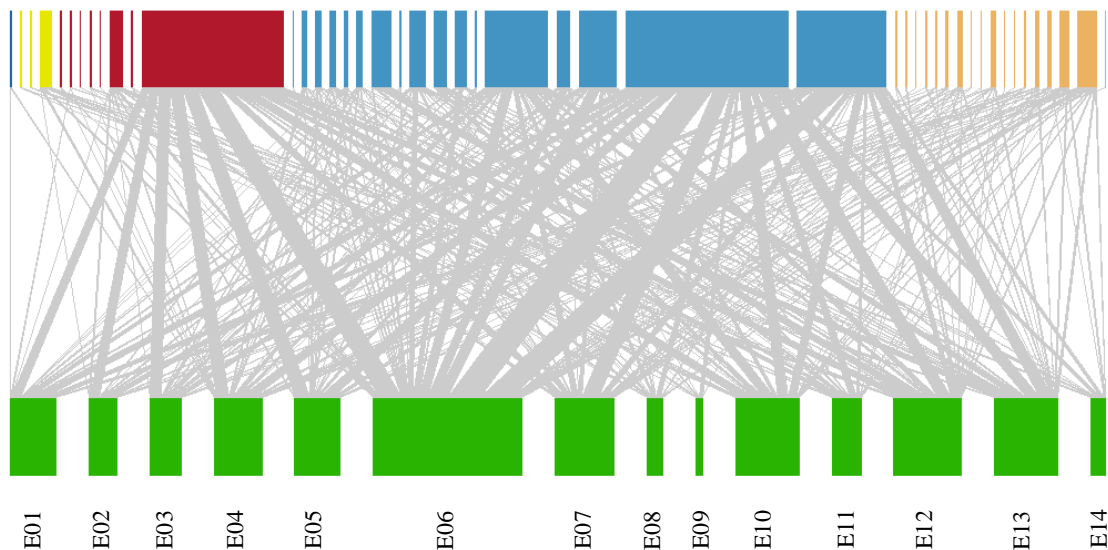


Figure 3.3. Pollination network with the pool of pollinators observed (upper side) on the 14 *Echium candicans* at Site 1 (lower side). The size of each box on the upper part is proportional to the number of visits performed by each group of pollinators. The different groups are organized from left to right as: Blattodea (Dark Blue), Coleoptera (Yellow), Lepidoptera (Red), Hymenoptera (Blue), Diptera (Orange) and Squamata (Light Blue). In the lower part, the bar width is proportional to the number of visits done to each individual *E. candicans*. The link width shows the number of visits done by a group of pollinators on an individual *E. candicans*.

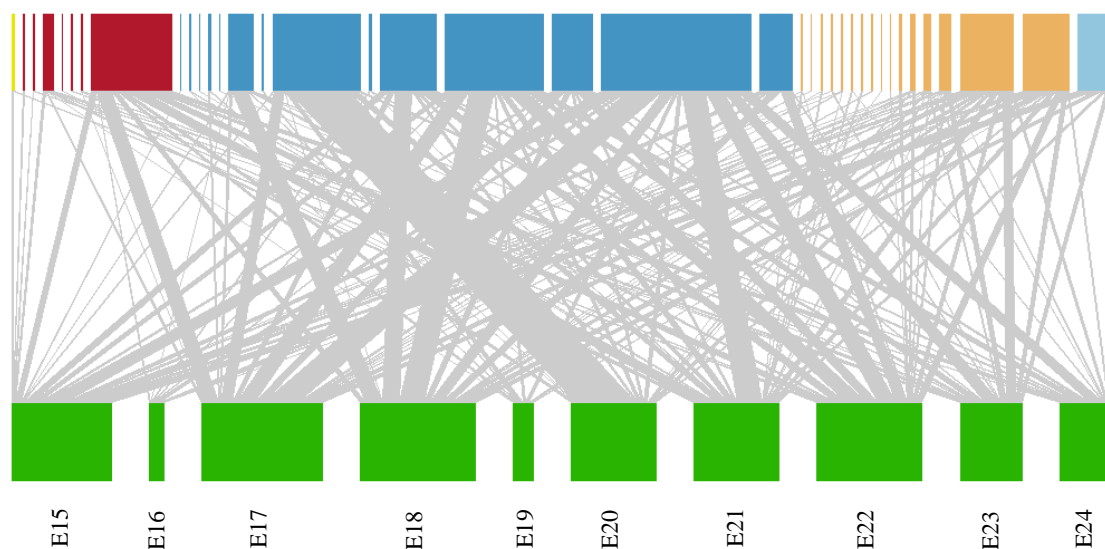


Figure 3.4. Pollination network with the pool of pollinators observed (upper side) on the 10 *Echium candicans* at Site 2 (lower side). The size of each box on the upper part is proportional to the number of visits performed by each group of pollinators. The different groups are organized from left to right as: Coleoptera (Yellow), Lepidoptera (Red), Hymenoptera (Blue), Diptera (Orange) and Squamata (Light Blue). In the lower side, the bar width is proportional to the number of visits done to each individual *E. candicans*. The link width shows the number of visits done by a group of pollinators on an individual *E. candicans*.

Table 3.1. Differences in the number of visits by different pollinator species and groups on the two study sites (S1 and S2). Data are presented as mean \pm SD per individual *E. candicans* (* different between sites – $p < 0.05$).

