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Mgr. Kristýna Vazačová

EVOLUCE VLASTNOSTÍ ROSTLIN TÝKAJÍCÍCH SE JEJICH SCHOPNOSTI DISPERZE

THE EVOLUTION OF DISPERSAL TRAITS IN PLANTS

Disertační práce

Školitelka/Supervisor: Doc. RNDr. Zuzana Münzbergová, Ph.D.

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Table of contents

Abstrakt

Tato disertační práce je zaměřena na studium evolučního potenciálu vlastností rostlin týkajících se jejich schopnosti disperze na ostrovech jako analogie fragmentovaného systému. Toto téma jsem zkoumala na několika úrovních. Za prvé jsem srovnala vlastnosti mezi blízce příbuznými endemickými a neendemickými druhy za účelem zjištění změn v jejich schopnosti se šířit (**článek 2**). Za druhé jsem hledala vlastnosti předurčující schopnost druhů kolonizovat ostrovy a přežívat na nich (**článek 3**). Za třetí jsem se soustředila na studium fylogeneze vlastností týkajících se schopnosti šíření a přežívání na ostrovech u skupiny druhů vzniklých adaptivní radiací (**článek 4**). Práce byla prováděna na druzích Kanárských ostrovů. Vlastnosti týkající se schopnosti disperze byly zjišťovány přímo prostřednictvím disperzních experimentů zaměřených na anemo-, hydro-, exo- a endozoochorii (**články 2, 3, 4**). V **článku 4** byly také měřeny morfologické parametry semen. Metodika k endozoochorii byla vytvořena v **článku 1**.

Srovnání vlastností týkajících se schopnosti šíření mezi endemickými a neendemickými druhy (**článek 2**) přímo nepotvrdilo hypotézu o ztrátě schopnosti šířit se u ostrovních endemitů. To ukazuje, že tuto hypotézu nelze zcela generalizovat. V mnoha případech se endemité vyznačovali stejnou nebo lepší schopností disperze než neendemičtí příslušníci stejného rodu. Udržování schopnosti se šířit je zřejmě spojeno s dalším šířením mezi ostrovy, neboť bylo zjištěno, že endemité jsou rozšířeni na více ostrovech než neendemité.

Článek 3 se zabýval významem vlastností spojených se schopností disperze pro šíření mezi ostrovy a kolonizaci nových stanovišť. Srovnání vlastností blízce příbuzných druhů lišících se v rozšíření mezi ostrovy odhalilo, že žádná z vlastností týkajících se schopnosti disperze není sama osobě schopna vysvětlit rozšíření druhů na ostrovech. Přítomnost druhů na ostrovech je výsledkem kombinace dobré schopnosti disperze, vlastností spojených se schopností druhů přežít na lokalitě (velikost semen, délka životního cyklu) a hojnosti druhů na ostrovech.

Evoluční potenciál těchto vlastností byl studován v **článku 4**. Jako modelová skupina druhů byl vybrán subtribus Sonchinae, který diverzifikoval v mnoho růstových forem a který se dále liší v rozšíření na ostrovech a dimorfismu chmýru. Výsledky testování fylogenetického signálu ukázaly, že vlastnosti spojené s přežíváním a určující rozšíření druhů jsou více fylogeneticky „konzervované“ než vlastnosti týkající se schopnosti disperze. Také jsem zjistila, že existence fylogenetického signálu může být zastřena rychle a snadno

probíhajícími evolučními změnami, jak bylo zjištěno v případě délky chmýru, která vykázala velkou morfologickou variabilitu. Naopak přímo měřené disperzní vlastnosti ukázaly nízkou variabilitu, což poukazuje na vysokou míru nikového konzervatismu.

Výše uvedené výsledky ukázaly, že morfologické znaky semen jsou lepším prediktorem disperze než přímo měřené disperzní vlastnosti. Dále lze říci, že vlastnosti spojené s disperzí semen dosahují menšího významu než vlastnosti týkající se přežívání a vlastnosti určující rozšíření druhů. Disperzní vlastnosti je tedy vhodné studovat v kontextu dalších vlastností spojených s přežíváním druhů a determinantů jejich rozšíření, neboť všechny tyto vlastnosti a determinanty se spolupodílejí na schopnosti druhu kolonizovat stanoviště a přežívat tam. Závěry této práce jsou potenciálně aplikovatelné na další fragmentované systémy včetně fragmentované krajiny na pevnině.

Abstract

This thesis focused on evolutionary potential of dispersal traits of species in fragmented system represented by oceanic islands. I aimed to look on this topic on different levels. First, I compared traits between closely related endemic and non-endemic species to test for the existence of changes in their dispersal ability (**Paper 2**). Second, I searched for the traits predetermining species ability to colonize islands and to persist there (**Paper 3**). Third, I focused on the evolution of traits related to dispersal and persistence by inferring phylogenetic history of species group arising by adaptive radiation (**Paper 4**). All the objectives were explored on species of the Canary Islands. Dispersal traits were tested as direct dispersal abilities by anemo-, hydro-, exo- and endozoochory (**Paper 2, 3, 4**), in **Paper 4** we also measured seed morphological parameters. The methodology for endozoochorous dispersal was developed in **Paper 1**.

The comparison of dispersal traits between endemic and non-endemic species (**Paper 2**) did not explicitly support the hypothesis about the loss of dispersal ability in island endemics showing that reduction of dispersal ability on islands may not be as general as previously expected. In many cases, endemic species had the same or better dispersal ability than their non-endemic congeners. I suggest that maintenance of good dispersal ability is probably related to species subsequent dispersal among islands as endemic species dispersing better than their non-endemic congeners were reported to occupy more islands within the archipelago.

The importance of dispersal traits for further species dispersal among islands and colonization of new habitats was examined in **Paper 3**. The comparison of traits o closely related species differing in their distribution among islands revealed that no dispersal trait alone can explain the distribution of species among islands. Species presence on islands is the result of combination of both good dispersal ability and traits related to species ability to persist at the locality (e.g. seed size, longevity) as well as abundance of the species in the archipelago.

The evolutionary potential of these traits (**Paper 4**) was examined on subtribe Sonchinae, which has experienced a great radiation in traits related to species persistence (e.g. growth habit, woodiness), in distribution as well as in pappus dimorphism. The results of testing for phylogenetic signal of traits revealed that traits related to species persistence and determinants of distribution are more phylogenetically conserved than dispersal related traits. I also showed that the existence of phylogenetic signal can be constrained by rapid and easy

evolutionary changes as it was demonstrated in the case of pappus length showing high variability in morphology. In contrast, the directly measured dispersal traits showed low variability indicating high level of niche conservatism.

Overall, direct dispersal traits showed fewer patterns and had lower variation than morphological traits related to species dispersal. In addition, all the dispersal traits seem to be of lower importance than traits describing species persistence and distribution. All this indicates that dispersal traits should be studied in the context of other traits related to species persistence and distribution as all the traits act in species colonization ability and its long-term survival on habitats. The conclusions of this thesis are potentially applicable to other fragmented systems including fragmented systems on the mainland.

General introduction

Understanding species traits responsible for species distribution and persistence on habitats is one of the major challenges of present-day ecology, especially in fragmented landscape where populations are isolated from each other (Hanski 1999, Saunders et al. 1991). Dispersal ability is thought to be one of the key factors responsible for species distribution on such habitats, as it maintains habitat connectivity by exchange of genes among populations and re-colonization of empty patches (Hanski 1999, Ozinga et al. 2009, Riba et al. 2009). Many studies have evidenced a close relationship between habitat occupancy and species dispersal ability (e.g. Ackerman et al. 1996, Ehrlén and Eriksson 2000, Eriksson 1996, Tremlová and Münzbergová 2007) indicating that traits such as terminal velocity or seed size may be important determinants of species response to habitat size and isolation. Furthermore, Alados et al. (2009) and Navarro et al. (2009) showed in highly fragmented landscape that species with good dispersal ability are less sensitive to fragmentation than species with restricted dispersal.

Understanding species dispersal ability in highly variable landscape is important not only for predicting species presence in fragmented habitats, but also for inferring species ability to respond to ongoing habitat changes. The knowledge on evolutionary dynamics of dispersal traits can be thus crucial for predicting species ability to disperse among fragmented patches to escape from unsuitable habitats or to colonize newly emerged ones.

Changes in landscape structure in combination with climate change are the major threats to species diversity in many parts of the world (Solé et al. 2004, Tilman 1994, Travis 2003) and understanding the mechanisms of species ability to respond to these changes is thus a great challenge for our ability to protect diversity in the landscape in the future (Alados et al. 2009). Previous studies have shown that fragmented habitats exert strong selection pressure upon dispersal traits of the plant species (Blanquart et al. 2014, Bourne et al. 2014). While there are many theoretical studies predicting evolutionary response of species dispersal ability to changes in landscape structure (e.g. Cunze et al. 2013, Hargreaves and Eckert 2014, Hughes et al. 2007), empirical evidence to test these predictions is scarce. In one of the few studies on this topic, it has been shown that plants can respond to such pressure by reducing or increasing dispersal due to their adaptive plasticity. This could be done e.g. by producing different types of diaspores as it was shown for *Rumex bucephalophorus* by Talavera et al. (2012) and for *Crepis sancta* by Cheptou et al. (2008) in patchy environments. Deeper knowledge on the evolutionary potential of dispersal traits under changing environment is, however, still limited. Specifically, there is little information which dispersal related traits are phylogenetically conserved and how fast the plants are able to respond to environmental pressures.

As obtaining direct evidence on evolutionary change in a specific trait is difficult, indirect approaches are often used to fulfill this aim. One of the possibilities to do this is to infer the evolutionary history of different traits from species phylogenetic history (see Ackerly et al. 2000, Alcantara and Lohmann 2011, Kembel and Cahill 2005, Pagel 1997). Nowadays, with the advance of various molecular techniques it is possible to obtain well-supported phylogenetic trees, which can be used as a basis for testing for phylogenetic signal of traits, i.e. to inspect whether ecological similarity of species is related to their phylogenetic relatedness (Blomberg et al. 2003). By this approach we can separate phylogenetically conserved (stable within clades in spite of possible variation of environment) and non-conserved (consistent within environments and independent of clades) traits (Ackerly 2009, Schmitt and Riviere 2002). Such approach has been already applied to traits related to plant morphology (Alcantara and Lohmann 2011, Harder and Barrett 2006, Nogueira et al. 2012), ecology (Ackerly et al. 2000, Cornwell and Ackerly 2009, Mayfield et al. 2009, Prinzing 2001) and plant fitness (Herben et al. 2014, Verdú and Traveset 2005). In contrasts, studies exploring the evolution of species dispersal traits are relatively scarce (Barker et al. 2004, Herrera 2002, Hollander et al. 2010, Talavera et al. 2012). The importance of such studies can be evident e.g. from paper by Hollander et al. (2010), who demonstrated on phylogeny of genus *Ephedra* that new means of dispersal can evolve from different, ancestral forms of seed dispersal.

Most researchers observing the evolutionary changes in dispersal traits use easy to measure morphological traits such as pappus length or seed surface as a proxy of dispersal abilities (e.g. Bernardello et al. 2006, Cody and Overton 1996, Darling et al. 2008, Hollander et al. 2010, Hughes et al. 1994, Lavergne et al. 2004). It was, however, repeatedly shown, that changes in morphological parameters cannot be easily translated into real dispersal distances (Anderson 1993, Willson et al. 1990). As Sheldon and Burrows (1973) demonstrated on 18 species of the family Compositae, the differences in dispersal ability are given more likely in fine details of diaspore morphology such as the possession of hairs on achenes which are, however, not easily measurable. In fact, species with no apparent morphological adaptation can be also successfully dispersed (Couvreur et al. 2004, Fischer et al. 1996, Heinken and Raudnitschka, 2002 and Vargas et al. 2012). Moreover, in contrast to morphological parameters, direct determination of diaspore dispersal ability enables the estimation of the dispersal ability of all species by multiple vectors reducing the risk of overlooking an important dispersal agent in each particular species. In spite of this, studies on direct dispersal traits measuring real dispersal distances are not so common (Darling et al. 2008, Fresnillo and Ehlers 2008, Kudoh et al. 2013, Riba et al. 2009), although the methodology for estimating direct dispersal abilities is relatively well-elaborated, especially for some dispersal modes (e.g. anemochory, Tackenberg et al. 2003, or hydrochory, Knevel et al. 2005).

Studies exploring species response to habitat changes often show that species response is not related only to current habitat changes, but also to the changes, which happened in the past (Chýlová and Münzbergová 2008, Saar et al. 2012). Strong species response to past landscape structure can be attributed to slow growth dynamics of many perennial species in combination with relatively fast changes in the current landscape (Linborg 2007, Purschke et al. 2012). Due to dispersal limitation (Herben et al. 2006, Münzbergová et al. 2013) and extinction debt (Hylander and Ehrlén 2013, Piqueray et al. 2011) the distribution of these species may reflect historical habitat configuration instead of their long-term ability to successfully colonize habitats and to survive there. As a result the traits identified as the major determinants of species presence at the locality can be different from those in more stable landscape, which remains fragmented for longer evolutionary time (Parisod and Bonvin 2008, Parisod and Christin 2008).

Due to human intensive activity it is rather difficult to identify habitat fragments, which remained unchanged for a long time period, on which we could study species ability to colonize new habitats and to evolve there on long time scales. Suitable candidates of such systems are habitats which are naturally fragmented such as mountain tops or oceanic islands. In contrast to continental landscape, such fragments are not a result of human activity and remained almost unchanged in size and number since their origin. Thus the islands are generally thought to be more stable in time as they are fragmented and isolated for much longer time periods. Analogy between oceanic islands and continental fragments comes from island biogeographic theory and has its supporters (e.g. Cook et al. 2002, Haila 2002, Watson 2002) as well as its opponents (e.g. Laurance 2008). The relevance of such a theory for understanding fragmented ecosystems has been criticized due to the fact that island biogeographic theory does not consider e.g. changes of community composition in fragments over time or ignore edge effects or effects of vegetation in surrounding matrix (Laurance 2008). In contrast, other studies do not attribute such relevancy to these aspects. For instance, in study by Prevedello et al. (2010), patch size and isolation were shown to be more important determinants of species biodiversity than matrix type. Regarding dispersal, Riba et al. (2009)

demonstrated on *Mycelis muralis* that similar pattern of the decline of wind dispersal ability of species presented by Darwin on islands (so called “Darwin´s wind hypothesis”) is also visible on fragmented habitats on the mainland.

Dispersal and evolution on oceanic islands

Oceanic dispersal is thought to be fundamental to biogeography and the evolution of biodiversity on oceanic islands (Cowie and Holland 2006). Oceanic islands were never connected to larger land masses, thus the species persisting on islands were able to disperse there or evolved from ancestors which were able to colonize islands from the mainland.

Species on islands are expected to undergo selection against dispersal to reduce the risk of loss of seeds in the sea (Carlquist 1965). According to this hypothesis, it could be predicted that selection will favor species with propagules of lower dispersability than was the dispersability of their ancestors when colonizing the islands from the continent. Although the direct continental ancestors may have gone extinct, the reduction of dispersability can be studied from comparison of dispersal abilities between island endemics and their continental relatives as it was shown e.g. by Carlquist on morphological parameters of species of *Bidens* (Carlquist 1966). In contrast, studies looking at this phenomenon by comparing direct dispersal traits are almost lacking. The results of existing papers (e.g. Fresnillo and Ehlers 2008, Kudoh et al. 2013) indicated that the reduction of dispersal abilities visible on morphological parameters is on some species detectable also on directly assessed dispersal traits. However, for proper survey on this phenomenon it is necessary to involve more species in the study and to take into account different dispersal modes acting on islands including e.g. wind, water and both external and internal bird dispersal.

Oceanic islands are also suitable system for studying the evolutionary potential of dispersal traits as they host large groups of species that have recently undergone speciation. Such process known as adaptive radiation is usually driven by intense competition among closely related species resulting in extensive divergence in morphological as well as ecological traits, but showing yet relatively little divergence in molecular sequences, crossability (Givnish et al. 2004) or other traits (e.g. chromosome number, Ardévol-Gonzáles et al. 1993). They thus provide a unique opportunity to separate evolutionarily conserved traits from those that can change easily in evolution to bring us an information on evolutionary dynamics of such traits.

Studies on island plants arising by adaptive radiation have already explored evolution on changes in growth form (e.g. the Canary Island genus *Aeonium*, Mes and Hart 1996, or

Hawaiian silversword alliance, Robichaux et al. 1990) or in ecophysiological traits (e.g. Hawaiian lobelioids, Montgomery and Givnish 2008). However, studies on adaptive changes in dispersal traits of island species are far less common (but see Givnish et al. 2009, Schenk 2013, Talavera et al. 2012). Givnish et al. (2009) revealed from phylogeny of Hawaian lobeliads, that some fleshy fruits evolved several times probably in consequence of species colonization of rainforest understoreys. The mechanism of repeated evolution of similar dispersal types in consequence of habitat shifts is a good example how species can respond to habitat changes to make dispersal mechanisms more effective.

It can be expected that reduction in dispersal abilities of island species can be accompanied by selection for traits enabling species to persist at a locality. Thus for studying the evolutionary potential of species it is meaningful to observe also traits related to species long-term persistence on islands (e.g. growth habit, woodiness, seed size) as well as determinants of species distribution (e.g. number of islands occupied by a species, number of vegetation zones). As shown by Maurer et al. (2003) and Saar et al. (2012) for calcareous grasslands and Kavanagh and Burns (2014) for islands surrounding New Zealand, at certain conditions (e.g. when fragments are far from each other) traits enhancing persistence can be even more important for occurrence of species on habitats that traits affecting dispersal. In my Ph.D. thesis I called for simplicity all the variables observed as traits, although some of them do not match the exact definition of a trait (e.g., they are not measured at the individual level or independently of environmental conditions, Violle et al. 2007).

Ph.D. Thesis: study site, aims and results

Study site

My Ph.D. thesis was conducted on species present on the Canary Islands (except for 2 *Sonchus* species from Madeira, **Paper 4**). This Macaronesian archipelago is an ideal system for exploring evolutionary potential of different dispersal traits for several reasons. It is a group of seven major volcanic islands which have never been connected to the continent, thus their species must have evolved from ancestors which colonized islands by dispersing from the mainland. The islands differ in size and age (Afonso 1988). The age of the islands decreases with increasing distance from the closest mainland (Africa) and from east to west; the easternmost islands are the oldest, while the westernmost the youngest. Species on islands had thus different time to migrate to different islands and to evolve there. The spatial and temporal diversity, the proximity to the mainland (around 100 km from Fuerteventura to Morocco, Juan et al. 2000) and climatic stability are the main factors responsible high percentage of island endemic flora (around 20% from 2100 species, Arechavaleta et al. 2010). The phylogenetic history of the many island species groups is well studied (e.g. Barber et al. 2002, Francisco-Ortega et al. 2002, Goodson et al. 2006, Lee et al. 2005), especially of those which arose by adaptive radiation (e.g. *Aeonium*, Mes and Hart 1996, *Descurainia*, Goodson et al. 2006, *Sonchus*, Kim et al. 2007). All this allows detecting groups of species with known phylogenetic relationships and thus comparing dispersal traits among closely related species.

Even though nowadays some parts of the islands are quite heavily inhabited, we suppose that the main dispersal events happened before human´s strong influence. Our selection of species excluded purely ruderal species and focused on those occurring in some (semi-) natural habitats such as laurel forests and canary pine woodlands. Such communities obviously suffer from human destructive activities (e.g. fragmentation and reduction in area), but species extinctions on single islands occur only rarely and were not reported of any of our studied species (Caujapé-Castells et al. 2010).

Aims of the thesis

In my PhD thesis I studied the evolution of dispersal related traits on islands at various levels. First, I compared traits between closely related endemic and non-endemic species to test for the existence of changes in their dispersal ability (**Paper 2: Dispersal ability of island endemic plants: What can we learn using multiple dispersal traits?**). I decided to use the pairs of endemic and non-endemic species, because I suppose that the reduction in dispersal ability can be visible from comparison of these two types of closely related taxa. Second, I searched for the traits predetermining species ability to colonize islands and to persist there (**Paper 3: The importance of species traits for species distribution on oceanic islands**). To do this I compared species dispersal ability and traits related to persistence and distribution of species pairs differing in their distribution among islands. Third, I focused on the evolution of traits related to dispersal and persistence by inferring phylogenetic history of species group arising by adaptive radiation (**Paper 4: The evolutionary potential of different traits in subtribe Sonchinae**). To do this I used species of subtribe Sonchinae, which were reported to differ in morphological as well as in ecological traits and in their distribution among islands. In this way it is possible to understand the evolutionary changes in species traits both at finer scale within a single group (**Paper 4**), as well as to obtain a coarser comparison that allow us to generate the conclusions to a wide range of species (**Paper 2** and **3**).

To properly encompass all the above mentioned issues, the studied species were tested for direct abilities to disperse by all the four dispersal modes acting in long-distance dispersal on islands: dispersal by wind, water and birds (both exo- and endozoochory). While the methodology for anemo-, hydro- and exozoochorous experiments is relatively well-elaborated, the methodology for bird internal transport of seeds was missing. Thus in the very first step of my PhD study I developed a methodology simulating the seed passage through pigeon gut **(Paper 1: Simulation of seed digestion by birds: How does it reflect the real passage through a pigeon´s gut?**).