Pollinator species and groups	S1	S2
<i>A. quadrifasciata</i> *	5.1 \pm 11.9	24.4 \pm 18.9
<i>A. mellifera</i> *	35.5 \pm 42.5	8.1 \pm 10.2
<i>B. ruderatus</i>	64.6 \pm 40.5	37.4 \pm 23.7
<i>B. terrestris</i>	14.9 \pm 16.1	10.2 \pm 8.9
<i>L. wollastoni</i>	24.8 \pm 17.7	14.0 \pm 12.7
<i>E. tenax</i>	7.1 \pm 7.7	11.6 \pm 7.8
<i>S. pyrastris</i> *	3.4 \pm 3.6	13.4 \pm 5.7
<i>C. croceus</i>	5.0 \pm 5.4	2.4 \pm 2.2
<i>H. maderensis</i> *	56.5 \pm 28.8	20.1 \pm 12.2
<i>T. dugesii</i> *	0.1 \pm 0.3	9.1 \pm 13.1
Bees (5 spp.)	152.9 \pm 123.4	95.2 \pm 65.6
Hoverflies (4 spp.)*	14.0 \pm 12.2	31.3 \pm 14.7
Butterflies (2 spp.)*	62.6 \pm 32.8	23.5 \pm 14.5

3.3. Plant characteristics and isolation as drivers of flower visitation rates and network metrics

The number of flowers per plant and the average size of the inflorescences proved to be a good predictor of the diversity of pollinators (SR, Number of visits and EShan), as well as with visitation network properties (Table 3.2). These variables were positively related with SR, Number of visits and EShan, with EShan also showing a negative relation with isolation (Table 3.2). Concerning the species level network metrics, Species strength, Normalised degree and Weighted closeness were positively related with the number of flowers per plant and the average size of the inflorescences (Table 3.2). Nested rank only showed a small negative relation with the average size of the inflorescences. Despite these relations, the *E. candicans* that showed the highest values of the mentioned variables (E06), except in the case of Nested rank which was the lowest, wasn't the one with the highest number of flowers or size of the inflorescences. Finally, of the Modularity metrics, z was positively related with the number of flowers per plant, and c was not related with any explanatory variable (Table 3.2), being the only of these metrics to diverge between sites ($p < 0.05$).

Of the three insect groups analysed, bees were the only positively related with both the number of flowers per plant and the average size of the inflorescences, while butterflies and hoverflies only responded to average size of the inflorescences and number of flowers per plant, respectively (Table 3.3). Of the ten species selected, six (*A. mellifera*, *A. quadrifasciata*, *B. ruderatus*, *B. terrestris*, *E. tenax* and *S. pyrastris*) had a positive relation with the number of flowers per plant, five species had a positive relation with average size of the inflorescences (*A. mellifera*, *B. ruderatus*, *B. terrestris*, *L. wollastoni*, and *H. maderensis*), and three had a negative one (*A. quadrifasciata*, *S. pyrastris*, and *T. dugesii*) (Table 3.3). Only three species (*A. quadrifasciata*, *E. tenax*, and *T. dugesii*) were less observed on the more isolated plants (Table 3.3). For a single species, *C. croceus*, the number of visits was not related with any of the explanatory variables.

Table 3.2. Association between plant characteristics with the diversity of pollinators and modularity and species level metrics. The standardized regression coefficients are shown jointly with its significance (ns stands for non-significant; *, ** and ***, stand for $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively).

	Plant traits		
	Number of flowers per plant	Average size of inflorescences	Isolation
SR	0.32**	0.36**	ns
Number of visits	0.65**	0.59*	ns
EShan	2.84***	ns	-1.53*
Normalised degree	0.01**	0.10**	ns
Species strength	1.07**	0.77*	ns
Weighted closeness	0.01*	0.01*	ns
Nested rank	ns	-0.02*	ns
z (within-module degree)	0.81*	ns	ns

Table 3.3. Association between the plant characteristics with the number of visits of selected species and groups. The standardized regression coefficients are shown jointly with its significance (ns stands for non-significant; *,** and ***, stand for $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively).

	Plant traits		
	Number of flowers per plant	Average size of inflorescences	Isolation
TOTAL (51 spp.)	0.65**	0.59*	ns
<i>B. ruderatus</i>	0.68**	0.83***	ns
<i>B. terrestris</i>	1.09**	0.97*	ns
<i>A. mellifera</i>	ns	2.62***	ns
<i>A. quadrifasciata</i>	1.79***	-1.42*	-2.05***
<i>L. wollastoni</i>	ns	0.93**	ns
<i>E. tenax</i>	1.18***	ns	-0.67*
<i>S. pyrastris</i>	0.97***	-1.20***	ns
<i>H. maderensis</i>	ns	0.99***	ns
<i>T. dugesii</i>	ns	-2.07*	-3.39*
Bees (5 spp.)	0.89***	0.90***	ns
Hoverflies (4 spp.)	1.13***	ns	ns
Butterflies (2 spp.)	ns	0.95***	ns

3.4. Differences in the behaviour of flower visitors

Of all behaviour variables, the visitation rate was the one that showed the largest differences between the studied species (Figure 3.5). Three of the larger bees (*A. quadrifasciata*, *A. mellifera* and *B. ruderatus*) had a significantly higher visitation rate than most all the other pollinators. Only *B. terrestris* visitation rate did not differ significantly from the other three large bees and hoverflies (Figure 3.5). The four larger bees, as well as the hoverfly *S. pyrastris*, visited a significantly higher number of flowers than *C. croceus*, *L. wollastoni* and *T. dugesii* (Table 3.4). Looking at the number of inflorescences visited, once again the four larger bees and *S. pyrastris* visited a significantly higher number than the butterfly *H. maderensis* and *T. dugesii*. The number of inflorescences visited by *A. mellifera* and *A. quadrifasciata* was also higher than *C. croceus*, as well as the number visited by *A. quadrifasciata* compared to *L. wollastoni* (Table 3.4). Finally, the time spent on the plant was only significantly higher for *S. pyrastris*, when compared to *A. quadrifasciata* and *C. croceus* (Table 3.4).

Table 3.4. Differences in the behaviour of selected flower visitors of *Echium candicans*. Data are presented as mean \pm SD.

Species	Visitation Time (seconds)	Number of Inflorescences visited	Number of Flowers visited
<i>A. quadrifasciata</i>	118 \pm 100	11.7 \pm 8.9	79.8 \pm 71.1
<i>A. mellifera</i>	237 \pm 193	10.1 \pm 7.3	87.3 \pm 68.9
<i>B. ruderatus</i>	149 \pm 100	7.5 \pm 5.2	68.1 \pm 45.4
<i>B. terrestris</i>	216 \pm 177	6.7 \pm 5.8	66.1 \pm 60.6
<i>L. wollastoni</i>	150 \pm 129	4.1 \pm 3.7	9.8 \pm 9.3
<i>C. croceus</i>	129 \pm 120	3.4 \pm 2.4	9.6 \pm 8.5
<i>H. maderensis</i>	248 \pm 207	2.1 \pm 1.2	23.8 \pm 18.7
<i>E. tenax</i>	292 \pm 244	4.5 \pm 2.4	31.1 \pm 26.3
<i>S. pyrastris</i>	328 \pm 206	7.2 \pm 4.4	45.9 \pm 22.0
<i>T. dugesii</i>	142 \pm 114	2.0 \pm 1.3	8.1 \pm 6.0

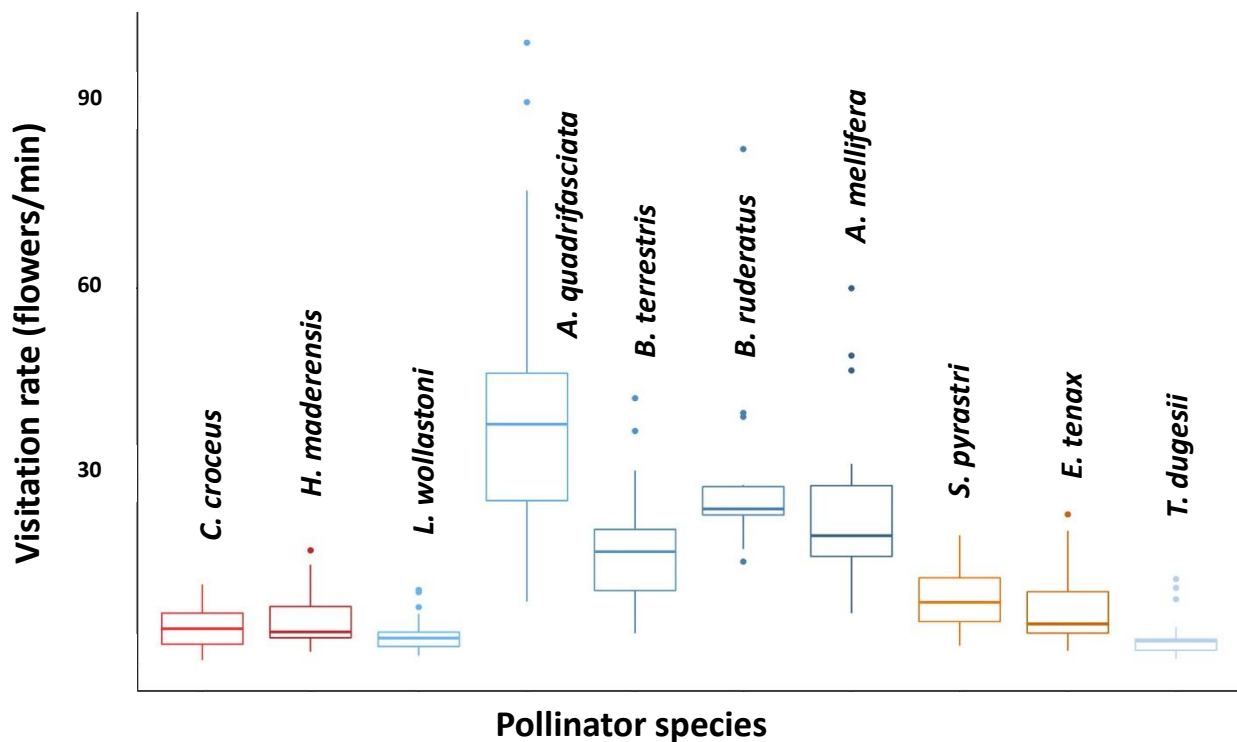


Figure 3.5. Visitation rate of the ten species selected for the behaviour study. Data are presented as boxplots with descriptive values (minimum, first quartile, median, third quartile, maximum) and outliers. The different colour gradients represent distinct pollinator groups (butterflies in red, bees in dark blue, hoverflies in yellow and the lizard in light blue). The boxplots were built using R package “ggplot2” (Wickman, 2016).

3.5. Flower visitors in the study area

Of all the main groups of flower-visitors (bees, hoverflies and butterflies), bees were the only one with species (five) in the community that were not observed on *E. candicans* (Figure 3.6a). One of those species, *Megachile versicolor*, was quite common on the flowers of other plant species, particularly in Asteraeae (Appendix 4). In contrast, *E. candicans* were visited by two species of butterflies and two of hoverflies that were not observed on the surrounding heterospecific flowers (Figures 3.6b and 3.6c). Furthermore, some species (the bee *Halictus frontalis*, the beetle *Psilothrix illustris*, the hoverfly *Sphaerophoria scripta*) were also more commonly observed on plants other than *Echium candicans* (Appendix 4).