Results of the thesis

In **Paper 1** of my PhD thesis, I searched for the best methodology for simulating of seed passage through a pigeon´s gut. Seeds of 20 different Canary Island species were subjected to 7 different simulations consisting of seed scarification and acid immersion and to the real gut passage. The results showed that selecting one type of simulation as a good predictor of seed survival after gut passage is difficult due to the species-specific fit between direct digestion by the pigeon and the different simulation treatments. Still the strongest simulation (24 h-scarification and 240-min acid immersion) was the best predictor of pigeon´s internal dispersal and it was further used as a proxy of endozoochorous dispersal ability for species tested in **Paper 2, 3** and **4.**

In **Paper 2** I compared directly measured dispersal traits of 27 pairs of closely related endemic and non-endemic species. Endemic species are supposed to have experienced reduction in their dispersal potential to prevent their seeds from falling out to the sea. In contrast to similar studies on seed morphological parameters (Carlquist 1966, 1974) the results did not explicitly support the hypothesis about the loss of dispersal ability of island species showing that reduction of dispersal ability of islands may not be as general as previously expected. In many cases, endemic species had the same or better dispersal ability than their non-endemic congeners. I suggest that maintenance of good dispersal ability is probably related to species subsequent dispersal among islands as endemic species dispersing better than their non-endemic congeners were reported to occupy more islands within the archipelago. The theory of evolution of better dispersal in consequence of colonization of other islands is consistent with stepping stones` theory (Darwin, 1859), in which the islands are not merely target areas for dispersal, but they can work as transient sites for dispersal to other subsequently emerging islands (Carlquist, 2009, Harbaugh et al. 2009).

The importance of dispersal traits for species dispersal among islands and colonization of new habitats was examined in **Paper 3**. Within this study I compared the values of dispersal traits among 18 species pairs differing in their distribution within the Canary archipelago. As it was previously demonstrated that species distribution may be influenced also by other traits related to species persistence and abundance, I decided to test also these traits. Due to the close relatedness between examined species and the possibility of sharing similar ecological traits, the tests were applied both with and without phylogenetic correction. The results revealed that no dispersal trait alone can explain the distribution of the species among islands. They, however, also suggest that species with better dispersal compared to their close relatives are better colonizers. Similarly, abundance of species in the archipelago (measured as number of islands) seems to be an important predictor of species colonization ability only when comparing closely related species. All this indicates that both types of analyzes should be combined when testing closely related species to fully understand the importance of various plant traits for species distribution. Contrasting results of analysis with and without phylogenetic correction were found in previous studies for dispersal traits (Tremlová and Münzbergová 2007) or traits related to plant growth (Lanta et al. 2011) and for flower longevity (Stratton 1989).

In **Paper 4** I looked on the evolutionary potential of traits related to species dispersal and persistence to find out which traits are conserved and did not change during evolution and which traits have a potential to evolve and thus can play a key role in species response to landscape changes. For this purpose I chose 25 species of subtribe Sonchinae containing both strictly endemic species having arisen by adaptive radiation (woody *Sonchus* Alliance*,* Kim et al. 1996) and species which are present also on the mainland. The species in the group differ in many traits such as growth habit, woodiness, endemism and distribution. All these traits as well as traits related to dispersal potential (both directly measured traits and seed morphological parameters) were tested for phylogenetic signal. The results showed that traits related to persistence and distribution are more phylogenetically conserved than dispersal related traits. The conservatism of persistence traits such as woodiness and longevity was reported also in other groups arising by adaptive radiation (e.g. *Echium,* Böhle et al. 1996, *Pericallis,* Swenson and Manns 2003). While woodiness of species is supposed to evolve easily and is a typical feature of species arising by adaptive radiation, other traits such as fruit types have not undergone dramatic changes in these species during their evolutionary history (García-Verdugo et al. 2013).

The results also demonstrated that the existence of phylogenetic signal can be constrained by rapid and easy evolutionary changes. This was probably the case of pappus length which showed high variability in the group. The evolutionary potential of pappus length has been repeatedly reported also by other studies including those on oceanic islands (e.g. Carlquist 1966, Cody and Overton 1996).

Summary

The outputs of my Ph.D. study are based on research on oceanic islands, but can be potentially applicable to the fragmented landscape on the mainland, which is to a certain degree analogous to the system of oceanic islands. In contrast to studies conducted on fragmented systems on the mainland, our results do not suffer from unstable history of the fragments and their potential fast changes over time.

The results of my papers surprisingly revealed dispersal traits to be of lower importance than traits describing species persistence and distribution. This could indicate that species in fragmented habitats may be more limited e.g. by the amount of seeds available for dispersal or by availability of suitable habitats and ability to establish than by dispersal ability. The lack of diaspores was probably the reason for higher importance of persistence traits in fragmented landscape represented by calcareous grasslands in Maurer et al. (2003).
There are other scenarios under which the lower importance of dispersal traits can happen.
One possibility is that species dispersal takes place regularly and there is no problem with seed saturation. Alternatively, the patches are so far away from each other that dispersal could

not be effective (Maurer et al. 2003). Regarding Canary archipelago, its proximity to the closest mainland and taking into account the fact that we do not explicitly support the hypothesis about the loss of dispersal ability on islands, the first scenario could be considered as the most probable.

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Paper 1

SIMULATION OF SEED DIGESTION BY BIRDS: HOW DOES IT REFLECT THE REAL PASSAGE THROUGH A PIGEON'S GUT?

Kristýna Vazačová and Zuzana Münzbergová

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Simulation of Seed Digestion by Birds: How Does It Reflect the Real Passage Through a Pigeon's Gut?

Kristýna Vazačová · Zuzana Münzbergová

Abstract Simulation of seed passage through a bird's gut is an important tool for comparing the effect of bird digestion and thus the potential for plant dispersal by endozoochory. However, sufficient methodology is missing. Thus, we subjected seeds of 20 plant species to seven different simulations of gut passage and to the real passage through a pigeon's gut to determine which simulation type best reflects the effects of real bird digestion. We also measured various seed traits to identify the traits responsible for differences between species. Results show that four out of seven simulations were significant predictors of seed survival after gut passage. The fit between direct digestion by the pigeon and the different simulation treatments was, however, species-specific and depends not only on the commonly tested traits such as seed mass and water permeability, but also on other unmeasured traits. Seed mass was the best predictor of differences between real digestion and simulation. Selecting one type of simulation to be a good predictor of seed survival after gut passage is difficult. The strongest simulation (24-h scarification and 240-min acid immersion) is the best predictor and may be used to compare the ability of seeds to be dispersed by bird endozoochory. Such knowledge can be included in databases of species traits, as is currently done for many other species traits.

Keywords Canary Islands · Endozoochory · Scarification · Seed dispersal · Sulphuric acid

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K. Vazačová (✉) · Z. Münzbergová
Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 01 Prague 2, Czech Republic
e-mail: vazacova@seznam.cz

K. Vazačová · Z. Münzbergová
Institute of Botany, Academy of Sciences of the Czech Republic, CZ-252 43 Průhonice, Czech Republic

Introduction

Internal transport of seeds by birds (endozoochory in general) is an important long-distance dispersal mechanism (Ridley 1930; Wilkinson 1997). Besides dispersal itself, endozoochory is thought to enhance seed germination (Spence et al. 1971; Teltscherová and Hejný 1973; Janzen 1983; Willson 1983; Izhaki and Safriel 1990; Yagihashi et al. 1998) due to cleaning of pulp, which may contain germination inhibitors (Evenari 1949; McDiarmid et al. 1977; Izhaki and Safriel 1990; Barnea et al. 1991) or due to abrasion of the seed coat increasing permeability to water and gasses (Barnea et al. 1990; Clergeau 1992; Sánchez et al. 2006).

Endozoochory by birds is generally studied by comparing seeds/fruits that have passed through a bird's gut to seeds/fruits that did not. Most authors examine differences in germinability (Krefting and Roe 1949; Meyer and Witmer 1997; Santamaría et al. 2002; Soons et al. 2008; Wongsriphuek et al. 2008; Brochet et al. 2010), germination rate (Midya and Brahmachary 1991; Clout and Tilley 1992; Figuerola et al. 2002) or weight of ingested *vs* non-ingested seeds (Nogales et al. 2001; Paulsen and Högstedt 2002; Rodríguez-Pérez et al. 2005). Because such studies are done on different bird and plant species, the results are very heterogeneous and mostly incomparable.

To test the effect of bird and plant species, seeds of one plant species are ingested by several bird species (Barnea et al. 1990; Murphy et al. 1993; Traveset et al. 2001; Charalambidou et al. 2003) and *vice versa* (Wahaj et al. 1998; Rodríguez-Pérez et al. 2005). While the differences among bird species are usually explained by variability in gut morphology (e.g., the presence of gizzard, length of the digestive tract influencing gut retention time, Murray et al. 1994; Wahaj et al. 1998), the differences among plant species are attributed to the variability in fruit type or seed traits such as seed size, coat thickness and water permeability (Levey and Grajal 1991; Murray et al. 1993; Meyer and Witmer 1997; Soons et al. 2008; Traveset et al. 2008). Because most birds eat only some types of seeds/fruits or prefer seeds/fruits of certain plant species, research on one bird species is limited to a few species. Hence there are only a few studies using more than 10 plant species (e.g., De Vlaming and Proctor 1968; Lieberman and Lieberman 1986; Rodríguez-Pérez et al. 2005; Soons et al. 2008). To test the effect of seed gut passage on many different species a simulation of seed gut passage is required (De Vlaming and Proctor 1968). The main advantage of such simulation is that it is much easier to achieve uniform conditions of digestive processes for all the species. Although the simulation cannot fully substitute the effect of real gut passage, it enables distinguishing seeds that are definitely able to survive bird gut passage from the seeds that are damaged in the gut and thus are not able to germinate. We are aware that such results are too rough for studying the close interaction between bird and particular species, but they are sufficient for comparing many species and among different studies (cf. Knevel et al. 2005).

A methodology for simulating endozoochory is well elaborated for large mammals (e.g., Simao Neto and Jones 1987; Ocumpaugh and Swakon 1993; Bonn 2004; Römermann et al. 2005) and data on this type of endozoochory are included in the LEDA database containing data on a large set of plant species from Europe (Kleyer et al. 2008). In contrast, appropriate methods for birds are still missing. To our knowledge the only published papers examining simulation of seed gut passage and comparing its effect to the real gut passage are McDiarmid et al. (1977), Glyphis et al. (1981) and Santamaría et al. (2002). In all these studies the authors used the seeds of only one plant species (*Stemmadenia donnell-smithii*, *Acacia cyclops* and *Potamogeton pectinatus*, respectively) and exposed them to gut passage of one (McDiarmid et al. 1977) or several bird species (Glyphis et al. 1981 and Santamaría et al. 2002). Scarification of seeds and acid or enzyme treatments were used to simulate endozoochory. While McDiarmid et al. (1977) and Glyphis et al. (1981) showed significant differences in germination between bird ingested and artificially treated seeds (with seed gut passage having weaker effect on viability of seeds than most of the treatments), Santamaría et al. (2002) did not find any differences. Considering that each author used different plant and bird species and a different methodology for the simulation treatments (e.g., type of acid, duration of scarification) such heterogeneous results are not surprising. For application of results to other plant species it is thus necessary to unify the methodology, especially to find out how intensive the simulations should be to sufficiently reflect the real passage of various types of seeds through a bird's gut. This can be done using seeds of a wide range of plant species and exposing them to the digestion by one bird species and different intensities of simulations.

This study aims to estimate the effect of different types of simulated endozoochory by bird on viability of seeds from 20 plant species and compare it to the real gut passage by a single bird species, the pigeon. As bird endozoochory is supposed to act especially in dispersal for long distances during colonization of oceanic islands (Carlquist 1974; Bucher and Bocco 2009), we used plants from the Canary Islands as the model species. Specifically, we asked the following questions: *i*) how do different types of simulations of birdseed gut passage reflect the real passage through the pigeon's gut? and *ii*) what are the main seed parameters responsible for differences among species? We predict that species adapted to endozoochorous dispersal will have higher proportion of intact seeds after their passage through a bird's gut than species that are not adapted to endozoochory. We also expect that the stronger the simulation, the higher proportion of non-viable seeds.

Material and Methods

Study Species

For our purpose we used seeds (fruits or seeds, Table 1) of 20 different plant species from the Canary Islands (Spain). Seeds of each species were collected from at least eight individuals either in the field (Gran Canaria, Tenerife, La Palma and La Gomera) or in the Botanical Garden "Jardín Canario Viera y Clavijo", Las Palmas (Gran Canaria, Table 1) during the seed-setting period in 2007. The seeds were stored under dry conditions. Because most species of the Canary Islands produce non-fleshly fruits, we decided to use a granivorous bird commonly occurring on these islands, the pigeon. Both experiments (simulation and seed gut passage) took place ca. 6 months after seed collection.

Table 1 List of species used in the experiments

Species name[a]	Family	Analyzed propagule	Collection site
Andryala pinnatifida Aiton	Asteraceae	fruit	T
Reichardia ligulata (Vent.) G. Kunkel & Sunding	Asteraceae	fruit	T
Atalanthus regis-jubae (Pit.) A. Hansen & Sunding	Asteraceae	fruit	G
Echium plantagineum L.	Boraginaceae	fruit	C
Crambe strigosa L´Hér.	Brassicaceae	fruit	T
Descurainia artemisioides Svent.	Brassicaceae	seed	BG
Cistus monspeliensis L.	Cistaceae	seed	BG
Carex canariensis Kük.	Cyperaceae	fruit	P
Lotus arinagensis Bramwell	Fabaceae	seed	C
Hypericum canariense L.	Hypericaceae	seed	BG
Salvia canariensis L.	Lamiaceae	seed	BG
Sideritis discolor Bolle	Lamiaceae	seed	BG
Lavatera cretica L.	Malvaceae	fruit	P
Plantago arborescens Poir.	Plantaginaceae	seed	T
Plantago lagopus L.	Plantaginaceae	seed	C
Limonium pectinatum (Aiton) Kuntze	Plumbaginaceae	seed	BG
Brachypodium arbuscula Knoche	Poaceae	fruit	BG
Melica minuta ssp. *latifolia* (Coss.) Hempel	Poaceae	seed	T
Rumex vesicarius L.	Polygonaceae	seed	C
Reseda luteola L.	Resedaceae	seed	C

BG – Botanical garden, C – Gran Canaria, G – La Gomera, P – La Palma, T – Tenerife.

[a] According to Arechavaleta et al. (2010).

Seed Parameters

For each plant species we measured seed length, width and height (to calculate seed volume), thickness of seed coat, seed mass and water permeability (Table S1 in Electronic Supplementary Material). Length, width and height of seed and thickness of seed coat were measured on 10 seeds of each species using a Nikon SZX12 stereomicroscope and software M.I.S. Quick Photo Micro 2.2. The mean of 10 measurements was used in the analyses.

The length of a seed was regarded as the longest dimension, no matter if it was equivalent to the morphological length. The width was defined as the widest axis perpendicular to the length axis (Götzenberger 2005). For measuring seed height and thickness of seed coat, seeds were transversely halved. Because the seed coat thickness varied within the same seed, the final value was the mean of two perpendicular measurements. In the case of a whole fruit, pericarp thickness was measured as well and both values were taken into account. Seed volume was calculated from seed length, width and height by assuming the closest matching geometrical shape (Soons et al. 2008).

Water permeability of seeds was determined indirectly as an increase of seed weight after 24-hour soaking in water. Water permeability was expressed as seed weight increase proportional to the weight of soaked seed. For each species 10 replicates were made (each replicate had 5 or 10 seeds depending on seed size). The same number of replicates was made for dry seed mass.

A Pigeon's Digestive Tract

A pigeon's digestive tract consists of bill, mouth with tongue, pharynx, esophagus, crop, two-chambered stomach, small intestine, caeca, rectum and cloaca. The pharynx and esophagus help with swallowing food and with its passage to the crop, which serves as a reservoir for quickly eaten food. It does not secrete any acids and enzymes. It contains only water and bacteria supporting food decomposition (Ferianc et al. 1982). The first stomach chamber, the proventriculus, secretes hydrochloric acid (pH around 0.2) to break down food. The second part, the gizzard, consists of powerful muscles that together with small stones (grit) and various glands pulverize food. The intestines and caeca help to absorb nutrients into the body. The waste is stored in the rectum and excreted through the cloaca (Baumel 1993).

According to Clout and Tilley (1992) gut passage time ranges from 55 to 140 minutes for a pigeon. However, the time depends on many factors such as nutritional level of the diet, consistency, hardness, water content or amount of food (Clench and Mathias 1992; Traveset 1998).

Seed Passage Through a Pigeon's Digestive Tract

In our experiment, we used a King pigeon, a utility breed with poor flight ability that is amenable to our experiments due to its easy manipulation. We are aware that using a captive bird could be potentially problematic because the digestive tract of captive birds could differ from the digestive tract of wild birds (Clench and Mathias 1995). However, it is a sufficient model for our experiment. If we used a wild pigeon, it could suffer from the stressful conditions connected with its caging that could influence the effect of digestion.

Before the experiment the pigeon was housed outdoors and fed on a stable diet of commercial grain mixture (Columuni Anima CZ). The mixture was removed two days before starting the experiment, only water and grit (small stones eaten by birds to enhance digestion, commercial mixture for pigeons) were available. During the experiment the pigeon was caged in the outdoor roofed aviary (2 m × 1 m × 1 m), the floor was covered with paper. The pigeon was fed with seeds from 20 species, 30 seeds per species. Bigger seeds (5 species) were given to the pigeon directly, and small ones (15 species) were incorporated into skinned commercial sunflower seeds. A few skinned sunflower seeds were provided as extra feeding during the experiment. During feeding the pigeon was observed to make sure that all seeds had been eaten. No regurgitated seeds were found. The paper bedding was changed when necessary and stored in dry conditions. The last one was removed ca. 48 hours after the last tested seeds were fed to the pigeon. According to the published average time for seed passage through pigeon gut (Clout and Tilley 1992) 48 hours should be sufficient for digestion of all the analyzed seeds. Dried feces were scraped off the paper, then soaked in the water and rinsed through 1 mm sieve. Intact seeds were retrieved, counted and dyed with 0.1 % solution of 2,3,5-triphenyl-2H-tetrazolium chloride (Cottrell 1947) to test seed viability.

This method provides the same accuracy in estimating seed viability as the germination tests (Lakon 1949). According to our experience the viability of seeds estimated using the tetrazolium test largely matches visual estimation of seed viability. Untreated seeds were tested for viability following the same procedure and used as controls.

Laboratory Simulation of Seed Gut Passage

Each species was subjected to seven different treatment combinations of length of seed scarification and length of chemical treatment with sulphuric acid (Table 2). During scarification 250-ml plastic flasks filled with 20 g of wet pigeon grit and 30 seeds were shaken for 2 or 12 hours in an electric orbital shaker (200 shakes per min). Then seeds were separated from the grit, rinsed and immersed in 5 ml of 1 M H_2SO_4 (pH≈0.3) for 5, 30 or 120 min. We used the same intervals and acid concentration as Santamaría et al. (2002) for ducks because we had no prior knowledge on simulation of seed gut passage for pigeons. To extend the range of treatments we made another simulation with 24-h seed shaking (200 shakes per min) and 240-min immersion in sulphuric acid. Intact seeds were retrieved, counted and tested for viability. Untreated seeds were used as a control.

Data Analysis

To estimate the explanatory power of the individual simulation treatments for predicting seed survival after pigeon digestion, we used a generalized linear model with binomial distribution. Seed viability (estimated as the proportion of viable seeds of the total number of ingested seeds) after one type of simulation was used as the independent variable. As the dependent variable we used the number of viable seeds after seed gut passage linked to the number of non-viable seeds by cbind function (Crawley 2002). This analysis was done for each simulation separately.

To test the effect of seed traits on seed viability after seed gut passage and after simulation of gut passage, we used a generalized linear model with binomial distribution. All measured seed traits were used as independent variables. As the dependent variable we used the number of viable seeds after seed gut passage or simulation

Table 2 Duration of scarification and acid treatments used for different types of simulation in the study and the codes used to refer to the different types of simulation

		Scarification (hours)		
		2	12	24
Acid immersion (minutes)	5	2/5	12/5	
	30	2/30	12/30	
	120	2/120	12/120	
	240			24/240

linked to number of non-viable seeds by cbind function (Crawley 2002). Control viability was used as a covariate.

To explain the differences between simulation and real gut passage we used a generalized linear model with binomial distribution. Treatment (simulation vs real gut passage), seed viability of the control seeds and the seed traits were independent variables. As the dependent variable we used the number of viable seeds linked to the number of non-viable seeds by cbind function (Crawley 2002). The test was done for each simulation type separately. The model also included interactions between individual seed traits and treatment as additional independent variables (model 1). To explore the proportion of variation between species not explained by seed traits we developed another model that also included species and interaction between species and treatment as independent variables in addition to all factors in model 1 (model 2).

To compare the effect of scarification with that of acid treatment, we used scarification and acid treatment duration as two independent variables and seed viability after simulation as a dependent variable. The model also included species and interactions between scarification and acid treatment duration and species as additional independent variables.

In all cases, the tests were done in S-Plus 6.2 Professional (MathSoft, Inc. 2000).

Results

Four different simulations were significant predictors of seed viability after gut passage through a bird. The scarification was between 2 and 24 hours and acid immersion 30 and 240 (treatments 2/120, 12/30, 12/120 and 24/240, Table 2). The two strongest simulations (12/120 and 24/240) were the best predictors (Table 3).

Almost all the measured seed traits had significant effects on the viability of seeds after different types of simulation. The strongest effect on seed viability was shown for water permeability and seed mass (Table 4). While the effect of water permeability was negative, seed mass had positive effect on seed viability in all types of simulation. In contrast, only seed mass had significant positive effect on the viability of seeds, which passed through the bird's gut (Table 4).