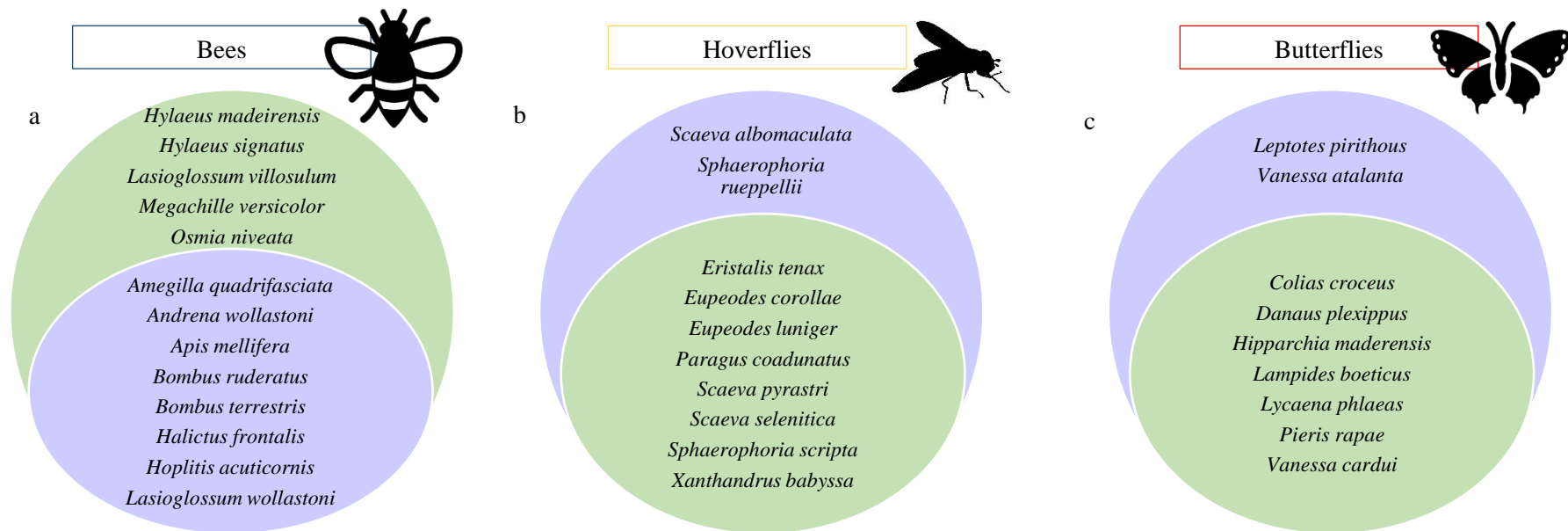


Figure 3.6. Species captured by the different sampling methodologies. Purple represents the ones found on *Echium candicans*, while green represents those sampled by transects plus pan-traps in the surrounding community. The upper part of each figure represents the species sampled by only one of the sampling strategies while the lower part is common to both sampling strategies. Data is presented for bees (a), hoverflies (b) and butterflies (c).

4. Discussion

4.1. The *Echium candicans* pollinators

The flowers of *E. candicans* are visited by different pollinator groups, including butterflies, hoverflies and vertebrates, but bees were the most commonly observed pollinators. This was expected, as bees have been commonly reported as abundant flower-visitors on both Madeiran and Canarian *Echium* (Dupont and Skov, 2004, Valido and Olesen, 2010, Kratochwil and Schwabe, 2018, Jaca et al., 2019b, Kratochwil et al., 2019a, Valido et al., 2019), with these plants being important providers of both pollen and nectar resources (Dupont et al., 2003, Kratochwil and Schwabe, 2018). Interestingly, *E. candicans* showed an equivalent number of bee visitors (8 species) (Appendix 1) compared to previous studies on the Canarian *E. wildpretii* and *E. simplex* (Valido et al., 2002, Jaca et al., 2019b), but these numbers seem to reflect the poor diversity of bees on Macaronesian islands, especially in the Madeiran case, due to filtering effects (Borges et al., 2008, Arechavaleta et al., 2010).

It is also important to mention that the bumblebee *Bombus ruderatus*, the most commonly observed species in our study, may have been introduced in Madeira (Kratochwil et al., 2018). Despite having been described from specimens collected in Madeira (Fabricius, 1775), *Bombus ruderatus* is potentially introduced in the Azores, and it is considered introduced in Tenerife, Canary Islands (Pérez and Macías-Hernández, 2012, Weissmann et al., 2017). So, a potential introduction of *Bombus ruderatus* in Madeira from the Iberian Peninsula, where it also occurs, cannot be discarded (Kratochwil et al., 2018). So far there are no recorded impacts of this species on native plants and pollinators on Macaronesia archipelagos (Pérez and Macías-Hernández, 2012, Kratochwil et al., 2019a), in contrast with other areas where it was introduced (e.g. Morales, 2007). Although in Madeira *Bombus ruderatus* has been mostly observed on Fabaceae, some records have also been made in *Echium nervosum* (Kratochwil et al., 2018). Nevertheless, our findings showed that *Bombus ruderatus* did not seem to affect the presence and abundance of other pollinator species that co-visited the *E. candicans* plants.

Another potentially problematic species is the European honeybee *Apis mellifera*. The impact of the European honeybee has been widely studied on many archipelagos (e.g. Kato et al., 1999, Olesen et al., 2002, Valido et al., 2019), with studies demonstrating its the negative effects on native pollination networks structure and composition (e.g. Valido et al., 2019). Despite this, in our study the presence of the honeybee did not seem to affect the number of visits by other pollinators in the same plants. Further studies are needed to assess the ecological impact of both *A. mellifera* and *B. ruderatus* on Madeira plant-pollinator communities.

In contrast with Jaca et al. (2019b), who found a high abundance of beetles (Coleoptera) on *E. simplex*, we did not record either a high diversity or abundance of this order on the flowers of *E. candicans*. On the other hand, both hoverflies and butterflies (particularly *Hipparchia maderensis*) were found in higher diversity and abundance, when compared to the findings from the Canarian *Echium* (Valido et al., 2002, Jaca et al., 2019b, Valido et al., 2019). So far, hoverflies and butterflies have been considered of minor importance for Macaronesia *Echium*, with bees being considered the most important pollinators (Dupont and Skov, 2004, Valido et al., 2002, Jaca et al., 2019b). However, a recent study already documented another Madeiran endemic, *Echium nervosum*, as an important plant for butterflies in Porto Santo (Kratochwil et al., 2019b).