Table 3 The effect of single types of simulation on seed viability after real digestion by bird, general linear model, d.f.=1

Type of simulation	*P*-value	R^2
2/5	0.105	-
2/30	0.245	-
2/120	0.048	0.077
12/5	0.068	-
12/30	0.024	0.101
12/120	0.017	0.112
24/240	0.021	0.105

Table 4 The effect of seed traits on seed viability after digestion by pigeon and after different types of simulation (logistic regression). The values in the table are R^2 values of the significant effects, N=30

		Type of simulation						
	Digestion	2/5	2/30	2/120	12/5	12/30	12/120	24/240
Control viability	n.s.	0.093^{***}	0.140^{***}	0.102^{***}	0.048^{***}	0.066^{***}	0.091^{***}	0.087^{***}
Seed coat thickness	n.s.	0.012^{**}	0.017^{***}	0.015^{***}	n.s.	n.s.	n.s.	n.s.
Water permeability	n.s.	0.045^{***}	0.068^{***}	0.100^{***}	0.080^{***}	0.082^{***}	0.120^{***}	0.136^{***}
Seed mass	0.029^{*}	0.078^{***}	0.066^{***}	0.043^{***}	0.069^{***}	0.035^{***}	0.035^{***}	0.054^{***}
Seed volume	n.s.	0.009^{**}	n.s.	0.013^{**}	0.012^{**}	0.019^{***}	0.039^{***}	0.043^{***}

Type of simulation – duration of shaking in hours/duration of immersion in acid in minutes.

n.s. – non-significant. *** – $P<0.001$; ** – $P<0.01$; * – $P<0.05$.

Seed viability after all types of simulation significantly differed from viability of seeds that passed through the bird's digestive tract. Even in the case of the strongest simulation (24/240) the viability was higher than after passage through the gut (Table 5). Seeds of 6 out of 20 species remained viable after passage through the pigeon (*Carex canariensis*, *Cistus monspelliensis*, *Lavatera cretica*, *Plantago arborescens*, *Reseda luteola*, *Salvia canariensis*, Table S2 in Electronic Supplementary

Table 5 The results of logistic regression comparing digestion by bird with one type of simulation of digestion, in each case. The values in the table are R^2 values of the significant effects, N=30

Type of simulation		2/5	2/30	2/120	12/5	12/30	12/120	24/240
Model 1	Control viability	0.045^{***}	0.068^{***}	0.05^{***}	0.023^{***}	0.032^{***}	0.043^{***}	0.04^{***}
	Seed-coat thickness	0.005^{*}	0.007^{*}	0.006^{*}	n.s.	n.s.	n.s.	n.s.
	Water permeability	0.021^{***}	0.032^{***}	0.047^{***}	0.039^{***}	0.04^{***}	0.057^{***}	0.063^{***}
	Seed mass	0.05^{***}	0.044^{***}	0.03^{***}	0.047^{***}	0.027^{***}	0.025^{***}	0.033^{***}
	Seed volume	0.007^{**}	n.s.	0.01^{**}	0.009^{**}	0.015^{***}	0.031^{***}	0.033^{***}
	Treatment	0.184^{***}	0.158^{***}	0.165^{***}	0.160^{***}	0.135^{***}	0.094^{***}	0.084^{***}
	Seed-coat thickness× treatment	n.s.	0.005^{*}	0.005^{*}	n.s.	n.s.	n.s.	n.s.
	Water permeability× treatment	0.006^{*}	0.011^{***}	0.015^{***}	0.012^{***}	0.014^{***}	0.027^{***}	0.034^{***}
	Seed mass × treatment	0.008^{**}	0.01^{*}	0.007^{*}	0.005^{**}	n.s.	0.008^{*}	0.014^{**}
	Seed volume × treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Sum of model 1	0.326	0.335	0.335	0.295	0.263	0.285	0.301
Model 2	Sum of model 1 + Species + species× treatment	0.594^{***}	0.609^{***}	0.593^{***}	0.549^{***}	0.552^{***}	0.561^{***}	0.557^{***}

Type of simulation – duration of shaking in hours/duration of immersion in acid in minutes.

n.s. – non-significant *** – $P<0.001$; ** – $P<0.01$; * – $P<0.05$.

Material). The number of viable seeds after their passage through the bird's tract ranged from 1 (*Salvia canariensis*) to 5 seeds (*Carex canariensis*). While seeds of only 6 species survived the strongest simulation (24/240, 13 %–87 % viability) seeds of 13 species remained viable after the weakest simulation (2/5, 3 %–97 % viability, Table S2 in Electronic Supplementary Material). All seeds of *Crambe strigosa*, *Hypericum canariense*, *Limonium pectinatum* and *Plantago lagopus* were destroyed both by simulations and real gut passage. In the case of three species (*Carex canariensis*, *Plantago arborescens* and *Salvia canariensis*) more seeds remained viable after gut passage than after simulations (Table S2 in Electronic Supplementary Material).

The differences between each type of simulation and real gut passage can partly be explained by interactions between measured seed traits and treatment, such as water permeability and seed mass (Table 5). Despite the significant interactions between seed traits and treatment, adding the interaction between species and treatment into the model significantly increased the explained variation (explaining additional 25.4 %–28.9 %, Table 5). This indicates that other species-specific traits that were not measured influence the relationship between simulation and real gut passage.

Intensity of scarification had stronger effect on seed viability than duration of acid treatment ($P<0.001$, $R^2=0.020$ and $P<0.001$, $R^2=0.004$, respectively). The effect of seed scarification was also much more species specific (interaction of scarification and species $P<0.001$, $R^2=0.041$) than duration of acid treatment ($P<0.001$, $R^2=0.016$). However, there was no significant interaction between scarification and duration of acid treatment ($P=0.303$). There was, however, marginally significant three-way interaction among scarification, acid treatment and species ($P=0.052$).

The correlation matrices (Table S3 in Electronic Supplementary Material) comparing relationship between seed parameters showed significant correlations between seed volume and seed-coat thickness ($P<0.001$, $R^2=0.46$) and between seed volume and seed weight ($P<0.001$, $R^2=0.62$). A preliminary step-wise selection model applied to the data indicated that seed volume has additional effects to those of the other seed parameters (not shown) and it was thus kept in our models.

No other significant correlations between seed traits were found.

Discussion

Seed viability after all types of simulation significantly differed from viability of seeds after the passage through a bird's gut in our dataset. These results are consistent with conclusions of McDiarmid et al. (1977) and Glyphis et al. (1981). Also in their study, results of simulation treatments differed significantly from a bird's ingestion, with scarification being an exception.

In contrast, no differences between bird-ingested seeds and seeds processed by simulation were shown by Santamaría et al. (2002). However, in the paper by Santamaría et al. (2002) germination percentage was counted only from seeds that survived passage through a bird's gut and not from all the seeds ingested by a bird as it was done in McDiarmid et al. (1977), Glyphis et al. (1981) and in our study. As in Santamaría et al. (2002), average retrieval of intact seeds did not exceed 29 % for both bird species, and the results would have probably been different if the authors had involved all the bird-ingested seeds.

Many studies comparing the effect of seed gut passage (e.g., De Vlaming and Proctor 1968; Lieberman and Lieberman 1986; Barnea et al. 1991; Soons et al. 2008) or the effect of simulation of seed gut passage (e.g., Teltscherová and Hejný 1973) within a large group of plant species showed that each species responded differently to the same type of treatment. Moreover, in our study we demonstrated that this response differs also between real gut passage and all types of simulations. This rather complicates the selection of the most appropriate type of simulation.

Among different simulation treatments, the strongest simulation (24/240) was still the most similar to real seed gut passage. In 10 out of 20 species it has the same effect of seed viability as the real seed gut passage. Six species (*Carex canariensis*, *Cistus monspelliensis*, *Lavatera cretica*, *Plantago arborescens*, *Reseda luteola*, *Salvia canariensis*) are supposed to have an adaptation to the dispersal by birds as a certain number of their seeds survived the passage through a bird's digestive tract. Such results are in agreement with Figuerola and Green (2002) who reported *Carex* seeds to be transported in the digestive tract of mallards. *Plantago* and *Salvia* contain special substances in the seed coat, which change into mucilage when wet, protecting seeds from the destruction in the bird's gut. A small proportion of seeds of *Cistus monspelliensis* and *Reseda luteola* also survived the passage through a bird's gut, probably due to their small size (Bramwell 1985).

The direct indication of endozoochorous dispersal of other species used in the study is almost lacking. Only Bramwell (1985) assumes that the genus *Lotus*, *Crambe* and *Rumex* were transported to the Canary Islands probably in the gut of pigeons or doves, which is in contrast to our results.

However, the lack of information about endozoochorous dispersal of our study species does not necessarily mean that they do not definitively disperse by such dispersal mode because many species have the potential to be dispersed by more that one dispersal vector (Hughes et al. 1994). However, the literature about plant traits usually contains information only about the most probable dispersal mode and ignores other possibly important modes.

Seed traits explained the most variability between species in the strongest simulation (24/240). This could indicate that some seed traits such as water permeability and seed weight become more important for seed survival after exposing seeds to stronger simulation. The differences in the variation explained by seed traits were, however, relatively low among 24/240 simulation and other types of simulation.

The only seed parameter that explained at least some variation in seed viability after gut passage was seed mass. This result is in contrast to Wongsriphuek et al. (2008), who did not find any significant relationship between seed mass and seed viability after real gut passage. The variability in seed mass between species was, however, very low in Wongsriphuek et al. (2008).

Seed mass was also an important predictor of the differences between real digestion and simulation suggesting that the use of different birds may produce different results because of the difference in gut morphology (Murray et al. 1994; Wahaj et al. 1998).

Although water permeability did not explain any variation in seed viability after their gut passage, it was the best predictor of seed survival after the different types of simulations. It was also one of the best predictors (together with seed weight) of the interaction between species and treatments.

The effect of seed scarification was much more species specific than duration of acid treatment. This means that parameters such as water permeability affect much more the response of species to scarification than to duration of acid immersion.

We are aware that our methods of searching for the best simulation of seed passage through a pigeon's digestive tract did not bring fully satisfactory results. The very specific response of each species to different treatment complicates our intention to unify the methodology of simulation for the group of different species. Our approach, however, should be sufficient to differentiate species that are definitively not able to disperse by endozoochory from those that have a good potential to survive the passage through a bird's digestive tract.

Conclusions

Results of this study show that the correspondence between results based on direct seed gut passage through a pigeon's digestive tract and the different simulation treatments was species specific and depends not only on the commonly tested traits such as seed weight and water permeability, but also on other unmeasured traits (e.g., fiber content, Wongsriphuek et al. 2008, seed coat resistance to mechanical damage). Furthermore, different traits explain the difference between real gut passage and different simulations. The only common trait is seed mass. All this suggests that selecting one type of simulation as a good predictor of seed survival after gut passage is difficult.

Still the strongest simulation (24/240) is the best predictor of all and we thus suggest that this type of simulation may be used to compare ability to disperse by endozoochory for a larger set of species.

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Paper 2

DISPERSAL ABILITY OF ISLAND ENDEMIC PLANTS: WHAT CAN WE LEARN USING MULTIPLE DISPERSAL TRAITS?

Kristýna Vazačová and Zuzana Münzbergová

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Paper 2: Disperal ability of island endemics

Dispersal ability of island endemic plants: what can we learn using multiple dispersal traits?

Kristýna Vazačová[1, 2*] and Zuzana Münzbergová[1, 2]

[1] Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, Prague 2, CZ-128 01, Czech Republic

[2] Institute of Botany, Academy of Sciences of the Czech Republic, Lesní 322, Průhonice, CZ-252 43, Czech Republic

[*] Author for correspondence, email: vazacova@seznam.cz, tel: +420271015332

Abstract

Island endemic species are expected to have lower dispersal ability than their non-endemic congeners**.** Several studies have demonstrated differences in diaspore morphology between endemic species and their non-endemic congeners. It is, however, relatively difficult to translate the differences in morphology of the diaspores into differences in dispersal ability. To avoid this problem, we measured directly dispersal values (anemo-, hydro-, exo- and endozochory) of 27 pairs of closely related endemic and non-endemic species from Canary Islands. We did not explicitly support the hypothesis about the loss of dispersal ability of island species. The comparison of pairs of endemic and non-endemic species showed the reduction in dispersal potential only for endozoochory. In many cases, endemic species had in fact the same or better dispersal ability than their non-endemic congeners. Higher dispersal ability of endemic species could have been evolved as a consequence of species subsequent dispersal to neighboring islands. As a support for this we found that the endemic species dispersing better than their non-endemic congeners also occupy more islands within the archipelago. We conclude that reduction of dispersal ability of species on islands may not be as general as previously expected and we need to take into account multiple species traits to understand the possible evolution of species dispersal potential.

Key words: dispersal mode; long-distance dispersal; Macaronesia; terminal velocity.

Introduction

Oceanic islands have always fascinated scientists because of the various evolutionary phenomena arising from their specific conditions. One such phenomenon is the loss of dispersal ability of plant and animal species, which has been observed on many Pacific (Carlquist, 1965) and Atlantic islands (Talavera et al., 2012; Talavera et al., 2013). According to Carlquist (1967) the main reason for the reduction of dispersal ability of island species is lower probability of propagules to be dispersed out to the sea. Additionally, due to relative stability of island habitats over time, species colonizing islands have no need to escape from such suitable sites (Cody and Overton, 1996; Kuno, 1981; McPeek and Holt, 1992).

According to these hypotheses it could be predicted that selection will favor species with propagules of lower dispersability than was the dispersability of their ancestors when colonizing the island from the continent. Although the direct continental ancestors may not exist any longer, the reduction of dispersability can be studied from comparison of dispersal abilities between island endemics and their continental relatives, as it was done by Carlquist (1974) on diaspores of various Hawaiian species. Carlquist (1974) demonstrated the loss of dispersal ability mainly as a reduction of seed morphological parameters enabling species to disperse.

Well-known is an example of reduction of the awn length in island species of *Bidens* when compared to their mainland relatives (Carlquist, 1966). Assessing dispersal abilities from diaspore morphological parameters is a common method used in many studies on species dispersal (e.g. Bernardello et al., 2006; Cody and Overton, 1996; Hughes et al., 1994; Matlack, 1987; Ridley, 1930; van der Pijl, 1982; Willson et al., 1990). However, diaspore morphology may not always be a sufficient predictor of dispersal ability (Willson et al., 1990). This could be the reason, why Lavergne et al. (2004) did not find any differences between dispersal ability (estimated from parameters such as pappus length and surface) of 6 congeneric pairs of endemic and non-endemic species from the French Mediterranean region. As Sheldon and Burrows (1973) demonstrated on 18 species of the family Compositae, the differences in dispersal ability are given more likely in fine details of diaspore morphology such as the possession of hairs on achenes which are, however, not easily measurable. In fact, species with no apparent morphological adaptation can be also successfully dispersed as shown by e.g. Courveur et al. (2004), Fischer et al. (1996), Heinken and Raudnitschka (2002), and Vargas et al. (2012). As a consequence, the results based on diaspore morphological parameters can differ from the results based on direct measurements of diaspore dispersal ability as demonstrated by Fresnillo and Ehlers (2008) on mainland and island populations.

In contrast to morphological parameters, direct determination of diaspore dispersal ability enables the estimation of the dispersal ability of all species by multiple vectors reducing the risk of overlooking an important dispersal agent in each particular species. Unlike morphological parameters the values describing dispersal ability by different vectors are transferable into dispersal distances (e.g. Tackenberg et al., 2003; Will and Tackenberg, 2008) and thus are more appropriate for studying large groups of species with a large variation in diaspore types. Although the methodology for estimating dispersal ability of species by different modes (anemo-, hydro-, exo-, endozoochory, Knevel et al., 2005; Vazačová and Münzbergová, 2013) is well-elaborated, most studies are focused usually on one, most easily quantifiable vector. Such approach may, however, underestimate the real dispersal ability of the studied species because they usually do not disperse by only a single dispersal vector (Nathan, 2007; Vargas et al., 2012).

The comparison of traits between endemic and non-endemic species can also shed light on the evolutionary potential of different traits. The papers exploring the evolution of woodiness, growth form or floral type of island plants demonstrated that some traits are conserved remaining almost unchanged during evolution and others have changed in a short time period (e.g. Böhle et al., 1996; Emerson, 2002; Helfgott et al., 2000; Kim et al., 1996; Lavergne et al., 2004; Panero et al., 1999; Tkach et al., 2008). Studies looking at the evolution of species dispersal abilities have mostly tested differences in morphological parameters (e.g. Caputo et al., 2004; Eriksson et al., 2000; Givnish et al., 2009; Su et al., 2008). To our knowledge, only Darling et al. (2008), Fresnillo and Ehlers (2008), Kudoh et al. (2013) and Riba et al. (2009) looked at the evolution of dispersal abilities using more directly measured traits (wind- and water-dispersal potential). However, these traits were studied only on a few species (*Abronia umbellata* in Darling et al., 2008, *Cirsium arvense, C. hirsutum* and *Epilobium angustifolium* in Fresnillo and Ehlers, 2008, *Hibiscus glaber* and *H. tiliaceus* in Kudoh et al., 2013 and *Mycelis muralis* in Riba et al., 2009).

While it is expected that island endemic species will have reduced dispersal ability, the dispersal ability of island species can also increase if the ability to disperse among multiple islands within an archipelago is an important ability under selection. This may happen if new islands are continuously arising in the system.

The aim of the study is to answer the following questions. 1) What are the differences in dispersal traits between closely related endemic and non-endemic species? 2) Which dispersal modes show the strongest difference between endemic and non-endemic congeners? 3) Does the relationship within pairs of endemic and non-endemic congeners reflect species distribution on islands?

To answer these questions, we collected seeds of 27 different pairs of endemic and non-endemic closely related species growing on Canary Islands and measured their dispersal ability by 4 dispersal modes likely acting in long-distance dispersal on islands: wind, water and bird dispersal (both external and internal). We used published sources complemented with our field experience to identify the most likely dispersal mode for each species pair.

We predict that endemic species on the Canary Islands will have reduced dispersal ability in the comparison with their non-endemic congeners also growing on the islands as it was already shown for species on Hawaiian Islands (Carlquist, 1974) which are similar to the Canaries in many features. Not all traits will, however, be under the same selection pressure. These differences may thus not be detected in all dispersal modes, but should be visible in the most frequently used dispersal mode in each species pair. We also predict that in species pairs in which endemic species occupy more islands than its non-endemic congener, endemic species will have better dispersal ability than their non-endemic congeners.

Materials and Methods

Study site

The Canary Islands are part of Macaronesia and are situated between 27°45′and 29°2′N and between 18°00′and 13°37′W. They consist of 7 main volcanic islands with different age (from 0.8 My for El Hierro up to 21 My for Fuerteventura, Afonso, 1988) and size (from 278 km^2 for El Hierro up to 2034 km^2 for Tenerife, Dlugosch and Parker, 2007).

The shortest distance between the islands and the mainland is around 100 km (from Fuerteventura to Morocco, Juan et al., 2000). The spatial and temporal diversity, the proximity of the mainland and climatic stability are the main factors responsible for the high diversity of island flora (ca. 2100 species with 20% of endemic species, Arechavaleta et al., 2010, but see Hobbom 2000).

Species selection

In our study we used only native species (according to Arechavaleta et al., 2010). In contrast to introduced species they are supposed to exist on the islands long enough to be influenced by island conditions.

We selected 54 species belonging to 27 genera and 18 families, grouped into pairs (Table 1). One species in the pair is endemic to Macaronesia (mainly only to Canary Islands), the non-endemic species occurs also on the mainland. All the species were collected on islands to ensure that they originated from the same environmental conditions. Thanks to this, the possible differences in their traits were thus a reflection of their differential evolutionary history rather than of the different environments in which they are currently occurring.

As most species grow on habitats which are present on the majority of islands, we suppose that their absence from some of the islands is due to their low dispersability and not due to the absence of suitable habitats. We, however, cannot exclude the option that the limited distribution of the endemic species may be due to previous preemption of the suitable niches by other species, i.e. the non-endemics (Silvertown et al., 2005).

Species pairs were selected within genera, which is for most Canary Island species currently the best estimate of phylogenetic relatedness. Such method of selection has already been used in other studies (e.g. Lavergne et al., 2004; Tremlová and Münzbergová, 2007). Where available, we also used phylogenetic data to estimate the phylogenetically closest species within a pair (Table 1) preferably with non-endemic species being phylogenetically older than the endemic species. In addition, species selection was further limited to species for which sufficient seed samples could be obtained. The selected pairs also excluded most species having fleshy fruits due to their limited storability for dispersal experiments.

Seed collection

Diaspores (fruits or seeds representing the most probable dispersal units, see Table 1) for each species were collected from at least 8 individuals in the Botanical Garden "Jardín Canario Viera y Clavijo", Gran Canaria or in natural populations (all Canary Islands). All species collected in the Botanical Garden originally came from island populations. The collection of diaspores from natural populations was done in cooperation with the Botanical Garden which possessed appropriate permissions for collecting diaspores for scientific purposes. In the natural populations we preferably sampled 3 populations per species. Each population was then tested for dispersal abilities separately. We used 20 diaspores per species and population for experiments with anemochory, hydrochory and exozoochory and 30 seeds for testing endozoochory, i.e. 60 and 90 diaspores for each species, respectively. We used the same number of diaspores in species collected from the garden. Such number of diaspores was a compromise between a large amount of species tested and number of diaspores used in literature (c.f. Knevel et al., 2005).

For testing other traits related to dispersal (i.e. seed mass and seed viability) we used simple seeds, not fruits. In dispersal modes, where we used fruits as dispersal units, but accounted also for seed viability (i.e. hydrochory and endozoochory), the number of all seeds extracted from the fruits was used as a baseline number of seeds.

Within pairs, we compared only diaspores collected at the same site type (i.e. in the Botanical Garden or in the field). In this way, we excluded a possibility that the diaspores within a pair would differ due to different seed source.

Anemochory

The ability of diaspores to disperse by wind was estimated as terminal velocity defined as the maximum rate of seed falling in still air (Thompson, 2005). It was measured as the flight time of a diaspore from predefined height (270 cm, Münzbergová, 2004). Mean dispersal distance D was expressed as:

$$D = (w \times h) / t$$

where w is wind speed (being constant for all species), h is average plant height and t is terminal velocity. Values of average plant height were obtained from the literature (Bramwell and Bramwell 2001; Castroviejo et al., 1986-2012; Schönfelder and Schönfelder 2002a, 2000b; Tutin et al., 1964-1980).

We are aware that our dispersal model is simplified. Nevertheless, it has been successfully used in other studies to characterize mean dispersal distance of diaspores (e.g. Herben et al., 2006; Münzbergová et al., 2005; Soons and Heil, 2002; Tremlová and Münzbergová, 2007) and is the easiest way to combine the three key variables affecting wind dispersal. We thus suggest that it is a useful proxy of potential wind dispersal distances for comparison among species.

In the analyses, we used both terminal velocity (m/s) and mean dispersal distance (m). In addition, we tested for the difference in plant height between endemic and non-endemic species to see to what extent the differences in dispersal distance are affected by differences in plant height.

Hydrochory

The potential of diaspores to disperse in salt water (buoyancy) was measured as the proportion of diaspores still floating after a defined time period. Diaspores were gently put into beakers filled with salt water having 3.7% salinity (i.e. average salinity of the Atlantic Ocean along the Canary Islands coast, web 1). The size of beakers was proportional to the size of diaspores. Sea waves were simulated by continual shaking in electric orbital shaker with frequency of 100 shakes per min. The number of diaspores floating on water surface was checked immediately after putting them into bins and then after 5 minutes of shaking, 1, 2, 6, 24 hours and 7 days of shaking (Römermann et al., 2005). The experiment was finished after 1 week of diaspore shaking as it is the minimal time a diaspore needs for reaching the Canary islands from mainland when taking into account average speed of water currents in the Atlantic Ocean (60-90 km per week, Zhou et al., 2000) and the distance between mainland and the closest island (Africa to Fuerteventura, ca. 100 km).

At the end of the experiment, the number of floating and the number of sunken diaspores was counted and the two groups of diaspores were then tested for viability. In the analyses, we used the proportion of viable seeds which kept floating until the end of the experiment from the total number of viable seeds before the experiment.

The diaspore buoyancy was also expressed as T_{50}, the number of days after which 50 percent of diaspores was still floating. This parameter is commonly used in other studies assessing hydrochory (Boedeltje et al., 2003; Römermann et al., 2005; van den Broek et al., 2005), however it does not take into account seed viability.

We also used the information on effect of salt water on viability of seeds expressed as the proportion of viable seeds after the experiment (both floating and sunk)/seed viability before the experiment. Viability of seeds was tested by dying the dissected seeds with 0.1% solution of 2,3,5-triphenyl-2H-tetrazolium chloride and inspecting the color change of the embryo (Cottrell, 1947). This method provides the same accuracy in estimating seed viability as the germination tests (Lakon, 1949). In contrast to germination tests, it is not dependent on selection of the right conditions for germination for each individual species and it is thus in fact more reliable for between species comparisons.

Zoochory

Birds are the most important long-distance island dispersers transporting diaspores both externally and internally. The main bird dispersers acting on the Canary Islands are blackbirds (*Turdus merula*), robins (*Erithacus rubecula*), blackcaps (*Sylvia atricapilla* and *S. melanocarpa*, Olesen and Valido, 2004), common ravens (*Corvus corax*, Nogales et al., 1999), gulls (*Larus cachinnans*, Nogales et al., 2001) and pigeons (*Columba livia*, *C. junoniae* and *C. bolli*).

Bird exozoochory (Epizoochory)

Bird exozoochory was tested as diaspore adhesion to bird feathers. As a model species we used a pigeon of the King breed, a utility breed with poor flight ability that is amenable to our experiments. Although this species is clearly not native to the Canary Islands, the functionality of its feathers for diaspore dispersal is readily comparable with native insular pigeon species.

As the seed coat of some species (e.g. *Plantago, Salvia*) contains substances which become sticky when wet, all the diaspores were moistened before the application into pigeon feathers. Moistened diaspores were gently incorporated on 4 different body parts (on bust, neck and back, under wing). After 1 hour of pigeon free movement in an aviary (2 × 2 × 1 m) we checked the numbers of diaspores still attached to feathers. Taking into account the average flight speed of a trained pigeon (80 km/h, Gessaman and Nagy, 1988) and the shortest distance between mainland and the closest island (ca. 100 km), diaspores which remained attached to feathers after 1 hour are potentially able to get to the islands by this type of dispersal.

In the analyses we tested the proportion of diaspores which kept attached to the feathers after 1 hour (we refer to this value as seed adhesion). This parameter lacks the effect of real bird flight as we do not take into account the air movement around feathers during the flight that can dry out diaspores and cause them to drop earlier than in our simulation. However, we still think that our data are sufficient for the purpose to differentiate among diaspores with different ability to disperse by exozoochory.

Bird endozoochory

Bird endozoochory was tested by simulating diaspore gut passage through pigeon digestive tract. The methodology was based on our previous study (Vazačová and Münzbergová, 2013) in which we compared the effect of real gut passage through pigeon digestive tract on seed viability to different simulation approaches. Specifically, plastic flasks filled up with diaspores were shaken with wet grit (small stones eaten by birds to enhance digestion, commercial mixture for pigeons) for 24 hours in electric orbital shaker (200 shakes per minute, Vazačová and Münzbergová, 2013). Then diaspores were separated from the grit, rinsed and immersed in 5 ml of 1M H_2SO_4 (pH ≈ 0.3, Santamaría et al., 2002) for 4 hours. Intact seeds were retrieved, counted and tested for viability. The proportion of number of viable seeds which survived the simulation to the number of seeds viability before the experiment was used in the analysis. Seed viability after simulation was tested as described above.

Seed mass

Altogether 90 seeds per species were weighted. For this purpose, they were divided into groups by 10 to 30 seeds per group (10 seeds in the group for the largest and 30 for the smallest seeds, to get reasonable size estimates given by the precision of the balance, 0.0001g). Seed mass is generally recognized as a rough proxy of seed dispersal ability and germination ability (e.g. Hewittt and Kellman, 2002; Sakai et al., 1998; Tremlová and Münzbergová, 2007). The same amount of seeds was used for viability testing of intact seeds.

Most likely dispersal mode

For all species pairs the most likely dispersal mode was estimated from available literature (Table 2). Where such data were missing, we estimated the dispersal mode according to our experience with dispersal and seed morphology of the species. By testing for the most likely dispersal mode we attempted to elude the problem that not all species disperse by all measured dispersal modes in reality. Species forming a pair always had the same dispersal vector. As a result, by including species pair in our models as a covariate and thus comparing dispersal ability only within each pair (see below), we are able to explore differences in dispersal ability by the most likely dispersal mode between all endemic and non-endemic species within a single model.

Species distribution

Species distribution was expressed as a number of occupied islands, according to Arechavaleta et al. (2010).

Data analysis

To test the differences in diaspore traits between endemic and non-endemic species, we used the values of traits as dependent variables and species pair and species category (endemic vs. non-endemic) as independent variables. Terminal velocity, dispersal distance, plant height, seed mass and the most likely dispersal mode were tested by ANOVA after logarithmic transformation used to improve normality of the residuals. The other traits (buoyancy, seed survival in salt water, seed adhesion to feathers, seed viability after simulation of bird gut and seed viability) were tested by logistic regression (number of viable, attached or floating seeds was linked to initial number of seeds using cbind function in S-plus). In case of diaspore buoyancy, seed survival in salt water and seed viability after gut passage, number of viable seeds before the experiment was used as the initial number of seeds. T_{50} was tested by non-parametric Wilcoxon matched pairs test due to non-normal data distribution.

To visualize the results we corrected values by the mean value of the genus. This correction is the simplest version of phylogenetic correction (PC, Felsenstein, 1985). Specifically, we applied the formula previously used by Tremlová and Münzbergová (2007) and calculated the corrected values PC:

$$PC = S - MP$$

where S is the trait value of a single species (either endemic or non-endemic species) and MP is the mean of the trait value for each species pair.

The hypothesis that endemic species will disperse better than their non-endemic congeners in the species pairs with wider distribution of the endemic species was tested by Chi-square test. Specifically, we built a 2 × 2 pivot table with number of species pairs in which trait value in endemic species was higher or equal to non-endemic species, and number of species pairs with an opposite pattern ($E \geq N$, $E < N$) representing the columns. The rows were represented by number of species pairs in which number of islands occupied by the endemic species was higher or equal to non-endemic species, and number of species pairs with an opposite pattern (E ≥ N, E < N). This analysis was done for each dispersal mode separately (including the most likely dispersal mode).

Box plots and non-parametric tests were done in Statistica 7.0 (Statsoft, 2013). All the other analyses were done in S-plus 6.2 Professional (S-plus, 2000).

Results

There were only very few significant correlations among the tested dependent variables (Table 3) and the results based on different measures of dispersal ability were thus largely independent on each other. We found significant differences between endemic and non-endemic species in plant height, buoyancy and seed viability after simulation of endozoochory (Table 4). Plant height showed the strongest difference between endemic and non-endemic species, but the trait values indicated higher dispersal ability of endemic species (Figure 1A). The same pattern was shown for buoyancy (Figure 1B). In contrast, in the case of seed viability after simulation of endozoochory, non-endemic species showed higher dispersal ability than endemic species (Figure 1C). Endemic and non-endemic species did not significantly differ in terminal velocity (even when seed mass was used as a covariate), dispersal distance, seed survival in salt water, diaspore adhesion, seed mass and seed viability (Table 4). No significant relationship between the two groups of species was found also when testing buoyancy as $T_{50.}$

When considering only the most likely dispersal mode of each species pair, we found no significant differences in the values of dispersal traits between endemic and non-endemic species ($p = 0.52$, $F = 0.434$). Anemochory (dispersal distance) was the most likely dispersal mode in 15 out of 27 pairs. Five species pairs had exo- and endozoochory as the most likely dispersal mode. Hydrochory was the most likely dispersal mode for only 2 pairs (Table 2).

For the most likely dispersal mode, 18 out of the 27 pairs showed that endemic species had the same or better dispersal ability than non-endemic species. The number of pairs in which the endemic species had the same or better dispersal ability than non-endemic species for the other dispersal modes ranged between 16 and 21. The hypothesis that endemic species will disperse better than their non-endemic congeners in the species pairs with wider distribution of the endemic species was supported only for the most likely dispersal mode ($p = 0.03$, Table 5). The relationship between dispersal ability and number of occupied islands for the other dispersal modes was not significant ($p > 0.069$ in all cases).

Discussion

In our study we explored possible evolutionary changes in dispersal abilities of island species. We tested for differences between pairs of endemic and non-endemic species for the single dispersal mode. In contrast to many previous studies (e.g. Cody and Overton, 1996; Fresnillo and Ehlers, 2008), all the species were collected on islands. Thanks to this, all the species originated from the same environmental conditions. The possible differences in their traits were thus a reflection of their differential evolutionary history rather than of the different environments in which they are currently occurring.

In contrast to our expectation and to conclusions of previous studies (Carlquist 1974, Kudoh et al. 2013) the reduction of dispersal ability between endemic and non-endemic species was demonstrated only for endozoochory. Hydrochory and plant height showed the opposite pattern, i.e. endemic species dispersed better than its non-endemic congeners.

The inconsistent results when testing single dispersal modes are most probably given by the fact that each species does not have the same effectiveness in all dispersal modes. By testing each dispersal mode for all species, we included species which probably do not use the tested dispersal mode in reality. A species can be hardly expected to undergo selection for reduced dispersal ability by the dispersal mode by which it does not disperse (Cody and Overton, 1996; Kiviniemi and Eriksson, 1999, Yang et al. 2012). Surprisingly, the only dispersal mode with reduced dispersal ability in endemic species was endozoochory, which is not well represented in our species selection due to the bad storability of fleshy fruited seeds.

In contrast to our expectation, we, however, did not find significant differences even when testing differences in the most likely dispersal mode for each species pair. A possible explanation of this could be the fact that the methodology for identifying the most likely dispersal mode is not unified. We found a considerable inaccuracy in the method description in studies detecting the most likely dispersal mode. For example, some authors did not properly mention whether the stated dispersal mode is really the most likely one or the only tested one (e.g. Médail and Quézel, 1999; Vargas, 2007). Thus the identified most likely dispersal mode may not be the most likely mode in reality in some cases. Moreover, for some species the most likely dispersal mode differs according to different authors (e.g. for *Artemisia* anemochory in Médail and Quézel, 1999 or exozoochory in Huang et al., 2000). However, even after changing the most likely dispersal mode of some species the endemic and non-endemic species did not significantly differ in their dispersal ability.

The most likely dispersal mode can vary also according to the spatial scale considered. For example, the *Cistus* species are expected to be dispersed mainly by myrmecochory (Guzmán and Vargas, 2009). However, endozoochory is expected to be the main dispersal mode acting at long distances (Malo et al., 2000). In this study, we did not consider the modes acting exclusively on small spatial scales (e.g. autochory, myrmecochory and zoochory mediated by lizards or mammals). All our conclusions are thus based on the modes which are likely allowing species to disperse to long distances.

Endemic species dispersed better than non-endemic species in more than half of tested pairs. This could be explained by evolution of better dispersal ability during species colonization of additional islands. As a support of this theory we found for the most likely dispersal mode that endemic species disperse better than their non-endemic congener in the species pairs with wider distribution of the endemic species. No such relationship was however found for the single dispersal modes.

The theory of evolution of better dispersal ability in consequence of colonization of other islands is consistent with Darwin´s thinking about islands as stepping stones (Darwin, 1859). Such model was proposed for Hawaiian Islands and is based on the assumption that the colonized islands are not merely final areas for dispersal, but they can work as transient sites for dispersal to other subsequently emerging islands (e.g. during glacial sea-level minima, Carlquist, 2009; Fernández-Palacios et al., 2011; Harbaugh et al., 2009; Hess et al., 2000). The example of such species which had most probably initially colonized the Canary Islands and after their diversification they settled also on other Macaronesian islands (e.g. Madeira and Cape Verde) are species of the genus *Echium* (Böhle et al,. 1996; García-Maroto et al., 2009), *Pericallis* (Panero et al., 1999), *Phagnalon* (Montes-Moreno et al., 2010) and *Sonchus* (Kim et al., 1996).

Another possible explanation of high dispersal ability of endemic species is the shift of the dispersal modes during species evolution. Such possibility has already been tested for some Canary Island (Vargas, 2007) and Galápagos (Vargas et al., 2012) plant species. However, on both archipelagos no shifts within a plant group have been confirmed from the phylogenies available.

The absence of reduction of dispersal ability for endemic species can be also due to the proximity to the African continent. The majority of species is potentially able to get over 100 km distance between Fuerteventura and Africa, but is not able to successfully colonize continental habitats due to their competition ability and the absence of free niches (Emerson, 2002; Francisco-Ortega, 2000; Silvertown, 2004, but see Herben et al., 2005).

In our paper we primarily suppose that endemic species evolved from their non-endemic congeners which arrived on islands from the mainland. Thus non-endemic species should occur on islands for longer period of time than the endemic species. However, we have also to take in account that non-endemic species from mainland populations support the island populations with continuous diaspores dispersal. The island populations can thus be a mixture of both island and mainland individuals (see the discussions in Silvertown, 2004, Silvertown et al., 2005 vs. Carine, 2005 and Herben et al., 2005).

In any case, according to Marrero and Francisco-Ortega (2001), the majority of island endemic lineages is of recent origin and hardly exceeds 4-5 million years. The recent colonization of the Canary Islands followed by rapid speciation is considered e.g. for the genus of *Cistus* (Guzmán and Vargas, 2010), *Echium* (Böhle et al., 1996), *Euphorbia* (Molero et al., 2002), *Lotus* (Allan et al., 2004) and *Tolpis* (Moore et al., 2002). The comprehensive investigation of the timing of species radiation within other Macaronesian clades and their mainland relatives is, however, still missing, despite the increase of number of molecular phylogenetic studies in recent years (Carine, 2005; Caujapé-Castells, 2011; Kim et al., 2008).

The rate of evolution of dispersal traits is another topic which has not been fully resolved yet and can differ between islands and mainland. While Jordano (1995) showed, that a large part of present day variation in dispersal traits of continental species has not changed over several million years, Cody and Overton (1996) demonstrated that the dispersal ability of island species can change within a few generations. Cheptou et al. (2008) obtained the same results for *Crepis sancta* in continental fragmented landscape.

Bramwell (1985) and Vargas (2007) presumed that endozoochory is the most successful mode in dispersal of species in the Canary Islands. In contrast, in our study anemochory was revealed as the most likely dispersal mode in majority of the species. Zoochory (both exo- and endo-) was also the most probable dispersal mode in Hawaii (73% of species, Carlquist, 1967) and in Galápagos (60% of species, Porter, 1983). The reason of such difference between the published evidence and our dataset is because we did not primarily analyze fleshy fruited species due to their limited storability. Additionally, such species are mostly tertiary endemic relicts, whose non-endemic ancestors have already been extinct. We are aware that our approach is simplified and it is not without obstacles. However, to our knowledge, it is the most extensive study of the evolution of plant dispersal ability as it encompasses 54 different species tested on 4 dispersal modes. In contrast to the previous studies on the evolution of dispersal ability on the islands, we did not support the hypothesis about the loss of dispersal ability of island species. The previous studies were, however, mostly based on much simpler design (usually one type of dispersal and a few species). By comparing larger number of dispersal traits over larger number of species, our study is likely providing more complicated, but much more realistic insights into the possible evolution of dispersal traits on islands.

Conclusions

Our results did not explicitly support the hypothesis about the loss of dispersal ability of the island species. We suggest that maintaining good dispersal ability is probably related to species subsequent dispersal among islands. We also demonstrate that we need to take into account multiple species traits to understand the possible evolution of its dispersal potential.

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Table 1: List of 27 species pairs used in the study (the first mentioned is endemic species)

Species name[1]	**Family**	**Phylogeny**	**Analysed propagule**
Artemisia thuscula Cav.	Asteraceae	Sanz et al., 2008	Seed
Artemisia reptans C. Sm. *in* Buch			
Asteriscus graveolens ssp. *stenophyllus* (Link) Greuter	Asteraceae	Goertzen et al., 2002	Achene
Asteriscus aquaticus (L.) Less.			(with pappus)
Atractylis preauxiana Sch. Bip.	Asteraceae	No	Achene
Atractylis cancellata L.			(with pappus)
Brachypodium arbuscula Knoche	Poaceae	Catalán and Olmstead, 2000	Seed
Brachypodium sylvaticum (Huds.) P. Beauv.			(with glumes)
Carex canariensis Kük.	Cyperaceae	Hendrichs et al., 2004	Seed
Carex divulsa ssp. *divulsa* Stokes			(with utricle)
Cistus ocreatus C. Sm. *in* L. von Buch	Cistaceae	Guzmán and Vargas, 2010	Seed
Cistus monspeliensis L.			
Echium wildpretii ssp. *wildpretii* Pearson *ex* Hook. *f.*	Boraginaceae	Böhle et al., 1996	Seed
Echium plantagineum L.			plus calyx

Species name[1]	**Family**	**Phylogeny**	**Analysed propagule**
Erica platycodon spp. *platycodon* (Webb & Berthel.) Rivas-Mart. & al. *Erica arborea* L.	Ericaceae	McGuire and Kron, 2005	Seed
Euphorbia atropurpurea (Brouss.) Webb & Berthel. *Euphorbia regis-jubae* Webb & Berthel.	Euphorbiaceae	Barres et al., 2011	Seed
Geranium reuteri Aedo & Muńoz Garm. *Geranium robertianum* L.	Geraniaceae	Based only on morphology Yeo, 1973	Seed
Helianthemum thymiphyllum Svent. *Helianthemum canariense* (Jacq.) Pers.	Cistaceae	No	Seed
Hypericum glandulosum Aiton *Hypericum perforatum* L.	Hypericaceae	No	Seed
Lotus tenellus Dryand. *in* Aiton *Lotus glinoides* Delile	Fabaceae	No	Seed

Species name[1]	Family	Phylogeny	Analysed propagule
Pancratium canariense Ker-Gawl.	Amaryllidaceae	No	Seed*
Pancratium maritimum L.			
Phagnalon umbelliforme DC.	Asteraceae	Montes-Moreno et al., 2010	Achene (with pappus)
Phagnalon saxatile (L.) Cass.			
Plantago arborescens ssp. *arborescens* Poir.	Plantaginaceae	Rahn, 1996	Seed
Plantago afra L.			
Polycarpaea aristata (Aiton) DC.	Caryophyllaceae	No	Seed
Polycarpaea nivea (Aiton) Webb			
Reichardia ligulata (Vent.) G. Kunkel & Sunding	Asteraceae	Kim et al.., 2007	Achene (with pappus)
Reichardia tingitana (L.) Roth			
Reseda crystallina Webb & Berthel.	Resedaceae	Martín-Bravo et al., 2007	Seed
Reseda luteola L.			
Rumex lunaria L.	Polygonaceae	Navajas-Pérez et al., 2005	Achene
Rumex vesicarius L.			