Our community sampling scheme (transects plus pan-trapping) showed that the community of pollinators present in Pico do Arieiro is quite diverse. Furthermore, despite being less intensively sampled when compared to observations on individual *E. candicans*, it revealed that some species (such as *Halictus frontalis* and *Psilothrix illustris*) do not commonly visit *Echium candicans*, despite being common in the area. This segregation of species is most evident in the case of bees,

where five species present in the area were not recorded on *E. candicans*. This suggests that factors such as pollinator preferences and plant-pollinator trait matching are necessary to interpret plant-pollinator interactions at the community level (Rumeu et al., 2018). By comparing our results with plant-pollinator communities from Tenerife (Canary Islands) that include the endemic *E. wildpretii* (Dupont et al., 2003, Valido et al., 2019), it is clear that, like its Canarian counterpart, *E. candicans* is visited by many of the recorded species in the area. Thus, our work clearly highlights the important role of *E. candicans* as a food resource for many insect groups at higher altitudes in Madeira. In addition, a larger number of insect groups visit *E. candicans* in the more humid Madeiran mountain habitats than on the high-altitude drier habitats of *E. wildpretii* on Tenerife (Valido et al., 2002, 2019). However, we can't exclude a possible effect from differences between sampling, as the Canarian studies sampled pollinators at multiple times during the flowering season (Dupont et al., 2003, Valido et al., 2019). This bigger picture of the pollinator community of *E. wildpretii* may include a higher diversity of species, compared to the one present at different periods during the flowering season. In contrast, the sampling done in *E. candicans* shows a smaller time window, and a more distributed sampling could capture a higher diversity of pollinators. Thus, variation in flower visitors between Macaronesian *Echium* species may reflect the differences on the pollinator species pool at spatial and temporal scales as well as pollinator species preferences.

Like on other *Echium* (*E. wildpretii* and *E. simplex*) (Valido et al., 2002, Jaca et al., 2019b, Valido et al., 2019), reptiles were also observed visiting *E. candicans*. The presence of the Madeiran lizard was expected, as this reptile is a well-known flower-visitor of many plants, including *Echium nervosum* (Elvers, 1977, Olesen et al., 2012b). Not only this species was the first insular lizard ever to be proposed as a potential pollinator (Elvers, 1977), as it has also been reported visiting flowers of different plant species in Madeira archipelago (M. Boeiro, pers. communication) and Selvagem Grande (F. Aguilar, pers. communication), as well as in the Azores, where it was introduced (Olesen et al., 2012b). In contrast with the observations of the Canarian lizard *Gallotia galloti* on Canary *Echium* (Valido et al., 2002, Jaca et al., 2019b), the Madeiran lizard showed a high frequency of visitation of *E. candicans*. This might be due to the small size of the Madeiran species when compared with *G. galloti* (Sadek, 1981, Martín, 1985), allowing it to better climb the *E. candicans* inflorescences. Accordingly, the Canarian lizards occasionally observed on *E. wildpretii* were juveniles (Valido et al., 2002). The density of *Teira dugesii* near Pico do Arieiro is also very high (Koleska et al., 2017), which may explain their higher frequency on the inflorescences of *E. candicans* in S2. However, most *E. candicans* visited by these lizards were close to rocks and walls, which the reptiles use for shelter and thermoregulation. So, the overall importance of the Madeiran lizard as a pollinator of *E. candicans* is probably reduced when compared to insects, which are frequent and abundant visitors on virtually all the plants.

4.2. Individual-based network analysis

Many studies have found an effect of plant traits on the diversity and abundance of pollinators visiting plants' flowers (e.g. Klinkhamer and Jong, 1990, Valido et al., 2002, Stang et al., 2006), and the same pattern has been found on some individual-based pollination network studies (Dupont et al., 2011, Kuppler et al., 2016). According to our expectations, the diversity and abundance of pollinators of *E. candicans* were associated with the number of flowers and size of the inflorescences of individual plants, and these plant traits had already been documented as important predictors of pollinator visitation of the Canarian *E. wildpretii* (Valido et al., 2002). Plant traits related with attractiveness and reward for pollination services, such as the number of available flowers, nectar quantity and quality, and the spatial distribution of those resources seem to drive plant-pollinator interactions in many different genera (e.g. Klinkhamer and Jong, 1990, Valido et al., 2002, Stang et al., 2006, Dupont et al., 2011, Kuppler et al., 2016).

Plants that have a large flower display commonly act as hubs in networks (e.g. Dupont et al., 2003, 2014). This was clearly observed in our results, with Normalised degree and Species strength increasing with the average number of flowers and the size of inflorescences of each plant. As these metrics are related with the number of partners and the average importance that *E. candicans* have for their pollinators, respectively (see Table 2.1), this shows the positive effect that flower display has on pollinator preferences. In fact, plants with more flowers overall attracted higher numbers of bees and hoverflies. Also, large inflorescence size seems to drive higher visitation by bees (*Bombus* spp, *Apis mellifera* and *L. wollastoni*) and butterflies (*H. maderensis*) while only two insects, *A. quadrifasciata* and *S. pyrastris*, and the Madeiran lizard showed a negative relation with the size of the inflorescences of the *E. candicans* they visited.

Weighted closeness, which indicates the centrality of individual *E. candicans* was also positively associated with the size of the inflorescences. Plants that occupy more central positions seem to receive not only higher quantities of pollen but also a higher diversity of pollinators compared with conspecifics, thus being more generalists in their interactions (González et al., 2010, Gómez and Perfectti, 2011). This pattern is confirmed by the increase of z with the number of flowers, as this metric shows how linked an individual *E. candicans* is to others in the same module. Furthermore, having a central position in a network has been shown to be related with higher plant individual fitness (Gómez and Perfectti, 2011).