Species name[1]	Family	Phylogeny	Analysed propagule
Salvia canariensis L.	Lamiaceae	Walker and Sytsma, 2007	Seed
Salvia aegyptiaca L.			
Scrophularia smithii ssp. *smithii* Hornem.	Scrophulariaceae	No	Seed
Scrophularia arguta Sol. *ex* Aiton			
Senecio palmensis (C. Sm. *in* Buch) Link	Asteraceae	No	Achene (with pappus)
Senecio glaucus ssp. *coronopifolius* (Maire) C. Alexander			
Solanum vespertilio Aiton	Solanaceae	No	Seed
Solanum nigrum L.			
Sonchus canariensis ssp. *canariensis* (Sch. Bip.) Boulos	Asteraceae	Kim et al., 1996	Achene (with pappus)
Sonchus oleraceus L.			
Tolpis laciniata (Sch. Bip. *ex* Webb & Berthel.) Webb	Asteraceae	Moore et al., 2002	Achene (with pappus)
Tolpis barbata (L.) Gaertn.			
Vicia cirrhosa C. Sm. *ex* Webb & Berthel.	Fabaceae	Leht, 2005	Seed
Vicia hirsuta (L.) S. F. Gray			

[1] The species names according to Arechavaleta et al. (2010)

[*] Diaspores from the Botanical Garden

Table 2: The main dispersal modes and species distribution of 27 species genera

Genus	Most likely dispersal mode[1]	relationship between dispersal values in E and N[2]	No. of occupied islands (E / N)[3]
Artemisia	ANEMO Médail and Quézel, 1999	E ≥ N	5 / 3
Asteriscus	ANEMO	E ≥ N	1 / 4
Atractylis	EXO Hensen, 1999	E < N	2 / 6
Brachypodium	ANEMO Bonet and Pausas, 2004	E < N	3 / 5
Carex	ANEMO Bonet and Pausas, 2004	E ≥ N	5 / 7
Cistus	ENDO Malo et al., 2000	E < N	1 / 5
Echium	ANEMO Bramwell, 1985	E ≥ N	1 / 5
Erica	ANEMO McGuire and Kron, 2005	E < N	3 / 7
Euphorbia	ENDO Carlquist, 1967	E ≥ N	1 / 5
Geranium	EXO Yeo, 1973	E ≥ N	5 / 5
Helianthemum	EXO Kiviniemi and Eriksson, 1999	E ≥ N	2 / 7
Hypericum	ANEMO Médail and Quézel, 1999	E ≥ N	2 / 5

Genus	**Most likely dispersal mode[1]**	**relationship between dispersal values in E and N[2]**	**No. of occupied islands (E / N)[3]**
Lotus	ENDO Bramwell, 1985	E < N	2 / 6
Pancratium	HYD	E ≥ N	7 / 2
Phagnalon	ANEMO Bonet and Pausas, 2004	E ≥ N	4 / 7
Plantago	EXO Bramwell, 1985	E < N	5 / 7
Polycarpaea	ANEMO	E ≥ N	3 / 6
Reichardia	ANEMO Bonet and Pausas, 2004	E ≥ N	6 / 6
Reseda	ANEMO Bonet and Pausas, 2004	E < N	3 / 6
Rumex	HYD Carlquist, 1967	E ≥ N	7 / 7
Salvia	EXO Melendo et al., 2003	E ≥ N	7 / 5
Scrophularia	ANEMO + BAL Van der Pijl, 1982	E ≥ N	1 / 7
Senecio	ANEMO Bonet and Pausas, 2004	E < N	2 / 6
Solanum	ZOO Zanin et al., 1997	E < N	1 / 7
Sonchus	ANEMO Bonet and Pausas, 2004	E ≥ N	2 / 7

Genus	Most likely dispersal mode[1]	relationship between dispersal values in E and N[2]	No. of occupied islands (E / N)[3]
Tolpis	ANEMO Bramwell, 1985	E ≥ N	4 / 7
Vicia	ENDO	E ≥ N	5 / 5

[1] most likely dispersal mode was obtained mainly from the literature, where the literary data are lacking, the mode was estimated according to our experience with dispersal and seed morphology, ANEMO – anemochory, HYD – hydrochory, EXO – exozoochory, ENDO – endozoochory, ZOO – zoochory, BAL – ballistic dispersal

[2] E = endemic species, N = non-endemic species

[3] distribution on islands according to Arechavaleta et al. (2010)

Table 3: Matrix of correlation coefficients (r values) between the measured traits, N = 54

	Plant height	**Seed viability after simulation**	**Dispersal distance**	**Seed adhesion**	**Buoyancy**	**Terminal velocity**	**Seed survival in salt water**	**Seed mass**
Seed viability after simulation	-0.08							
Dispersal distance	**0.73**	-0.11						
Seed adhesion	0.02	-0.11	-0.14					
Buoyancy	0.27	-0.11	0.14	0.19				
Terminal velocity	0.18	0.17	**-0.47**	0.19	0.01			
Seed survival in salt water	-0.11	0.04	0.05	0.03	**0.28**	-0.11		
Seed mass	0.19	0.10	-0.26	0.26	-0.17	**0.76**	-0.11	
Seed viability	-0.03	0.22	**-0.32**	0.11	-0.25	**0.52**	-0.12	**0.46**

Values in bold are significant at 0.05 p-level

Table 4: Analysis comparing differences in dispersal traits between pairs of endemic and non-endemic species. F and D, Z indicates if the given value represents value of F statistics (for ANOVA]), deviance explained (for logistic regression) or Wilcoxon match pair test. DF Error = 26.

Dispersal mode	**Measured trait**	**Species pair DF = 26**		**End. vs. non-end. DF = 1**	
		p	**F / Deviance**	**p**	**F / Deviance**
ANEMOCHORY	Terminal velocity	< 0.001	30.778 F	0.675	0.180 F
	seed mass as covariate	< 0.001	21.487 F	0.847	0.038 F
	Dispersal distance	< 0.001	4.434 F	0.073	3.480 F
HYDROCHORY	Buoyancy	< 0.001	265.011	< 0.001	12.014 D
	Seed survival in salt water	< 0.001	242.759	0.473	0.515 D
	T_{50}			0.156	1.419 Z
EXOZOOCHORY	Seed adhesion	< 0.001	109.340	0.111	2.534 D
ENDOZOOCHORY	Seed viability after simulation	< 0.001	450.701	0.016	9.498 D
Other traits	Seed mass	< 0.001	6.956	0.524	0.136 F
	Seed viability	< 0.001	1070.586	0.821	0.051 D
	Plant height	0.006	2.789	0.045	4.439 F

Table 5: 2 × 2 pivot table showing the number of species pairs with E≥N and E<N relationship in number of occupied islands (rows) and in trait values of most dispersal mode (columns), p=0.03, N=27.

		most likely dispersal mode		
		E ≥ N	E < N	total
no.of islands	E ≥ N	7	0	7
	E < N	11	9	20
	Total	18	9	27

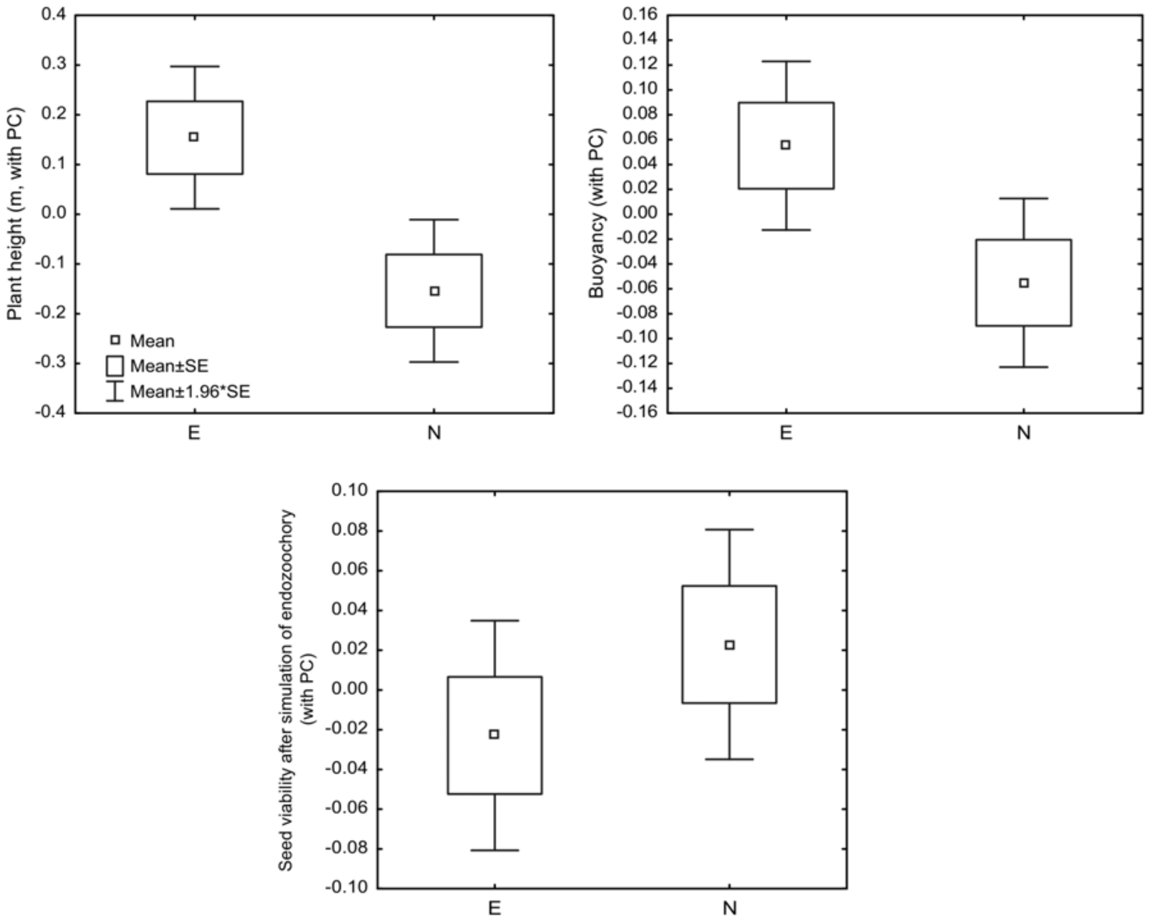

Figure 1: Box plots showing the differences between endemic and non-endemic species in (A) plant height ($p < 0.05$), (B) buoyancy ($p < 0.001$) and (C) seed viability after simulation of endozoochory ($p < 0.05$) after phylogenetic correction (PC).

Paper 3

THE IMPORTANCE OF SPECIES TRAITS FOR SPECIES DISTRIBUTION ON OCEANIC ISLANDS

Kristýna Vazačová and Zuzana Münzbergová

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Paper 3: Species traits influence its distribution on islands

The importance of species traits for species distribution on oceanic islands

Kristýna Vazačová[1, 2*] **and Zuzana Münzbergová**[1, 2]

[1] Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, Prague 2, CZ-128 01, Czech Republic

[2] Institute of Botany, Academy of Sciences of the Czech Republic, Lesní 1, Průhonice, CZ-252 43, Czech Republic

[*] Author for correspondence, email: vazacova@seznam.cz, fax: +420221951645

Abstract

Understanding species´ ability to colonize new habitats is a key knowledge allowing us to predict species´ survival in the changing landscapes. However, most studies exploring this topic observe distribution of species in landscapes which are under strong human influence being fragmented only recently and ignore the fact that the species distribution in these landscapes is far from equilibrium. Oceanic islands seem more appropriate systems for studying the relationship between species traits and its distribution as they are fragmented without human contribution and as they remained unchanged for a long evolutionary time. In our study we compared the values of dispersal as well as persistence traits among 18 species pairs from the Canary Islands differing in their distribution within the archipelago. The data were analyzed both with and without phylogenetic correction. The results demonstrate that no dispersal trait alone can explain the distribution of the species in the system. They, however, also suggest that species with better dispersal compared to their close relatives are better colonizers. Similarly, abundance of species in the archipelago seems to be an important predictor of species colonization ability only when comparing closely related species. This implies that analyses including phylogenetic correction may provide different insights than analyses without such a correction and both types of analyses should be combined to understand the importance of various plant traits for species colonization ability.

Introduction

Species ability to disperse and colonize new habitats is a key prerequisite for their response to ongoing landscape and climate changes [1, 2]. Understanding, which are the main traits responsible for this ability, is thus fundamental for prediction of future fates of different species [3, 4]. Many recent studies are attempting to understand the importance of species traits for species ability to colonize habitats of different size and isolation (e.g. [5, 6]). Most of these studies are done in various fragmented landscapes, predominantly in grasslands and forests. Often these studies demonstrate that species distribution is not only determined by current landscape structure, but is largely a result of landscape structure in the past (e.g. [7, 8]).

Strong species response to past landscape structure can be attributed to slow growth dynamics of many perennial species in combination with relatively fast changes in the current landscapes [9, 10]. Due to dispersal limitation [11, 12] and extinction debt [13, 14] the distribution of these species may reflect historical habitat configuration. Species distribution in the landscape may then not reflect species long-term ability to successfully colonize habitats and to survive there. Thus the traits driving species distribution on young habitat fragments in a changing landscape can be different from those in the landscapes fragmented for longer evolutionary time [15, 16].

Due to intensive human activity all over the world, it is rather difficult to identify fragmented habitats which remained unchanged for a long time period, on which we could study species ability to colonize new habitats on long time scales. Oceanic islands seem to be suitable candidates of such systems [17, 18]. In contrast to continental landscape, oceanic fragments are not a result of human activity and remained almost unchanged in size and number since their origin. Thus the islands are generally thought to be more stable in time as they are fragmented and isolated for much longer time periods. For these reasons they are suitable systems for studying the importance of dispersal traits for species occurrence on isolated patches. Similarly to the studies on habitat fragments on the mainland (e.g. [19, 20]), we can predict that species occurring on the youngest and the most isolated islands will have higher dispersal ability than species present on older and more connected islands.

In this study, we analyzed species traits determining distribution of selected native species on the Canary Islands. The Canary archipelago is a suitable model system as it consists of islands differing in their age, size and isolation as well as in species composition.

Specifically, we attempted to understand the determinants of species presence on the newest, smallest and most isolated island (El Hierro).

Because closely related species often share a wide range of biological traits, distribution of a species may be related to the traits under study or to other traits correlated with these traits that are characteristic for the whole clade to which the species belongs [21, 22]. Comparison of results of analyses with and without phylogenetic correction can help in distinguishing between the traits that are really responsible for a pattern and traits correlated with these within larger species groups. The necessity of phylogenetic correction is a highly debated issue (e.g.[21, 22, 23]) and it has been suggested that the phylogenetic and ecological explanations for species distribution in a landscape are not mutually exclusive (see also [24]). Separating the phylogenetic and ecological explanations for species distribution is thus difficult. It is, however, generally recognized that both of these types of analyzes should be considered when trying to explain the effect of species traits on species distribution (e.g. [25]).

To consider species phylogeny in this study, we compared dispersal values of 18 pairs of closely related species differing in their presence on El Hierro. In addition, we used the same species to test the relationship between species traits and number of occupied islands in the archipelago. For each species, we assessed the dispersal ability by all possible dispersal vectors acting on islands, i.e. wind, water and animals (anemo-, hydro-, exo- and endozoochory). We also used published sources complemented with our field experience to identify the most likely dispersal mode for each species pair.

Even though nowadays some parts of the islands are quiet heavily inhabited, we suppose that the main dispersal events happened before human´s strong influence. Also none of the studied species is purely ruderal. All the species occur in some (semi)-natural habitats such as laurel forests and canary pine woodlands. Such communities obviously suffer from human destructive activities being fragmented and reduced in area, but species extinctions on single islands occur only rarely and were not reported for any of our model species [26] .

Although dispersal ability is widely considered as a major determinant of species distribution on islands due to their isolation, other traits, especially those related to species persistence on habitats should not be overlooked as was shown in studies e.g. by Maurer et al. [27] and Saar et al. [8]. For this reason we also tested traits related to species survival and persistence on the islands (i.e. species longevity and woodiness) and traits characterizing species distribution serving as a proxy for amount of seeds available (number of vegetation zones and number of islands occupied by a species). As a number of occupied islands itself can be a function of plant traits, we also explored the life history traits associated with number of occupied islands.

Specifically, we asked the following questions: 1) Which life history traits explain species presence on El Hierro? 2) Which life history traits explain the number of islands occupied by the species? 3) How do the conclusions change when applying phylogenetic correction?

We predict that species occurring on El Hierro will have better dispersal ability and will occupy more islands than species not occurring there. We also expect that species occupying more islands will be more likely r-strategists possessing traits, which enable rapid colonization of free space on islands (i.e. non-woody annuals occupying more vegetation zones).

Materials and Methods

Ethic statement

To test exozoochorous dispersal, we used a pigeon of the King breed, purchased from a local breeder. To minimize subjection to stress during the experiment, the animal was caged in its home aviary (2 × 1.5 × 1 m) and had free access to commercial diet and water. The bird was not subjected to any invasive intervention which could cause him suffering. As he was tamed since his youth, his manipulation during seed incorporation into feather did not cause him extreme stress. The manipulation with pigeon was approved by Ministry of Education, youth and sport of the Czech Republic (permission no. 24773/2008-10001) and complied with the relevant legislation of the Czech Republic (article 11, regulation no. 207/2004).

Study site

The Canary Islands are part of the Macaronesian archipelago situated between 27°45´and 29°2´N and between 18°00´and 13°37´W. They consist of 7 main volcanic islands differing in age and size (Figure 1). The age of the islands decreases with increasing distance from the closest mainland (Africa) and from east to west; the easternmost islands are the oldest, while the westernmost are the youngest. Vegetation composition and habitat diversity on islands is highly influenced by altitudinal gradients in combination with predominant north-eastern trade winds [28]. The oldest and most eroded islands Lanzarote and Fuerteventura lack forests, other, steeper and roughed islands (Gran Canaria, Tenerife, La Palma, La Gomera, El Hierro) are covered by thermo-sclerophyllous woodlands, evergreen laurel forests and canary pine woodlands. The highest parts of Tenerife and La Palma host meso-oromediterranean summit broom scrubs [29].

Species selection

We selected 36 species belonging to 22 genera and 15 families, all native to the Canary Islands [30]. The species were grouped into pairs (Table 1). The species within the pair usually belong to the same genera. In three pairs, the two species in the pair represented closely related genera from the same family. Within each pair, the species differed in occurrence on El Hierro, on the youngest Canary Island, but they both were present on the adjacent islands (at least on Tenerife and La Palma or Tenerife and La Gomera). We chose Tenerife as it is considered as a centre of biodiversity of the area and thus can play a key role as a source for species dispersal to the westernmost islands ([31], but see [32]). La Gomera and La Palma were chosen because of their relative proximity to El Hierro and due to their similar size. All the three islands are also similar to El Hierro in the main vegetation zones including *Euphorbia* scrubs, thermo-sclerophyllous woodlands, evergreen laurel forests and canary pine woodlands. Due to these similarities we can suppose that species present on Tenerife and La Palma or Tenerife and La Gomera and not on El Hierro are those which have not been able to reach El Hierro due to dispersal limitation and not due to ecological barriers related to the absence of habitat [31].

We are aware that species presence/absence on El Hierro could be potentially mediated also by human activities. However, this island is less inhabited than the other Canary Islands. While some of the selected genera may occur in ruderal habitats (e.g. the genus of *Reseda*, *Senecio*, *Trifolium*), all of these occur also in some (semi)-natural habitats such as laurel forests and canary pine woodlands and could thus be distributed on the islands prior to increased human activities. We thus suppose that the main dispersal events happened before human´s strong influence.

Species selection was further limited to species for which sufficient seed samples could be obtained. For this reason we had to exclude all the previously considered species pairs having fleshy fruits.

Diaspore collection

Diaspores (fruits or seeds representing the most probable dispersal units, see Table 1) for each species were collected in natural populations on the islands except for *Limonium* species. Diaspores of *Limonium* were obtained from the populations in the Botanical Garden “Jardín Canario Viera y Clavijo”, Gran Canaria. The garden populations originally come from the island populations.

The collection from protected areas was done in cooperation with the Botanical Garden “Jardín Canario Viera y Clavijo”, Gran Canaria which obtained appropriate permission for collecting seeds for scientific purposes. The permission was issued by Consejería de Medio Ambiente y Aguas, Islas Canarias. The permission for seed collection from unprotected areas was not required.

In the field we preferably sampled 3 populations per species. For each population, we aimed to collect diaspores from at least 8 individuals. Each population was then tested for dispersal abilities separately. Garden collection was considered as one population and we sampled seeds from 8 individuals in the garden. To have the same number of measurements for the species with seeds collected from the field and from the garden, we had 3 replicates for each dispersal experiment for diaspores collected in the garden.

We used 20 diaspores per species and population for experiments with anemochory, hydrochory and exozoochory and 30 diaspores for testing endozoochory, i.e. 60 and 90 diaspores, respectively. Such number was a compromise between a large amount of species tested and number of seeds used in the literature (c.f. [33]).

For testing other traits related to dispersal (i.e. seed mass and seed viability) we used simple seeds, not fruits. In dispersal modes, where we used fruits as dispersal units, but accounted also for seed viability (i.e. hydrochory and endozoochory), the number of all seeds extracted from the fruits was used as a baseline number of seeds.

Data on all traits used in the study are provided in Supplementary Information (Table S4 and S5).

Traits related to dispersal

Anemochory

The ability of diaspores to disperse by wind was estimated as terminal velocity defined as the maximum rate of seed falling in still air [34]. It was measured as the flight time of a diaspore from predefined height (270 cm [35]). Mean dispersal distance D was expressed as:

$$D = (w \times h) / t$$

where w is the wind speed (being constant for all species), h is the average plant height and t is the terminal velocity. Values of average plant height were obtained from the literature [36, 37, 38, 39, 40]

We are aware that our dispersal model is simplified. Nevertheless, it has been successfully used in other studies to characterize mean dispersal distance of diaspores (e.g.[11, 6]) and is the easiest way to combine the three key variables affecting wind dispersal. We thus suggest that it is a useful proxy of potential wind dispersal distances for comparison among species.

In the analyses, we used both terminal velocity (m/s) and mean dispersal distance (m). In addition, we tested for the difference in plant height between species present on El Hierro and species absent from El Hierro to see to what extent are the differences in dispersal distance affected by differences in plant height.

Hydrochory

The potential of diaspores to disperse in salt water (buoyancy) was measured as the proportion of diasporess still floating after a defined time period. Diaspores were gently put into beakers filled with salt water having 3.7% salinity (i.e. average salinity of the Atlantic

Ocean along the Canary Islands coast). The size of beakers was proportional to the size of diaspores. Sea waves were simulated by continual shaking in electric orbital shaker with frequency of 100 shakes per min. The number of diaspores floating on water surface was checked immediately after putting them into bins and then after 5 minutes of shaking, 1, 2, 6, 24 hours and 7 days of shaking [41]. The experiment was finished after 1 week of diaspores shaking as it is the minimal time a diaspore needs for reaching the Canary islands from mainland when taking into account average speed of water currents in the Atlantic Ocean (60-90 km per week [42]) and the distance between mainland and the closest island (Africa to Fuerteventura, 96 km).