In contrast with other metrics, Nested rank was negatively related with the size of the inflorescences of the *E. candicans*, despite not being correlated with the number of flowers. This plant trait has been shown to positively influence pollinator attraction in Canarian monocarpic *Echium wildpretii* (Valido et al., 2002). As lower values of Nested rank are related with a higher generalisation of the plant on pollinators (see Table 2.1), it is expected that taller plants or those with larger inflorescences will have lower values, as they tend to attract a more diverse community of pollinators (Valido et al., 2002, Dupont et al., 2011).

Following the classification proposed by Olesen et al (2007), in our study, no individual *E. candicans* revealed a value of z high enough to be considered a module hub and only five plants (E08, E11, E13, E14 and E24) had a c value high enough to be considered connectors (Appendix 2 and 3). This might be due to the fact that the modules obtained mirror the distribution of the plants between the sampling sites, as well as within S1 (Appendix 5), which might explain the higher c values of S1 plants. In a recent study, carried out at a smaller scale than ours, the module organization of a network was related with the spatial distribution of the sampled plants (Dupont et al., 2014). In our study, there was a clear difference between the numbers of particular pollinator groups observed at the two sites (Table 3.1). Overall, butterflies seemed to be more common at S1 (where *H. maderensis* was more abundant) while hoverflies were mostly observed at S2, especially *S. pyrastris*. Despite no differences found for the overall number of bee observations, two species clearly varied between the sites, with *A. mellifera* being mostly observed at S1 while *A. quadrifasciata* was more common at S2. However, these differences did not correspond to an exclusion between the two species at the individual plant level. This probably means that either the populations of these species vary spatially, for example as a consequence of altitudinal segregation, or that the two species prefer different patches of plants to forage. This seems likely, at least in the case of the honeybees, which are good flyers and known to forage as far as 6 km from their nests (Visscher and Seeley, 1982).

Despite the mentioned community differences, in terms of plant traits, the two sites only seemed to diverge significantly in the average size of *E. candicans* inflorescences (Appendix 6). This might explain why the species found in higher numbers at S2 (*A. quadrifasciata*, *S. pyrastris* and *T. dugesii*) showed a negative relation with the average size of the inflorescences (Table 3.3), as the *E. candicans* observed near Pico do Arieiro revealed a significantly lower size compared to the ones at S1. However, a better sampling of plant characteristics and their communities at both sites would be necessary to exclude the possibility of species preference, as other variables that

were not taken into account, such as the number of flowers in near conspecifics, can influence the abundance of particular pollinator species (e.g. Valido et al., 2002).

The negative effect of plant isolation on pollinator visitation was only recorded in three of the most observed species (*A. quadrifasciata*, *E. tenax* and *T. dugesii*) (Table 3.3). In the case *A. quadrifasciata* and *E. tenax* the negative response may be explained by these species having the highest relations with the number of flowers. This might indicate that they prefer patches with a higher overall density of plants, which have been previously reported as being more attractive for pollinators (Klinkhamer and Jong, 1990, Dupont et al., 2011). As for *T. dugesii*, this result might be due to the fact that the *E. candicans* closer to each other were also the ones near the walls where high numbers of these lizards were also present. The negative relation of EShan with isolation seems to indicate that, despite the visitation of most common species observations not decreasing with isolation, this might be happening with some rarer species and groups, like *Polistes dominulus* or *Sphaerophoria scripta*, which represent a much larger number of animal interactions in the network compared to the common species.

It is important to note that the individual that showed the highest diversity of visitors, as well as the highest number of visits (E06), did not show the highest number of flowers or larger size of inflorescences. The extremely high values, showed by this individual, of Normalised degree, Species strength and Weighted closeness, and extremely low in the case of Nested rank, might be related with the isolation of the patch of *E. candicans* that included the plant. The S1 site was mostly composed of groups of plants, in contrast of S2. As some pollinators seem to prefer to forage on patches that have a higher amount of resources overall, this might have enhanced the attractability of the E06 individual.

4.3. Pollinator behaviour comparisons

Large bees such as *Bombus* and *Apis mellifera* have been reported to minimize the distances they travel when foraging (Herrera, 1987, Montaner et al., 2001), preferring to visit close and concentrated patches of floral resources (Klinkhamer and Jong, 1990, Dupont et al., 2004). Our behavioural results showed that large bees (*Amegilla quadrifasciata*, *Apis mellifera* and both *Bombus* species) have a higher visitation-rate to *E. candicans* flowers than most of the other pollinators, with the exception of *B. terrestris* which was similar to both hoverflies (Figure 3.5). Putting together these data with those on pollination networks, where high numbers of large bees were observed when compared to other pollinators, we can conclude that large bees probably provide a higher amount of pollination, like what has been reported for other plants and sites (Herrera 1989, Jaca et al., 2019b). However, we should note that, in terms of the number of visited flowers, hoverflies visited an equally high number when compared with large bees. Hoverflies have shown to be effective pollinators (Fontaine et al., 2006). Despite not being as abundant overall, these data indicate that at least some species might be as important as some large bees, especially in sites where they are equally abundant.