At the end of the experiment, the number of floating and number of sunk diaspores was counted and the two groups of diaspores were then tested for seed viability.

In the analyses, we used the proportion of viable seeds which kept floating until the end of the experiment from the total number of viable seeds before the experiment.

The diaspore buoyancy was also expressed as T_{50}, the number of minutes, after which 50 percent of diaspores was still floating. This parameter is commonly used in other studies assessing hydrochory [43, 41] however it does not take into account seed viability.

We also used the information on effect of salt water on viability of seeds expressed as the proportion of viable seeds after the experiment (both floating and sunk)/seed viability before the experiment. Viability of seeds was tested by dying the seeds with 0.1% solution of 2,3,5-triphenyl-2H-tetrazolium chloride [44]. In contrast to germination tests, it is not dependent on selection of the right conditions for germination for each individual species and it is thus in fact more reliable for between species comparisons.

Zoochory

Birds are the most important long-distance island dispersers transporting diaspores both externally and internally. The main bird dispersers acting on the Canary Islands are blackbirds (*Turdus merula*), robins (*Erithacus rubecula*), blackcaps (*Sylvia atricapilla* and *S. melanocarpa*, [45]), common ravens (*Corvus corax*, [46]), gulls (*Larus cachinnans*, [47]) and pigeons (*Columba livia*, *C. junoniae* and *C. bolli*).

Bird exozoochory (Epizoochory)

Bird exozoochory was tested as diaspore adhesion to bird feathers. As a model species we used a pigeon of the King breed, a utility breed with poor flight ability that is amenable to our experiments.

Although this species is clearly not native to Canary Islands, the functionality of its feathers for diaspore dispersal is readily comparable with native insular pigeon species.

As the seed coat of some species (e.g. *Plantago, Lobularia*) contains mucilaginous substances which become sticky when wet, all the diaspores were moistened before the application into pigeon feather. Moistened diaspores were gently incorporated into feathers on 4 different body parts (on bust, neck and back, under wing). After 1 hour of pigeon free movement in an aviary we checked the numbers of diaspores still attached to feathers. Taking into account the average flight speed of a trained pigeon (80 km/h [48]) and the shortest distance between mainland and the closest island (96 km), diaspores which remained attached to feathers after 1 hour are potentially able to get to the islands by this type of dispersal.

In the analyses we tested the proportion of diaspores which kept attached to feathers after 1 hour (we refer to this value as seed adhesion). This parameter lacks the effect of real bird flight as we do not take into account the air movement around feathers during the flight that can dry out diaspores and cause them to drop earlier than in our simulation. However some behavior of our pigeon during seed testing such as cleaning of feathers was similar to behavior of wild birds. Thus, we still think that our data are sufficient for the purpose to differentiate among diaspores with different ability to disperse by exozoochory.

Bird endozoochory

Bird endozoochory was tested by simulating diaspore gut passage through pigeon digestive tract. Plastic flasks filled up with diaspores were shaken with wet grit (small stones eaten by birds to enhance digestion, commercial mixture for pigeons) for 24 hours in electric orbital shaker (200 shakes per minute [49]). Then diaspores were separated from the grit, rinsed and immersed in 5 ml of 1M H_2SO_4 (pH ≈ 0.3 [50]) for 4 hours. Intact seeds were retrieved, counted and tested for viability. The proportion of number of viable seeds which survived the simulation to the number of seeds viability before the experiment was used in the analysis. Seed viability after simulation was tested as described above.

Seed mass

Altogether 90 seeds per species were weighted. For this purpose, they were divided to groups by 10 to 30 seeds per group (10 seeds in the group for the largest and 30 for the smallest seeds, to get reasonable size estimates given by the precision of the balance, 0.0001g). Seed mass is generally recognized as a rough proxy of seed dispersal ability and germination ability (e.g. [51, 6]). The same amount of seeds was used for viability testing of intact seeds.

Most likely dispersal mode

For all species pairs the most likely dispersal mode was estimated from available literature (Table 1). Where such data were missing, we estimated the dispersal mode according to our experience with dispersal and diaspore morphology of the species.

Traits related to persistence and distribution

Data on species longevity (short-lived vs. perennial), woodiness (woody vs. not woody) and the number of vegetation zones with species occurrence were gained from Bramwell and Bramwell [38] and Schönfelder and Schönfelder [39, 40].

Species distribution was expressed as a number of occupied islands, according to Arechavaleta et al. [30].

Data analysis

To test the importance of life history traits for species presence on El Hierro, we used a generalized linear model with binomial distribution. Species category (present on El Hierro vs. absent from El Hierro) was used as dependent variable and species traits as independent variables. In this analysis the number of islands occupied by a species was counted excluding El Hierro as the effect of El Hierro is already included in the dependent variable.

The importance of traits for species distribution among islands was tested by log-normal regression. Number of islands occupied by a species was used as dependent variable and species traits as independent variables.

The analyses were also performed with phylogenetic correction. Because the exact phylogenetic relationships between the studied species are unknown, we used the simplest version of phylogenetic correction based on comparison of species within the pairs (e.g.[6]). The corrected trait values PC were calculated by applying the formula:

$$PC = (S - MP) / MP$$

where S is the trait value of a single species (either present or absent from El Hierro) and MP is the mean of the trait value for each species pair. The phylogenetically corrected trait values were used in the tests as described above.

All the tests were done using two different approaches. First, we tested the effect of each trait separately. Afterwards, we combined all the traits in a single model and used forward step wise regression to select an optimal model.

To visualize the similarity between different species in their traits we used principal component analysis (PCA). The data on single species traits were treated as “species”, and data on each species represented “samples.” The analysis was centered and standardized by “species”; in this way all the traits were expressed in the same, relative, units.

Box plots were done in Statistica 7.0 [52], PCA was processed in CANOCO 4.5 [53]. All the other analyses were done in S-plus 6.2 Professional [54].

Results

Species present on El Hierro and species absent from El Hierro did not differ in any studied dispersal traits. There was, however, marginally significant effect on number of occupied islands (without El Hierro) (Table 2) with species present on El Hierro occupying more islands than species absent from El Hierro (Figure 2A). The results changed dramatically after incorporating phylogenetic correction into analyses. After phylogenetic correction, species presence on El Hierro was significantly influenced by dispersal distance, seed mass, species longevity and by the number of islands occupied by the species (Table 2). Species present on El Hierro dispersed further by wind, had smaller seeds, shorter life-span and occupied more islands than species absent from El Hierro (Figure 2B-D).

Number of islands occupied by a species was significantly influenced only by species longevity (Table 3). Species occupying more islands were more likely annuals than species occupying fewer islands. This trend remained the same even after phylogenetic correction. All the significant variables also remained in the model after stepwise regression showing that the traits are largely independent of each other (Tables 2 and 3).

Principal component analysis of dispersal traits showed that species within a pair are rather dissimilar in their traits (Figure 3). As seen in Figure 3, species are partly grouped according to the most likely dispersal mode.

Discussion

The results of the study indicated that species presence on El Hierro, the smallest, youngest and the most remote island is influenced by both dispersal and persistence traits as well as by the number of other Canary islands occupied by a species. This result was, however, found only after applying phylogenetic corrections. This suggests that the advantage of these traits is relative, and the traits thus play a role only after accounting for other possible differences between closely related species.

Contrasting results with and without phylogenetic correction were found previously also by e.g. Tremlová and Münzbergová [6] for dispersal traits, by Lanta et al. [55] for traits related to plant growth and by Stratton [56] for flower longevity pointing out the necessity for considering phylogenetic information in the analyses. The strong discrepancy between the two types of results is related to the stability of these traits within species phylogenies (e.g. [57, 58, 59]). The results obtained in this study should thus be interpreted not as the main effects of the given dispersal mode. In contrast, they e.g. suggest that within a given species group (sharing a wide range of biological traits) the species with relatively better dispersal are better colonizers.

Our expectation that species present on El Hierro disperse better than species absent from El Hierro holds only for wind dispersal mode. The importance of anemochory in dispersal among oceanic islands has been mentioned in classical islands studies [60, 61]. Regarding the Canary archipelago, seed transport from the eastern to the western islands (including El Hierro) can be mediated by northeasterly trade winds (which blew during arid Quaternary episodes [62]) as it was reported by e.g. Percy and Cronk [63] or Allan et al.[64]. However, when estimating dispersal distance using simply the data on terminal velocity, plant height and mean wind speed on islands (6.55 m/s [65]) and the nearest distance from El Hierro to neighboring island (La Gomera, 50 km), no species would be able to reach the island by wind. While such simple dispersal model is commonly used to approximate wind dispersal ability of species, such a model is rather simplified [66]. To estimate realistic dispersal distances of species we need to know also other parameters related to wind activity (mainly turbulence and updrafts) and island topography. Considering these types of data in the model is, however, beyond the scope of this study. Another indirect evidence for the importance of wind as an important dispersal mechanism on islands is that species present on El Hierro have smaller seeds (and thus more suitable for flying in the air) than species not present there. Generally, according to Lindborg et al. [25], species with smaller seeds are better dispersers, whereas those with large seeds are better recruiters and tend to have improved establishment in a wider range of habitats [67, 68] or when competing with neighbors (see [69]). However, the good competitive ability is not necessarily important for habitats on young volcanic islands arising de novo such as El Hierro. Additionally, the vegetation on El Hierro was repeatedly disturbed by volcanic activities causing extensive landslides further favoring good colonizers over good competitors.

No significant differences in other dispersal traits between species differing in the presence on El Hierro can signify that these species do not disperse by the tested dispersal modes in reality. For this reason we also tested the most likely dispersal mode, which was based on the selection of the most likely dispersal mode within species pair according to the literature. However, using the most-likely dispersal mode did not show significant differences between species present on El Hierro and absent from El Hierro. The use of such type of dispersal information from a variety of literary sources based on heterogeneous methodology for determining the most likely dispersal mode is questionable, but frequently practiced [70, 69]. As a result, the most likely dispersal mode differs according to different authors for some species (e.g. for *Euphorbia* hydrochory in Wald et al. 2005 [71] and endozoochory in Carlquist 1967 [72]). However, even after changing the most likely dispersal mode of some species there were no significant differences between species present and absent on El Hierro in their dispersal ability. Moreover, the only significant wind dispersal in our study was the most frequently chosen most likely dispersal mode. This suggests that the selection of the most likely dispersal mode is not so far from the reality.

According to our results, species present on El Hierro are distributed on more islands (excluding El Hierro) than species absent from El Hierro. This could be due to better wind dispersal ability of species on El Hierro. However, no dispersal trait significantly predicted number of islands occupied by the species. This suggests that better dispersal ability is not generally related to distribution on more islands as we could suppose. No relationship between dispersal and range size was shown e.g. by Kelly and Woodward [70], Goodwin et al. [73] and Lester et al. [74]. Lester et al. [74] assumed that dispersal may only influence species´ geographical distributions at certain spatial scales or in particular habitats or environment and/or within certain taxonomic groups, depending on how the mechanisms by which dispersal and range size are related.

The reason why the number of occupied islands is a good predictor of species´ presence on El Hierro could be that the number of occupied islands represents a measure of the amount of available sources (i.e. a proxy of number of plant populations or species abundance) for species´ colonization (e.g. [75]). Indeed, to properly measure the amount of available sources we should also know the species local abundances and seed production. Obtaining good information on these two characteristics is, however, rather complicated and such data are not available. Alternatively, number of occupied islands could also be linked to niche width as species with wider distribution range tend to have wider niches and thus more likely occupy a novel habitat (Knappová unpubl.).

Species longevity was another trait influencing species presence on El Hierro. Species on El Hierro were mainly short-lived (annuals and biennials) showing that short life span enabling rapid production of offspring can be an advantage for colonizing this westernmost island. Due to their ruderal strategy, short-lived species are usually able to grow on newly emerged or disturbed habitats indicating that the island vegetation is still developing. According to Kelly [76] and Kelly and Woodward [70] short-lived species are expected to have smaller ranges than perennials, which is in contrast to our results. We showed the opposite pattern; short-lived species have wider distribution among islands.

There are other possible traits such as seed bank longevity, seed production, pollination mode or detailed characteristics of species habitat requirements (e.g. in the form of indicator values) or species local abundance, which can influence species distribution as was shown e.g. by Pocock [23] and Gabrielová et al. [77]. These studies are mainly done on European species, where most of these data are available as a part of databases [78, 33] . No such complete data is, however, available for the whole flora of the Canary Islands.

Possible limitations of the study

Despite the above arguments explaining limited role of dispersal traits in species distribution we cannot exclude the possibility that the importance of species dispersal is undervalued due to our species selection, especially by excluding species with fleshy fruits. We excluded species with fleshy fruits primarily for practical reasons as we were not able collect sufficient number of fruits due to scarcity and the protection status of some of the potential species (e.g *Sambucus palmensis, Pleiomeris canariensis, Heberdenia excelsa*). However, as our species list involves mainly anemo- and exozoochorous species, addition of only few pairs of species with fleshy fruits would generate uneven distribution of dispersal modes resulting in few strong outliers. Such data could maybe lead to conclusion that dispersal is more important than we are suggesting based on the current results. On the other hand such a conclusion based on few outliers would not be very robust. We thus suggest that the limited species selection used in this study can also be viewed as an advantage as our study provides relatively robust conclusions for a wide range of anemo- and exozoochorous species.

Another possible critique of our study is that we are working with only 18 pairs of species. Species number was mainly limited due to the approach used to study dispersal, which was dependent on large number of seeds available for each species. Thanks to this approach, we were, however, able to obtain really detailed information on species dispersal by the main dispersal vectors acting among islands. In contrast, other dispersal studies dealing with more species are often based only on categorization of dispersal abilities inferred from the combination of seed visual observation and field experience [79, 80] or assessing dispersal by one dispersal mode only ([81]). Such approach enables to cover larger number of species, but species traits are only roughly assessed. As a result, the insights obtained in these studies are more general on one hand, but very rough on the other, not allowing to explore the importance of smaller differences in dispersal ability between different species. We suggest that the results obtained in our study are more likely to indicate possible long term fates of species in fragmented systems within sets of species of similar growth forms dispersing in similar ways.

Conclusions

The results demonstrated that the relationship between species distribution and species traits depends on the approach we use. Different results were obtained after incorporating phylogenetic relationship between species than when such correction was not used. Thus we suggest to combine both approaches when analyzing closely related species to understand the importance of various plant traits for species distribution.

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Table 1: List of 18 species pairs used in the study (the first mentioned is species absent from El Hierro)

Species name[1]	**Family**	**Analysed Propagule**	**Most likely dispersal mode**
Aeonium sedifolium (Webb *ex* Bolle) Pit. & Proust	Crassulaceae	Seed	ANEMO
Aeonium spathulatum (Hornem.) Praeger			
Carex perraudieriana Gay *ex* Bornm.	Cyperaceae	Seed	ANEMO [82]
Carex canariensis Kük.		(with utricle)	
Cistus symphytifolius Lam.	Cistaceae	Seed	ENDO [83]
Cistus monspeliensis L.			
Euphorbia segetalis L.	Euphorbiaceae	Seed	HYDRO [71][*]
Euphorbia lamarckii Sweet			ENDO [72]
Hypericum glandulosum Aiton	Hypericaceae	Seed	ANEMO [84]
Hypericum grandifolium Choisy			
Limonium imbricatum (Webb *ex* Girard) C.F.Hubb.	Plumbaginaceae	Seed	EXO
Limonium pectinatum (Aiton) Kuntze		(with corolla)	
Plantago ovata Forssk.	Plantaginaceae	Seed	EXO [85]
Plantago lagopus L.			

Species name[1]	**Family**	**Analysed Propagule**	**Most likely dispersal mode**
Polycarpaea aristata (Aiton) DC. *Polycarpaea nivea* (Aiton) Webb	Caryophyllaceae	Seed	ANEMO
Reichardia tingitana (L.) Roth *Reichardia ligulata* (Vent.) G. Kunkel & Sunding	Asteraceae	Achene (with pappus)	ANEMO [82]
Reseda scoparia Brouss. Ex Willd. *Reseda luteola* L.	Resedaceae	Seed	ANEMO [82]
Salvia aegyptiaca L. *Salvia canariensis* L.	Lamiaceae	Seed	EXO [86]
Scrophularia glabrata Aiton *Scrophularia arguta* Aiton	Scrophulariaceae	Seed	ANEMO +BAL [79]
Senecio leucanthemifolius Poir. *Senecio glaucus* L.	Asteraceae	Achene (with pappus)	ANEMO [82]
Tolpis lagopoda C.Sm. *in* Buch *Tolpis barbata* (L.) Gaertn.	Asteraceae	Achene (with pappus)	ANE [85]

Species name[1]	**Family**	**Analysed Propagule**	**Most likely dispersal mode**
Trifolium stellatum L.	Fabaceae	Seed	EXO [87]
Trifolium arvense L.		(with calyx)	
Emex spinosa (L.) Campd.	Polygonaceae	Seed	HYD [88] (*Emex*), [72] (*Rumex*)
Rumex bucephalophorus L.		(with spines)	EXO [89] (*Emex*)*, [90] (*Rumex*)*
Monanthes laxiflora (DC.) Bolle	Crassulaceae	Seed	ANEMO
Aichryson laxum (Haw.) Bramwell			
Descurainia millefolia (Jacq.) Webb & Berthel.	Brassicaceae	Seed	EXO [87] (*Descurainia*)
Arabis caucasica Schltdl.			[91] (*Arabis*)

[1] The species names according to Arechavaleta et al. [30]

* The dispersal mode used as the most likely dispersal mode in the analyses presented (the results do not change when using the other dispersal mode).

Table 2: Analysis of the relationship between the presence of species on El Hierro and the life history traits, the results are presented with and without phylogenetic correction (PC), Dev indicates deviance explained. DF Error = 35

		Without PC				**With PC**			
		separately		By stepwise		separately		By stepwise	
Dispersal mode	**Traits tested**	P	Dev	P	Dev	P	Dev	P	Dev
ANEMOCHORY	terminal velocity	0.728	0.121			0.326	0.967		
	dispersal distance	0.983	< 0.001			0.050	3.847	0.082	3.026
HYDROCHORY	buoyancy	0.927	0.009			0.553	0.353		
	seed surfoval in salt water	0.624	0.241			0.287	1.135		
	T50	0.686	0.164			0.384	0.759		
EXOZOOCHORY	seed adhesion	0.910	0.013			0.498	0.458		
ENDOZOOCHORY	seed viability after simulation	0.976	0.001			0.474	0.513		
MOST LIKELY DISPERSAL MODE		0.752	0.100			0.654	0.201		

		Without PC				**With PC**			
		separately		By stepwise		separately		By stepwise	
	Traits tested	P	Dev	P	Dev	P	Dev	P	Dev
Other traits	seed mass	0.317	1.000			0.007	7.338	0.021	5.340
	seed viability	0.990	< 0.001			0.849	0.036		
	plant height	0.540	0.375			0.108	2.585		
Persistence traits	longevity	0.154	2.029			0.001	11.089	0.132	2.263
	woodiness	0.315	1.008			0.103	2.657		
Distribution	no. of vegetation zones	0.331	0.945			0.177	1.824		
	no. of islands	0.062	3.484	0.062	3.484	< 0.001	15.140	0.002	9.487

Table 3: Analysis of the relationship between the number of occupied islands by a species and the life history traits, the results are presented with and without phylogenetic correction (PC), Dev indicates deviance explained. DF Error = 35

		Without PC				**With PC**			
		separately		By stepwise		separately		By stepwise	
Dispersal mode	**Traits tested**	P	Dev	P	Dev	P	Dev	P	Dev
ANEMOCHORY	terminal velocity	0.492	0.472			0.513	0.428		
	dispersal distance	0.967	0.002			0.781	0.077		
HYDROCHORY	buoyancy	0.501	0.453			0.382	0.763		
	seed surfoval in salt water	0.862	0.030			0.643	0.215		
	T50	0.312	1.021			0.985	< 0.001		
EXOZOOCHORY	seed adhesion	0.765	0.089			0.464	0.535		
ENDOZOOCHORY	seed viability after simulation	0.908	0.013			0.967	0.002		
MOST LIKELY DISPERSAL MODE		0.575	0.315			0.979	< 0.001		

		Without PC				**With PC**			
		separately		By stepwise		separately		By stepwise	
	Traits tested	P	Dev	P	Dev	P	Dev	P	Dev
Other traits	seed mass	0.594	0.284			0.280	1.168		
	seed viability	0.465	0.534			0.425	0.637		
	plant height	0.518	0.418			0.995	< 0.001		
Persistence traits	longevity	0.006	7.501	0.006	7.501	0.055	3.693	0.055	3.693
	woodiness	0.038	4.316			0.186	1.750		
Distribution	no. of vegetation zones	0.194	1.687			0.346	0.886		

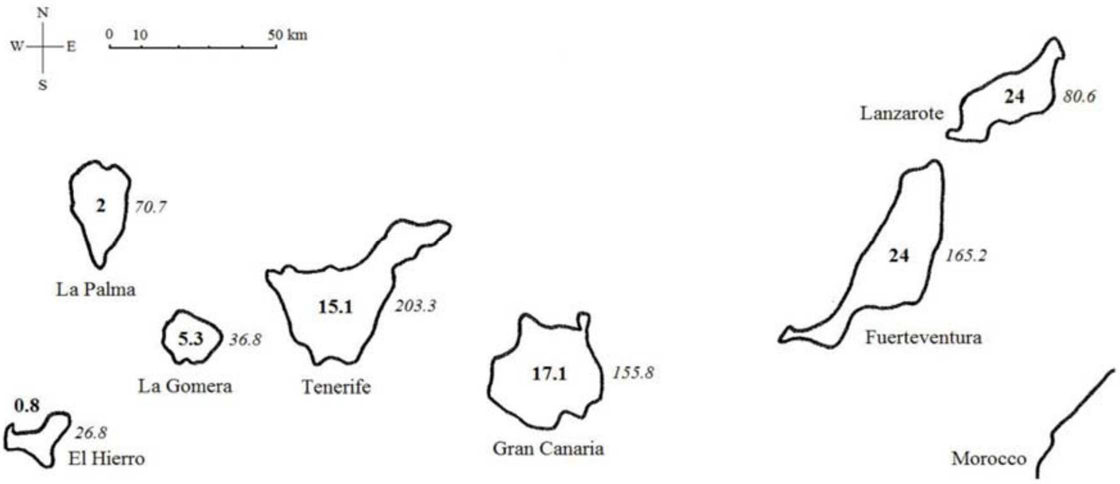

Figure 1: The Canary archipelago. Numbers in bold are island ages (in million years), numbers in italics are island areas (in hectares).

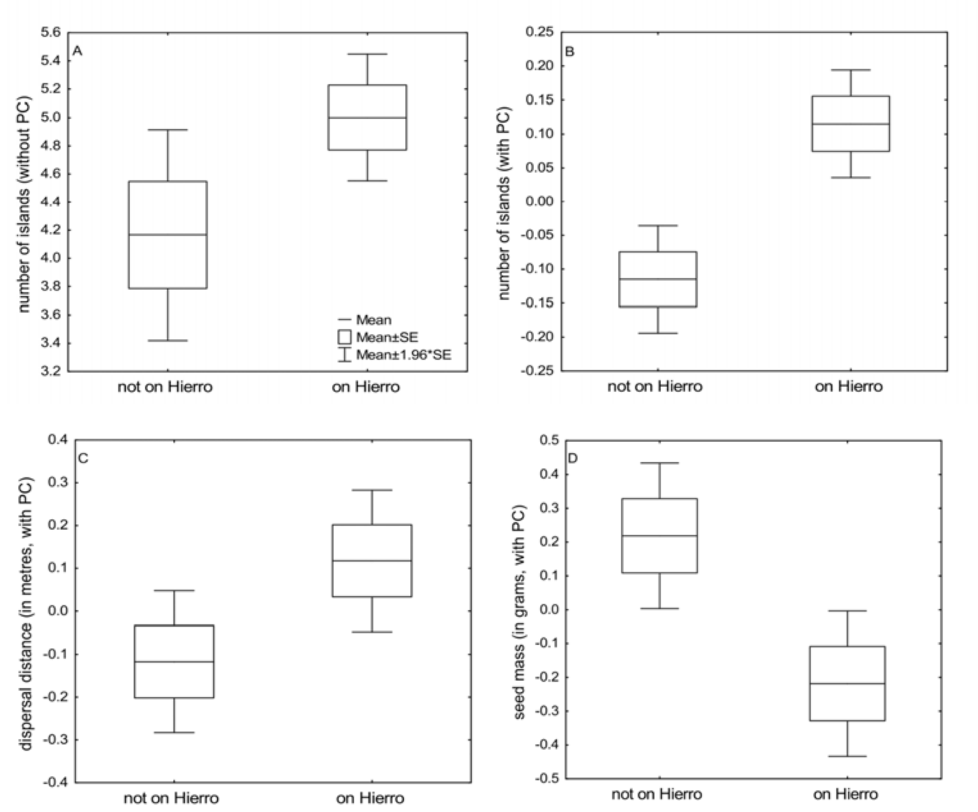

Figure 2: Box plots showing the differences between species present on El Hierro and species absent on El Hierro in number of islands occupied by a species without phylogenetic correction (PC, A, $p = 0.062$) and with PC (B, $p < 0.001$), dispersal distance with PC (C, $p = 0.05$) and seed mass with PC (D, $p = 0.007$).

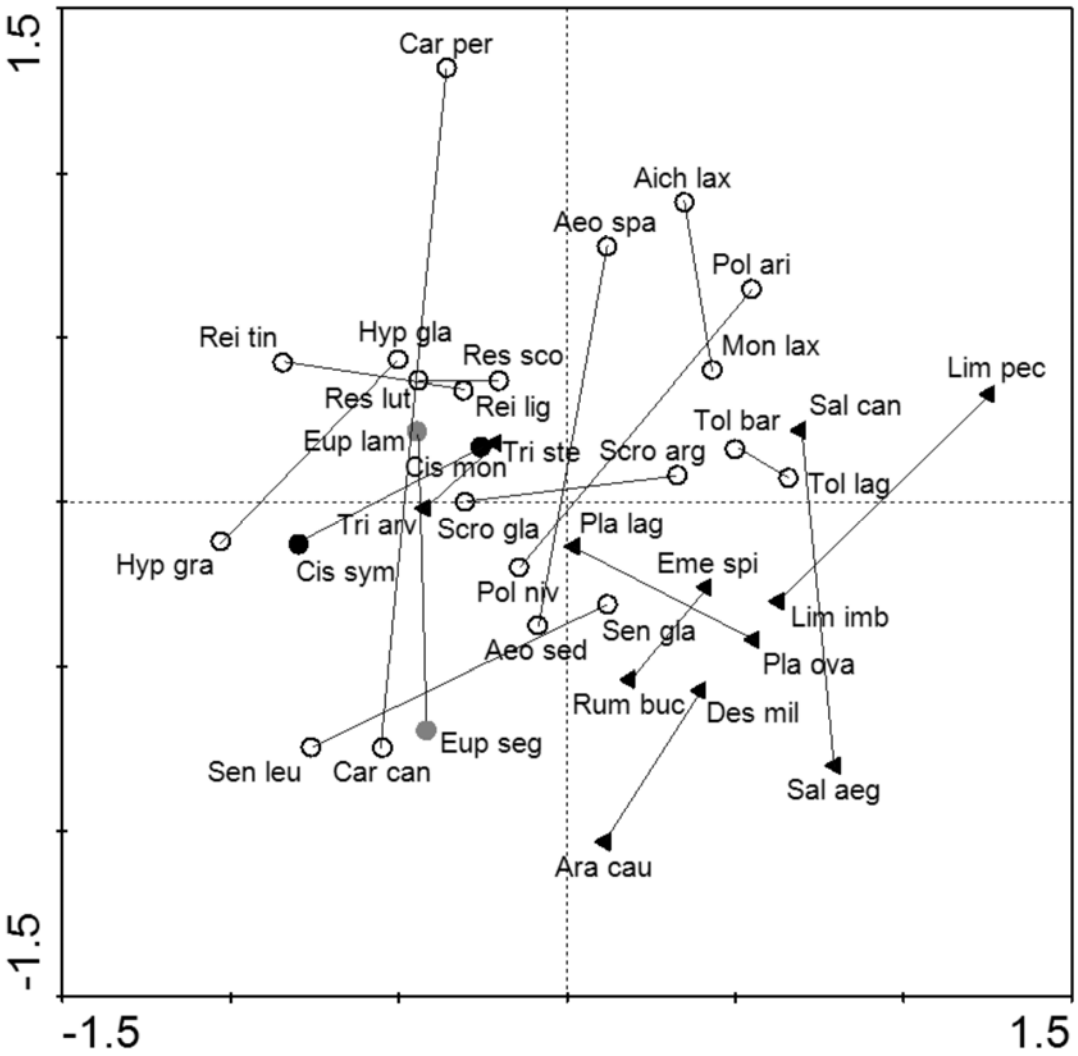

Figure 3: Relationship between individual species determined by principal component analysis (PCA) using trait data as dependent variables, the first axis explained 27.7 % of variability, the second axis explained 26.1 %. Different symbols indicate species most likely dispersal modes (according to literature): species with solid black circles are most likely dispersed by endozoochory, species with solid grey circles are dispersed by hydrochory, species with opened symbols are dispersed by anemochory and species with solid black triangles are dispersed by exozoochory. Species pairs are connected by lines.

Paper 4

THE EVOLUTIONARY POTENTIAL OF DIFFERENT TRAITS IN SUBTRIBE SONCHINAE

Kristýna Vazačová, Mária Šurinová and Zuzana Münzbergová

(Manuscript)

The evolutionary potential of different traits in subtribe Sonchinae

Kristýna Vazačová[1, 2*], **Mária Šurinová**[1,2] **and Zuzana Münzbergová**[1, 2]

[1] Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, Prague 2, CZ-128 01, Czech Republic

[2] Institute of Botany, Academy of Sciences of the Czech Republic, Lesní 322, Průhonice, CZ-252 43, Czech Republic

[*] Author for correspondence, email: vazacova@seznam.cz, tel: +420271015332

Abstract

The knowledge of evolutionary potential of different traits is a key prerequisite for understanding species ability to respond to landscape changes. Detailed studies on this topic, however, are still lacking. Specifically, there is little information on which traits are phylogenetically conserved and do not change during evolution and which traits have a potential to evolve. This topic can be studied on a group of closely related species arising by adaptive radiation on oceanic islands. For our study we chose 25 species from subtribe Sonchinae and tested the evolutionary potential for traits related to dispersal, persistence and as well as traits describing species distribution. Dispersal traits were estimated both as direct dispersal abilities by anemo-, hydro-, exo- and endozoochory and seed morphological parameters. The results of testing for phylogenetic signal of traits revealed that traits related to persistence and distribution are more phylogenetically conserved than dispersal related traits. We also showed that the existence of phylogenetic signal can be constrained by rapid and easy evolutionary changes as it was demonstrated in the case of pappus length showing high variability in morphology. In contrast, the directly measured dispersal traits showed low variability indicating high level of niche conservatism.

Introduction

Understanding species ability to disperse is a key prerequisite for predicting species ability to respond to landscape changes including changes in climate (Pearson and Dawson 2003, Thomson et al. 2010, Thuiller et al. 2008). Previous studies have evidenced that plant species can respond to environmental changes by reducing or increasing dispersal due to their adaptive plasticity (Imbert and Ronce 2001, Talavera et al. 2012). Deeper knowledge on the evolution of dispersal traits under changing environment is, however, still limited. Specifically, there is little information which dispersal related traits are phylogenetically conserved and how fast and by what dispersal related traits are the plants able to respond to environmental pressures (Barker et al. 2004, Hollander et al. 2010, Verdú and Traveset 2005).

Recent progress in molecular phylogeny comes up with various techniques for studying evolutionary history of different traits of a species within a phylogenetic tree (see Ackerly et al. 2000, Alcantara and Lohmann 2011, Kembel and Cahill 2005, Pagel 1997). Known and well-supported phylogeny can be used as a basis to test for phylogenetic signal of traits, i.e. to inspect whether ecological similarity of species is related to their phylogenetic relatedness (Blomberg et al. 2003). By this approach we can separate phylogenetically conserved (stable within clades in spite of possible variation of environment) and non-conserved (consistent within environments and independent of clades) traits (Ackerly 2009, Schmitt and Riviere 2002). Such approach has been already applied to different species traits related to plant morphology (Ackerly and Reich 1999, Alcantara and Lohmann 2011, Harder and Barrett 2006, Nogueira et al., 2012) as well as for traits related to ecology (Ackerly et al. 2000, Cornwell and Ackerly 2009, Mayfield et al. 2009, Prinzing 2001) and plant fitness (Herben et al. 2014, Verdú and Traveset, 2005). Studies exploring the evolution of species dispersal traits are, however, relatively scarce (Barker et al. 2004, Herrera 2002, Hollander et al. 2010, Riba et al. 2005).

Evolution of dispersal traits could be best studied on a group of closely related species that have recently undergone speciation. Best examples of such groups are closely related species which arose by adaptive radiation on oceanic islands. This evolutionary process is usually driven by intense competition among closely related species resulting in extensive divergence in morphological as well as ecological traits, but showing yet relatively little divergence in molecular sequences and crossability (Givnish et al. 2004). There are many known examples of adaptive radiation in plants in oceanic archipelagoes accompanied by changes in growth form (e.g. the Canary Island genus *Aeonium*, Mes and Hart 1996, or

Hawaiian silversword alliance, Robichaux et al. 1990) or in ecophysiological traits (e.g. Hawaiian lobelioids, Montgomery and Givnish 2008). Studies on adaptive changes in dispersal traits of island species are far less common (but see Givnish et al. 2009, Schenk 2013, Talavera et al. 2012).

On islands, species are expected to undergo selection against dispersal to reduce the loss of diaspores in the sea (Carlquist 1965, but see Vazačová and Münzbergová 2014). Decreasing dispersal ability then could result in increasing levels of speciation and single-island endemism, as it was documented by e.g. Givnish et al. (2009) on Hawaian lobelioids.

In our study we explored the evolution of dispersal traits of the subtribe Sonchinae on the Canary Islands and Madeira. This subtribe involves a group of strictly endemic species arising by adaptive radiation from common ancestor colonizing the islands (woody *Sonchus* Alliance, Kim et al. 1996) as well as species which are present also on the mainland. Thus it enables us to compare dispersal abilities of these two different groups and check for the reduction in dispersal ability in endemic species. Previous studies have already documented a great diversification in morphological, ecological and anatomical diversity of woody *Sonchus* group (Aldridge 1977, 1978, Kim et al. 2007, Lee et al. 2005) in contrast to other traits such as a uniform chromosome number (Ardévol Gonzáles et al. 1993). Morphological differences between species were also detected between traits related to dispersal ability (e.g. pappus deciduousness or dimorphism, Kim et al. 2007) suggesting some evolutionary potential in these traits. To get a deeper insight into the evolution of dispersal ability of this group it is, however, necessary to study not only their morphological parameters, but also dispersal potential by all the major possible dispersal modes acting on islands. Thus we measured several seed parameters of each studied species as well as their ability to disperse by wind, water and bird (both exo- and endozoochory).

Besides traits related to dispersal ability, phylogenetic signal was also tested in traits related to species persistence on islands as such traits are important for species survival and their long-term persistence on islands and thus cannot be simply ignored. Such traits as woodiness or growth habit have already been reported to be related to divergence of *Sonchus* species (Kim et al. 2007, Lee et al. 2005, Santiago and Kim 2009). We decided to extend our knowledge by adding other traits related to persistence such as species longevity, growth habit and woodiness. As species dispersal abilities can be reflected by species distribution, our study involved also determinants of species distribution (e.g. the number of islands occupied by a species, the level of endemism and the number of vegetation zones occupied by the species).

By testing for phylogenetic signal we are examining the relationship between ecological and phylogenetical similarities of species (Blomberg and Garland 2002). This method is, however, not sufficient to detect the existence of niche conservatism, i.e. the situation when closely related species are more ecologically similar that would be expected based on their phylogenetic relationships (Wiens and Graham 2005). This scenario can happen especially in novel habitats including newly emerging islands, which will be occupied by species that are already well adapted to similar conditions resulting in minimized evolutionary adaptive changes in these species (Ackerly 2009). For this reason we decided to extend our study using the approach of testing for niche conservatism of traits.

Specifically, we asked the following questions: 1) What is the degree of phylogenetic conservatism of the dispersal and persistence traits? 2) What is the degree of phylogenetic conservatism in traits describing species distribution? 3) What is the degree of niche conservatism in all these traits?

Materials and Methods

Study site

The Canary Islands and Madeira are part of the Macaronesian archipelago being located in Atlantic Ocean between 27°45′ and 32° 39′ N and between 13°37′ and 18°00′ W. The Canary Islands consist of seven major islands differing in their size (from 278 km^2 for El Hierro up to 2034 km^2 for Tenerife, Dlugosch and Parker, 2007) and age (from 0.8 My for El Hierro up to 21 My for Fuerteventura, Afonso, 1988). Madeira is of the same volcanic origin having arisen 5 My ago, its area reaches 797 km^2. It differs from the Canary Islands in the level of isolation (580 km from Madeira vs. 110 km from Fuerteventura to the closest mainland).

Similar geological history, humid climate dominated by trade winds and high altitudinal gradients resulting in a great environmental heterogeneity are the main factors responsible for adaptive radiation of some genera across these islands (e.g. genus of *Aeonium*, *Argyranthemum*, *Sonchus* and *Pericallis).*

Species selection

Our study involves 25 species from the subtribe Sonchinae including woody *Sonchus* Alliance *(Dendrosonchus)* endemic to the Macaronesian islands (except for *S. pinnatifidus*, 18 species) and widely distributed genera of *Reichardia* (2 species), *Launaea* (2 species) and members of subgenus *Sonchus* (3 species including *S. asper, S. oleraceus, S. tenerrimus*, Table 1). We followed species classification according to The Plant List (web 1).

Macaronesian woody *Sonchus* species are reported to be a monophyletic group (Kim et al. 1996, Kim et al. 2007, Lee et al. 2005) accounting for 1/3 of the whole genus (Mejías et al. 2013). According to Lee et al. (2005) common ancestor of the alliance was herbaceous or weakly suffrutescent and subsequently dispersed to the Canary Islands, most likely from Gran Canaria. It subsequently underwent radiation on the islands and then colonized Madeira. Due to the similar origin of Canary Island and Madeiran species, we also included two Madeiran endemics, *Sonchus ustulatus* spp. *maderensis* and *S. fruticosus* into our study.

Seed collection

All the studied species were sampled on the islands (the Canary Islands and Madeira) to ensure that they originated from the same environmental conditions. Diaspores (i.e. achenes with pappus) for each species were collected from at least 8 individuals in the Botanical Garden "Jardín Canario Viera y Clavijo", Gran Canaria or in natural populations. These two sources of diaspores enabled us to involve quite an extensive collection of species in the study including highly threatened species. We are aware that combining diaspores from populations in the field with those from the garden can be problematic due to the specific conditions in the garden. However, the Botanical Garden “Jardín Canario Viera y Clavijo” is situated on the Canary Island, i.e. in the same environment as natural populations, from which the garden plants originally came. Furthermore, the specific location of the garden across the valley offers a large spectra of habitats, thus each species can be planted under the conditions close to the natural ones. Species in the Garden are planted in communities including plants they usually grow with in the natural habitats.

In the natural populations we preferably sampled 3 populations per species. For each population, we aimed to collect diaspores from at least 8 individuals and considered these as a single population. Each population was then tested for dispersal abilities separately. To have the same number of measurements for the species with seeds collected from the field and from the garden, we had 3 replicates for each dispersal experiment for diaspores collected in the garden.

We used 20 diaspores per species and population for experiments with anemochory, hydrochory and exozoochory and 30 seeds for testing endozoochory, i.e. 60 and 90 diaspores for each species, respectively. Such number of diaspores was a compromise between a large amount of species tested and number of diaspores used in literature (c.f. Knevel et al., 2005).

For testing other traits related to dispersal (i.e. achene mass and seed viability) we used achenes without pappus.

Measured traits

In the study we estimated traits related to species dispersal as well as determinants of species distribution. For simplicity, we called all these variables as traits although some of them do not match the exact definition of a trait (e.g., they are not measured at the individual level or independently on environmental conditions (Violle et al. 2007)).

Traits related to dispersal

Anemochory

The ability of diaspores to disperse by wind was estimated as terminal velocity defined as the maximum rate of seed falling in still air (Thompson, 2005). It was measured as the flight time of a diaspore from predefined height (270 cm, Münzbergová, 2004). Mean dispersal distance D was expressed as:

$$D = (w \times h) / t$$

where w is wind speed (being constant for all species), h is average plant height and t is terminal velocity. Values of average plant height were obtained from the literature (Bramwell and Bramwell 2001; Castroviejo et al., 1986-2012; Franquinho and da Costa, 1987 Schönfelder and Schönfelder 2002a, 2000b; Tutin et al., 1964-1980).

We are aware that our dispersal model is simplified. Nevertheless, it has been successfully used in other studies to characterize mean dispersal distance of diaspores (e.g. Herben et al., 2006; Münzbergová et al., 2005; Soons and Heil, 2002; Tremlová and Münzbergová, 2007) and is the easiest way to combine the three key variables affecting wind dispersal. We thus suggest that it is a useful proxy of potential wind dispersal distances for comparison among species.

In the analyses, we used both terminal velocity (m/s) and mean dispersal distance (m). In addition, we tested for the difference in plant height between endemic and non-endemic species to see to what extent the differences in dispersal distance are affected by differences in plant height.

Hydrochory

The potential of diaspores to disperse in salt water (buoyancy) was measured as the proportion of diaspores still floating after a defined time period. Diaspores were gently put into beakers filled with salt water having 3.7% salinity (i.e. average salinity of the Atlantic Ocean along the Canary Islands coast, web 2). The size of beakers was proportional to the size of diaspores. Sea waves were simulated by continual shaking in electric orbital shaker with frequency of 100 shakes per min. The number of diaspores floating on water surface was checked immediately after putting them into bins and then after 5 minutes of shaking, 1, 2, 6, 24 hours and 7 days of shaking (Römermann et al., 2005). The experiment was finished after 1 week of diaspore shaking as it is the minimal time a diaspore needs for reaching the Canary islands from mainland when taking into account average speed of water currents in the Atlantic Ocean (60-90 km per week, Zhou et al., 2000) and the distance between mainland and the closest island (Africa to Fuerteventura, ca. 100 km).

At the end of the experiment, the number of floating and the number of sunken diaspores was counted and the two groups of diaspores were then tested for viability. In the analyses, we used the proportion of viable seeds which kept floating until the end of the experiment from the total number of viable seeds before the experiment.

The diaspore buoyancy was also expressed as T_{50}, the number of days after which 50 percent of diaspores was still floating. This parameter is commonly used in other studies assessing hydrochory (Boedeltje et al., 2003; Römermann et al., 2005; van den Broek et al., 2005), however it does not take into account seed viability.

We also used the information on effect of salt water on viability of seeds expressed as the proportion of viable seeds after the experiment (both floating and sunk)/seed viability before the experiment. Viability of seeds was tested by dying the dissected seeds with 0.1% solution of 2,3,5-triphenyl-2H-tetrazolium chloride and inspecting the color change of the embryo (Cottrell, 1947). This method provides the same accuracy in estimating seed viability as the germination tests (Lakon, 1949). In contrast to germination tests, it is not dependent on selection of the right conditions for germination for each individual species and it is thus in fact more reliable for between species comparisons.