Species that have a higher visitation rate and occur on high numbers, such as *Apis mellifera*, have been shown to reduce plant reproductive success in several plant species (Aizen et al., 2014, Valido et al., 2019), for example by favouring the occurrence of geitonogamy (Dupont et al., 2004). However, another commonly observed pollinator was *Hipparchia maderensis*, and these butterflies spent long times on the flowers they visited (pers. observation), preferring to move between flowers of the same inflorescence (Table 3.4). By spending more time in each flower, this butterfly might have a higher probability of success in collecting and transferring pollen, thus being a more effective pollinator. Another commonly observed species that also showed low visitation rates was the tiny endemic bee *Lasioglossum wollastoni*. Butterflies and smaller sized bees have been associated with better pollination, possibly due to higher levels of xenogamy, in

multiple studies (e.g. Herrera, 1987, Rogers et al., 2013). Some research has suggested that different pollinators might fulfil different roles in the pollination of a particular plant (e.g. Hoehn et al., 2008, Rogers et al., 2013), which might be related with differences in their behaviour between visits (Herrera, 1987, Rogers et al., 2013) and during each visit (Hoehn et al., 2008). Furthermore, abundant species such as *A. mellifera* might contribute with a high number of pollinated flowers (Valido et al., 2019), while other pollinators deliver better quality pollen (Herrera, 1987, Rogers et al., 2013). To confirm this for *E. candicans*, future studies would have to evaluate the individual plant reproductive success, associated with the pollination of specific pollinator species, which should then be compared with their different foraging behaviour.

5. Conclusions

As far as we know, this is the first study of an insular plant-pollinator community using an individual-based design. *Echium candicans* is a key plant species in the Madeira mountain massif, visited by a diverse community of pollinators. The higher abundance of some groups, less found on the better studied Canary Islands species, shows that the importance for different pollinator groups of Macaronesia *Echium* might vary between plant species.

The plants with more flowers (larger reward for pollinators) are particularly important by supporting high numbers and diverse pollinators, mostly generalist insects, like *Bombus* spp., *Amegilla quadrifasciata* and *Eristalis tenax*, as well as the Madeiran lizard. This is also verified for the *E. candicans* with larger inflorescences, which are comparatively the most generalized. The plants with these traits showed a central position in the network, being well connected at least with conspecifics in the same module.

The more commonly observed pollinators showed different visitation rates to the flowers of *E. candicans*, with large bees showing the highest values. However, less abundant pollinators showed intermediate values and visited an equally high number of flowers when compared to large bees, which means they can be equally important in “quantity” of pollination. In contrast, the butterflies and the small bee *L. wollastoni*, have a much lower visitation rate. Despite this, these groups might be associated with higher “quality” of pollination, due to spending more time in the flowers and probably being more effective in depositing pollen.

The current status of *Echium candicans* populations is still unknown, as its habitat has been severely affected by wildfires and the spread of exotic species, such as *Cytisus scoparius*. Because of this, information about the state of its pollinator community is extremely important, as having a diverse and abundant community of pollinators might be vital for plant reproductive success in these populations. The organization of the plant-pollinator community, as well as pollinator preference and behaviour, is crucial information for restoration projects that focus on increasing the number of *Echium candicans* in the Madeira central mountain massif. Further studies are needed to more clearly understand the threats faced by *Echium candicans*, particularly the effects of exotic plants and pollinators, on the reproductive success of this unique plant.

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7. Appendix

Appendix 1. The pollinator species of *Echium candicans* at Pico do Arieiro, their conservation status and the overall number of visits to plants in the two study sites. Source: <https://www.iucnredlist.org>.

Species/Subspecies	Observations	Conservation status	Distribution Status
<i>Amegilla quadrifasciata maderae</i>	316	Least concern	Endemic
<i>Andrena wollastoni wollastoni</i>	110	Least concern	Endemic
<i>Apis mellifera</i>	578	Data deficient	Introduced
<i>Bombus ruderatus</i>	1278	Least concern	Introduced
<i>Bombus terrestris</i>	310	Least concern	Native
<i>Colias croceus</i>	94	Least concern	Native
<i>Danaus plexippus</i>	1	Least concern	Native
<i>Eristalis tenax</i>	216	Not evaluated	Native
<i>Halictus frontalis</i>	11	Least concern	Endemic
<i>Hipparchia maderensis</i>	992	Least concern	Endemic
<i>Hoplitis acuticornis</i>	2	Least concern	Native
<i>Lampides boeticus</i>	3	Least concern	Native
<i>Lasioglossum wollastoni</i>	487	Least concern	Endemic
<i>Lasius grandis</i>	279	Not evaluated	Native
<i>Leptotes pirithous</i>	3	Least concern	Native
<i>Lycaena phlaeas</i>	9	Least concern	Native
<i>Macroglossum stellatarum</i>	3	Not evaluated	Native
<i>Paragus coadunatus</i>	2	Not evaluated	Native
<i>Pieris rapae</i>	1	Least concern	Introduced
<i>Polistes dominulus</i>	144	Not evaluated	Native
<i>Psilothryx illustris</i>	63	Not evaluated	Native
<i>Scaeva albomaculata</i>	1	Not evaluated	Native
<i>Scaeva pyrastris</i>	182	Not evaluated	Native
<i>Scaeva selenitica</i>	21	Not evaluated	Native
<i>Sphaerophoria rueppellii</i>	1	Not evaluated	Native
<i>Sphaerophoria scripta</i>	37	Not evaluated	Native
<i>Stomorhina lunata</i>	6	Not evaluated	Native
<i>Tapinoma madeirense</i>	70	Not evaluated	Native
<i>Teira dugesii</i>	92	Least concern	Endemic
<i>Vanessa atalanta</i>	1	Least concern	Native
<i>Vanessa cardui</i>	8	Least concern	Native
<i>Xanthandrus babyssa</i>	1	Not evaluated	Endemic

Appendix 2. Values obtained for the metrics of each *E. candicans* at the S1 site (- means that the *E. candicans* did not have a z value because it was the only one in its module).

S1 <i>Echium candicans</i>	E01	E02	E03	E04	E05	E06	E07	E08	E09	E10	E11	E12	E13	E14
Normalized degree	0.426	0.298	0.340	0.532	0.362	0.723	0.489	0.340	0.149	0.404	0.404	0.489	0.532	0.234
Species strength	2.72	0.960	1.41	6.10	1.79	12.4	4.71	0.833	0.175	3.59	1.31	6.71	3.85	0.423
Nested rank	0.385	0.846	0.692	0.154	0.615	0	0.308	0.769	1	0.462	0.539	0.231	0.077	0.923
Weighted closeness	0.077	0.049	0.053	0.075	0.078	0.240	0.095	0.027	0.011	0.101	0.044	0.092	0.104	0.026
c	0.606	0.552	0.415	0.508	0.377	0.506	0.412	0.687	0.522	0.610	0.646	0.319	0.707	0.638
z	-0.387	-0.644	-0.389	1.36	-0.162	1.14	1.46	-1.16	-1.03	0.596	-0.596	-	0.559	-0.749

Appendix 3. Values obtained for the metrics of each *E. candicans* at the S2 site.

S2 <i>Echium candicans</i>	E15	E16	E17	E18	E19	E20	E21	E22	E23	E24
Normalized degree	0.564	0.282	0.462	0.359	0.231	0.359	0.564	0.436	0.487	0.359
Species strength	6.96	0.885	5.27	2.63	1.76	2.68	7.03	5.47	5.06	1.26
Nested rank	0	0.889	0.333	0.556	1	0.667	0.111	0.444	0.222	0.778
Weighted closeness	0.128	0.023	0.188	0.160	0.029	0.114	0.127	0.153	0.093	0.083
c	0.409	0.377	0.391	0.368	0.216	0.557	0.337	0.319	0.326	0.634
z	0.820	-1.11	0.313	-0.707	0.707	-0.707	0.806	0.707	0.295	-1.12

Appendix 4. Number of observations and captures of pollinators using the three different sampling methods in the two study sites.

Species	<i>E. candicans</i>	Transects	Pan-traps
<i>Amegilla quadrifasciata</i>	316	10	3
<i>Andrena wollastoni</i>	110	5	1
<i>Anthrax anthrax</i>	0	1	0
<i>Apis mellifera</i>	578	81	6
<i>Bombus ruderatus</i>	1278	39	61
<i>Bombus terrestris</i>	310	45	12
<i>Campiglossa producta</i>	0	6	2
<i>Colias croceus</i>	94	10	7
<i>Danaus plexippus</i>	1	1	0
<i>Dioxyna sororcula</i>	0	2	0
<i>Eristalis tenax</i>	216	23	0
<i>Eupeodes corollae</i>	8	7	0
<i>Eupeodes luniger</i>	5	4	0
<i>Halictus frontalis</i>	11	86	6
<i>Hipparchia maderensis</i>	992	85	12
<i>Hoplitis acuticornis</i>	2	5	5
<i>Hylaeus maderensis</i>	0	2	1
<i>Hylaeus signatus</i>	0	1	0
<i>Lampides boeticus</i>	3	2	1
<i>Lasioglossum villosulum</i>	0	8	4
<i>Lasioglossum wollastoni</i>	487	15	56
<i>Lasius grandis</i>	279	0	0
<i>Leptotes pirithous</i>	3	0	0
<i>Lycaena phlaeas</i>	9	9	1
<i>Macroglossum stellatarum</i>	3	0	0
<i>Megachile versicolor</i>	0	27	13
<i>Osmia niveata</i>	0	0	4
<i>Paragus coadunatus</i>	2	3	0
<i>Pieris rapae</i>	1	0	2
<i>Polistes dominulus</i>	144	4	6
<i>Psilothryx illustris</i>	63	87	35
<i>Scaeva albomaculata</i>	1	0	0
<i>Scaeva pyrastris</i>	182	14	0
<i>Scaeva selenitica</i>	21	4	0
<i>Sphaerophoria rueppellii</i>	1	0	0
<i>Sphaerophoria scripta</i>	37	44	3
<i>Stomorhina lunata</i>	6	0	0
<i>Tapinoma madeirense</i>	70	4	0
<i>Teira dugesii</i>	92	0	0
<i>Trupaneia insularum</i>	0	2	0
<i>Vanessa atalanta</i>	1	0	0
<i>Vanessa cardui</i>	8	0	1

<i>Xanthandrus babyssa</i>	1	3	0
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Appendix 5. Modules obtained for the studied *E. candicans* community.

Obtained Modules	1	2	3	4	5
Studied <i>E. candicans</i>	E02, E03, E04, E05, E07, E08, E09, E11	E19, E20	E1, E6, E10, E13, E14	E15, E16, E17, E18, E21, E22, E23, E24	E12

Appendix 6. Plant traits collected from the 24 *E. candicans*.

PlantID	Height (cm)	Diameter (cm)	Average size of inflorescences (cm)	Number of flowers	Isolation (m)
E01	146	284	27.2	39718	17.2
E02	108	119	23.4	1024	1.1
E03	96	130	29.4	4190	3.3
E04	131	181	31.6	6624	0.2
E05	110	150	31.8	9484	4.4
E06	134	298	27.6	19427	1.0
E07	80	130	24.0	7360	9.0
E08	86	143	28.3	650	5.4
E09	90	67	25.7	295	9.3
E10	190	340	27.2	15456	5.7
E11	108	162	34.8	2120	7.3
E12	173	257	31.4	16341	5.7
E13	159	266	31.0	10251	0.4
E14	114	94	21.6	1121	0.4
E15	240	420	23.2	54912	1.6
E16	91	88	21.2	1258	1.8
E17	101	211	21.6	40016	1.0
E18	146	236	21.8	16854	1.3
E19	77	103	15.0	946	6.0
E20	119	143	16.4	4180	11.0
E21	166	255	16.6	31304	5.3
E22	143	258	22.8	43738	7.8
E23	158	319	18.0	42990	2.0
E24	79	84	16.6	1229	0.4