Zoochory

Birds are the most important long-distance island dispersers transporting diaspores both externally and internally. The main bird dispersers acting on the Canary Islands are blackbirds (*Turdus merula*), robins (*Erithacus rubecula*), blackcaps (*Sylvia atricapilla* and *S. melanocarpa*, Olesen and Valido, 2004), common ravens (*Corvus corax*, Nogales et al., 1999), gulls (*Larus cachinnans*, Nogales et al., 2001) and pigeons (*Columba livia*, *C. junoniae* and *C. bolli*).

Bird exozoochory (Epizoochory)

Bird exozoochory was tested as diaspore adhesion to bird feathers. As a model species we used a pigeon of the King breed, a utility breed with poor flight ability that is amenable to our experiments. Although this species is clearly not native to the Canary Islands, the functionality of its feathers for diaspore dispersal is readily comparable with native insular pigeon species.

Moistened diaspores were gently incorporated on 4 different body parts (on bust, neck and back, under wing). After 1 hour of pigeon free movement in an aviary (2 × 2 × 1 m) we checked the numbers of diaspores still attached to feathers. Taking into account the average flight speed of a trained pigeon (80 km/h, Gessaman and Nagy, 1988) and the shortest distance between mainland and the closest island (ca. 100 km), diaspores which remained attached to feathers after 1 hour are potentially able to get to the islands by this type of dispersal.

In the analyses we tested the proportion of diaspores which kept attached to the feathers after 1 hour (we refer to this value as seed adhesion). This parameter lacks the effect of real bird flight as we do not take into account the air movement around feathers during the flight that can dry out diaspores and cause them to drop earlier than in our simulation. However, we still think that our data are sufficient for the purpose to differentiate among diaspores with different ability to disperse by exozoochory.

Bird endozoochory

Bird endozoochory was tested by simulating diaspore gut passage through pigeon digestive tract. The methodology was based on our previous study (Vazačová and Münzbergová, 2013) in which we compared the effect of real gut passage through pigeon digestive tract on seed viability to different simulation approaches. Specifically, plastic flasks filled up with diaspores were shaken with wet grit (small stones eaten by birds to enhance digestion, commercial mixture for pigeons) for 24 hours in electric orbital shaker (200 shakes per minute, Vazačová and Münzbergová, 2013). Then diaspores were separated from the grit, rinsed and immersed in 5 ml of 1M H_2SO_4 (pH $\approx$ 0.3, Santamaría et al., 2002) for 4 hours. Intact seeds were retrieved, counted and tested for viability. The proportion of number of viable seeds which survived the simulation to the number of seeds viability before the experiment was used in the analysis. Seed viability after simulation was tested as described above.

Seed traits

For each species we also measured achene length, width and height, pappus length and achene mass. Length, width and height of achene and pappus length were measured on 10 diaspores of each species using Olympus SZX12 stereomicroscope and software M.I.S. Quick Photo Micro 2.2 (Promicra, web 3). Mean of 10 measurements was used in the analyses. Achene length, width and height were further multiplied to calculate achene volume. Some species have dimorphic pappus, i.e. possessing short (persistent) and long (deciduous) pappus. Due to the imperfect storing conditions or diaspore quality we were, however, not able to keep both types of pappuses in all the species. Thus we decided to measure only the length of the short pappus. We included the information on pappus dimorphism (monomorphic vs. dimorphic) in our analyses.

Seed mass was measured by weighting 90 achenes per species. For this purpose, they were divided into 9 groups by 10 seeds per group to get reasonable size estimates given by the precision of the balance (0.0001g). Seed mass is generally recognized as a rough proxy of seed dispersal ability and germination ability (e.g. Hewittt and Kellman, 2002; Sakai et al., 1998; Tremlová and Münzbergová, 2007). The same amount of achenes was used for viability testing of intact seeds.

Traits related to persistence and distribution

Data on species longevity (short-lived vs. perennial), woodiness (woody vs. not woody), growth form (shrublets with basal rosettes on a short stem vs. shrubs with terminal leaf-rosettes on the branches) and the number of vegetation zones with species occurrence (one vs. more vegetation zones) were gained from Bramwell and Bramwell (2001) and Schönfelder and Schönfelder (2002a, 2002b).

Species distribution among islands (occupying up to 3 vs. more than 3 islands) was adopted from Arechavaleta et al. (2010). We also tested for the level of endemism (single island endemic, endemic to Macaronesian islands).

Phylogenetic approach

Sequence information for 25 species of the subtribe Sonchinae and outgroup species *Lactuca perennis* was obtained from the National Center for Biotechnology Information (web 4). For *Sonchus gomerensis* ITS sequences are not available. The sequences were aligned using CLUSTALW (Thompson et al., 1994) implemented in the BioEdit software. Phylogenetic analyses were performed on three data sets created from aligned sequences of chloroplasts *matK*- *trnK* and nuclear ITS1 and ITS2 regions (GenBank accession numbers are in Table 1). To obtain properly resolved tree we performed both Bayesian analyses and maximum likelihood analyses.

Bayesian analyses were performed using the Metropolis-coupled Markov chain Monte Carlo (MCMC) methods implemented in MrBayes vers. 3.2.2. (Ronquist et al. 2012) using GTR + Γ+ I model of six substitution types ($n = 6$) with a proportion of invariable sites. Each analysis consisted of two independent simultaneous runs. Analyses were initiated from a random starting tree, each chain was run for 6 million generations. The chain was sampled every 1,000 generations for a total 6001 tree samples per run. The average standard deviation of split frequencies should approximate zero, as simultaneous runs converged onto the stationary distribution. Convergence was established, when the standard deviation of split frequencies between simultaneous runs was less than 0.01, based MrBayes calculations. Additionally, the program Tracer vers. 1.5 (Rambaut and Drummond 2007) was used for trace files evaluation. First 25% sampled trees were discarded as burn-in.

Maximum likelihood analysis was done using PhyML version 3.0 (Guindon and Gascuel 2003), as implemented in Geneious version 6.1.8. Maximum-likelihood trees were constructed using a combination of nearest neighbor interchanges (NNIs) and subtree pruning and regrafting (SPR) algorithms. Node support was assessed by bootstrap analyses with 1,000 bootstrap replicates.

Data analyses

Phylogenetic signal of the continuous traits (Table 2) was assessed using Pagel´s λ (Freckleton et al. 2002). $\lambda = 0$ indicates evolution of traits that is independent of phylogeny, $\lambda = 1$ means that species´ traits are evolving according to Brownian motion on the given phylogeny, intermediate values of λ indicate that traits have evolved according to a process in which the effect of phylogeny is weaker than in the Brownian model (Freckleton et al. 2002). Before the analysis all the traits were tested for normality and transformed where necessary (Table 2). Pagel´s λ was calculated using function pgls from the package caper in R (Orme et al., 2013).

Some traits could not be normalized and in fact showed distribution that was largely bimodal. Such data were thus transformed to binary data. This was the case of T50 (50% diaspores buoyant less than 7 days vs. 50% buoyant for 7 days and more), species distribution among islands (species occupying up to 3 vs. more than 3 islands) and number of vegetation zones (one vs. more vegetation zones).

Phylogenetic signal of binary traits (Table 2) was examined by D statistics (Fritz and Purvis 2010). Values of D □ 0 are phylogenetically more conserved than under the Brownian model and values of D □ 1 are phylogenetically overdispersed (Fritz and Purvis 2010).

All the traits (both continuous and binomial) were also tested for phylogenetic signal by Mantel test in R package vegan (function mantel, Oksanen et al. 2013) comparing plylogenetic distance matrix and trait distance matrix. Phylogenetic distance matrix was created from the phylogenetic tree in Mesquite program by command patristic distances implied by the tree (Madison and Madison 2011). Distance matrix of traits was calculated as the Euclidean distance in Statistica (Statsoft 2013).

To test for niche conservatism of continuous traits, we calculated for each trait the variance of a set of standardized contrasts (c_i) as $\sum c_i^2 / C$, where $C = N - 1$ is the number of contrasts calculated for N taxa on a bifurcating tree (Ackerly 2009). The higher the variance, the higher the evolutionary rate of the trait and the lower niche conservatism. We also counted variance of the original trait values to see how variable are traits without estimating the phylogenetic relatedness of the species.

Niche conservatism of binary traits was counted as a number of reversals from one state to another proportional to the number of nodes within the phylogenetic tree (Ackerly

2009). The number of reversals was obtained from visual reconstruction of ancestral states of traits in Mesquite (Madison and Madison 2011). The higher the number of reversals the lower niche conservatism. To explore the variance in the original data, we also estimated the proportion of the most abundant trait states.

Results

Phylogenetic approach

Aligned sequence lengths were as follows: 260 bp for ITS1 region, 228 bp for ITS2 region, partial *trnK* 5′ and intergenic spacer 1–736 (736 bp), *matK* gene 737–2256 (1520 bp), intergenic spacer 2257–2605 (347 bp), *trnK* 3′ 2606–2641 (35 bp), partial *trnK-psbA* intergenic spacer 2642–2895 (253 bp). The combined alignment of ITS and plastid regions comprised 3383 constant characters, 383 variable but parsimony uninformative characters (11%), and 198 parsimony informative characters between ingroup and outgroup (5,8%) in the data matrix. Four major strongly supported clades are recognized (Figure 1). Phylogenetic analysis identified four clades, *Launaea* clade (1), *Reichardia* clade (2), the woody *Sonchus* Alliance clade (3) and subgenus *Sonchus* clade (4). Both, *Launaea* (1) and *Reichardia* (2) are monophyletic basal groups (weak support for the monophyly of *Reichardia*, <50% bootstrap value, <75% posterior probability). The clade of woody *Sonchus* (3) can be further separated into 2 subclades. These clades are well supported, but the phylogenetic positions for species *Sonchus brachylobus* and *Sonchus fauces- orci* are not well defined. The subgenus *Sonchus* clade (4) is monophyletic and strongly supported.

Trait evolution

Regarding dispersal traits, phylogenetic signal was found only for T50 related to species buoyancy, pappus dimorphism (Table 3) and achene height (marginally significant, Table 4). Traits related to persistence showed phylogenetic signal for longevity, woodiness, but not for growth form (Table 4). Phylogenetic signal was also found for all the traits related to species distribution (i.e. number of vegetation zones, number of occupied islands, single island endemism and endemism to Macaronesian islands). The results of D statistics slightly differed from Mantel´s test in two parameters, in pappus dimorphism and in vegetation zones, the first being significant in D statistics, the second one being significant in Mantel´s test.

High value of variance of standardized contrasts for pappus length (3.75, Table 3) indicates that this trait evolves easily and rapidly. Variance of other continuous traits ranging between 0.01 and 0.33 showed relatively high niche conservatism (Table 3). The proportion of reversals in trait states was the highest for growth form and vegetation zones showing low niche conservatism in these traits. The lowest values of the proportion of reversals were found for species distribution among islands, pappus dimorphism, longevity and woodiness. The latter four traits were also detected for phylogenetic signal indicating high level of phylogenetic conservatism.

Discussion

Phylogenetic approach

For this study we chose 25 species of subtribe Sonchinae. Eighteen of these species were already involved in phylogeny published by Kim et al. (2007) based on cpDNA matK and ITS nrDNA regions using parsimony based approach. We confirmed the same three major clades (*Launaea* clade, *Reichardia* clade and the subgenus *Sonchus* clade, Figure 1). We also confirmed basal position for *Launaea* clade and *Reichardia* clade within the subtribe and position of subgenus *Sonchus* clade. In contrast, the woody *Sonchus* Alliance clade is separated into 2 subclades with strongly supported groups within subclades. These differences may be caused by different data set of species used for phylogeny reconstruction and by using MCMC methods and maximum likelihood methods.

Trait evolution

Our results have shown that dispersal traits are less phylogenetically conserved than traits related to species persistence and distribution. In fact, phylogenetic signal was found only for achene height, pappus dimorphism and buoyancy represented by T50. Such results can indicate that traits related to dispersal are under strong selection pressure and do not evolve at all or conversely they evolve easily and rapidly. The latter could be especially the case of pappus length being shown to have high values of variance of standardized contrasts. Pappus length is generally supposed as a standard proxy of species dispersal ability (Lavergne et al. 2004, Riba et al. 2005, but see Sheldon and Burrows 1973) and its evolutionary potential has been repeatedly reported in many studies including those on oceanic islands (e.g. Carlquist 1966, Cody and Overton 1996). Evolutionary changes in pappus length can be related to processes resulting in the reduction of its size as it has been already shown by Carlquist (1966) for Pacific Compositace. The reduction in size was revealed in other seed parameters on e.g. genus *Bidens* on the Galapagoes (Carlquist 1966) or on *Hibiscus* on Bonin Islands (Kudoh et al. 2013).

No evolutionary changes can be expected in traits related to exo- or endozooochory, showing low variance of standardized contrasts. These dispersal modes are not supposed to play a crucial role in dispersal of *Sonchus* species, as animals are generally not considered as the main dispersal mediators of species possessing pappus. Dispersal modes, which are actually not used by species are thus assumed not to evolve.

Both achene height and pappus dimorphism are rather conserved within the phylogenetic tree. Achene height is not only a measure of dispersal potential, but could be a relatively good proxy for inferring the amount of reserves in the seed, which can be crucial for species survival and early growth in newly emerged habitats (Salisbury 1974, Fenner and Thompson 2005).

Phylogenetic signal in pappus dimorphism (i.e. possessing both short and long pappus) typical for woody *Sonchus* Alliance (Kim et al. 2007) can be an indication of some adaptive plasticity enabling species to respond to variable conditions by influencing their dispersal potential (Hollander et al 2010, Talavera et al. 2012). Different dispersal strategies within one species were reported by Talavera et al. (2013) also for *Rumex bucephalophorus*. The species possess two types of diaspores differing in dispersal potential and in presence on the Canary Islands according to their isolation; western and more isolated islands are settled exclusively by populations having short-dispersed propagules.

The importance of traits related to species persistence on islands can be explained by island isolation constraining species further dispersal. According to Donoghue´s theory (2008) it is easier for species to move to new environments than to evolve a competitive, stable phenotype that will persist through longer time. However, on islands, where it is nowhere else to disperse, species are under strong selection pressure for strategies enabling them to survive on the already occupied habitats. Longevity and woodiness showed low proportion of reversals indicating conservatism in these traits. Island endemics of *Sonchus* species are typically woody and long-lived and several hypotheses exist to explain the incidence for insular woodiness including better competitive ability or absence of herbivores (for review see Lens et al. 2013). In contrast, *Sonchus* species are able to change relatively easily in their growth form. Such tendency could be a predisposition for highly variable habitats on the Macaronesian islands being rich in geomorphology.

Phylogenetic signal was shown in all the traits related to species distribution as well as in the number of vegetation zones occupied by a species. The level of island endemism and the number of vegetation zones occupied represent a proxy of niche width of a species. The number of islands colonized by a species also reflect its ability for successful habitat colonization (Knappová et al. 2012).

The interpretation of results strongly depends on the scale the data are studied. For instance, when observing the evolution of woodiness on the Canary Islands on a scale of a few endemic groups arising by adaptive radiation, it was found out that this growth habit is widespread among different genera (e.g. *Sideritis* or *Argyranthemum* genera, García-Verdugo et al. 2014). In contrast, after involving other species-rich groups with similar history (e.g.

Echium or *Aeonium* genera), it has to be concluded that reversal in growth habit also occur during the evolution of lineages.

Conclusions

The results showed that the level of phylogenetic conservatism differs among different traits – traits related to persistence and distribution were reported to be more phylogenetically conserved than dispersal related traits. We also showed that for proper estimation of evolutionary potential of traits it is necessary to test for both phylogenetic signal and the level of niche conservatism.

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Web 4: http://www.ncbi.nlm.nih.gov/nuccore (last access: 2014-05-10)

Table 1: Species used in the study

Classification	Species name*	GenBank accession no.
The woody *Sonchus* Alliance	*Babcockia platylepis* (Webb) Boulos	DQ072470.1 (ITS1), DQ072471.1 (ITS2), DQ022966.1 (matK-trnK reg.)
	Sonchus acaulis Dum.Cours.	L48289.1 (ITS1), L48290.1 (ITS2), DQ023029.1 (matK-trnK reg.)
	Sonchus bornmuelleri Pit.	DQ072508.1 (ITS1), DQ072509.1 (ITS2), DQ023022.1 (matK-trnK reg.)
	Sonchus brachylobus Webb & Berthel.	DQ072510.1 (ITS1), DQ072511.1 (ITS2), DQ023014.1 (matK-trnK reg.)
	Sonchus canariensis (Sch.Bip.) Boulos	L48293.1 (ITS1), L48294.1 (ITS2), DQ022997.1 (matK-trnK reg.)
	Sonchus congestus Willd.	L48175.1 (ITS1), L48176.1 (ITS2), DQ023030.1 (matK-trnK reg.)
	Sonchus fauces-orci Knoche	DQ072476.1 (ITS1), DQ072477.1 (ITS2), DQ023007.1 (matK-trnK reg.)
	Sonchus fruticosus L.f.	DQ072484.1 (ITS1), DQ072485.1 (ITS2), DQ023002.1 (matK-trnK reg.)
	Sonchus gomerensis Boulos	DQ023031.1 (matK-trnK reg.)

Sonchus hierrensis (Pit.) Boulos	L48169.1 (ITS1), L48170.1 (ITS2), DQ023020.1 (matK-trnK reg.)
Sonchus leptocephalus Cass.	L48317.1 (ITS1), L48318.1 (ITS2), DQ023036.1 (matK-trnK reg.)
Sonchus ortunoi Svent.	DQ072490.1 (ITS1), DQ072491.1 (ITS2), DQ023032.1 (matK-trnK reg.)
Sonchus pendulus Sennikov	DQ072466.1 (ITS1), DQ072467.1 (ITS2), DQ022996.1 (matK-trnK reg.)
Sonchus pinnatifidus Cav.	L48131.1 (ITS1), L48132.1 (ITS2), DQ022973.1 (matK-trnK reg.)
Sonchus radicatus Aiton	DQ072496.1 (ITS), DQ072497.1 (ITS2), DQ023009.1 (matK-trnK reg.)
Sonchus sventenii U.Reifenb. & A.Reifenb.	DQ072526.1 (ITS1), DQ072527.1 (ITS2), DQ023033.1 (matK-trnK reg.)
Sonchus tectifolius Svent.	DQ072500.1 (ITS1), DQ072501.1 (ITS2), DQ023010.1 (matK-trnK reg.)
Sonchus ustulatus Lowe ssp. *maderensis* Aldridge	DQ072486.1 (ITS1), DQ072487.1 (ITS2), DQ023006.1 (matK-trnK reg.)

Subg. *Sonchus*	*Sonchus asper* (L.) Hill	AY458004.1 (ITS1+ITS2), DQ508004.1 (matK-trnK reg.)
	Sonchus oleraceus (L.) L.	AY458002.1 (ITS1+ITS2), DQ840449.1 (matK-trnK reg.)
	Sonchus tenerrimus L.	AY458000.1 (ITS1+ITS2), DQ507994.1 (matK-trnK reg.)
Launaea	*Launaea arborescens* (Batt.) Murb.	L48145.1 (ITS1), L48146.1 (ITS2), DQ840443.1 (matK-trnK reg.)
	Launaea nudicaulis (L.) Hook.f.	L48147.1 (ITS1), L48148.1 (ITS2), DQ840442.1 (matK-trnK reg.)
Reichardia	*Reichardia ligulata* (Vent.) G.Kunkel & Sunding	DQ507882.1 (ITS1), DQ507933.1 (ITS2), DQ840441.1 (matK-trnK reg.)
	Reichardia tingitana (L.) Roth	DQ507881.1 (ITS1), DQ507931.1 (ITS2), DQ507979.1 (matK-trnK reg.)
Lactuca	*Lactuca perennis* (L.) Schur	L48143.1 (ITS1), L48144.1 (ITS2), DQ840439.1 (matK-trnK reg.)

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[*] species classification according to The Plant list (2010)

Table 2: Traits used in the analyses

Traits related to	Trait´s name	Data type	Type of transformation
Persistence	longevity	binary	-
	woodiness	binary	-
	growth form	binary	-
Distribution	vegetation zones	binary	-
	distribution among islands	binary	-
	single island endemic	binary	-
	endemic to Macaronesian islands	binary	-
Dispersal	achene length	discrete	log
	achene width	discrete	log
	achene height	discrete	log
	pappus length	discrete	no
	achene volume	discrete	no
	achene mass	discrete	log
	pappus dimorphism	binary	-
	dispersal distance	discrete	log
	seed buoyancy	discrete	arcsin
	T50	binary	-
	seed survival in salt water	discrete	no
	seed adhesion	discrete	arcsin
	seed viability after simulation of endozoochory	discrete	no
	plant height	discrete	log

Table 3: The results of Mantel test and Pagel´s lambda for continuous traits, λ = 0 indicates no phylogenetic signal, λ = 1 means phylogenetic signal, λ < 1 indicates weak phylogenetic signal, significant values of p at 0.05 level are in bold

Continuous traits	**Mantel´s test** p	r	**Pagel´s lambda** λ	**Variance of standardized contrasts**	**Variance of trait values**
achene length	0.37	0.04	0	0.01	0.23
achene height	**0.06**	**0.24**	0.87	0.02	0.18
achene width	0.36	0.10	0	0.01	0.02
pappus length	0.71	-0.08	0	3.75	42.38
achene volume	0.83	-0.12	0	0.33	1.63
achene mass	0.77	-0.10	0	0.03	11.25
dispersal distance	0.98	-0.22	0	0.13	0.14
seed buoyancy	0.48	< -0.01	0	0.16	0.35
seed survival in salt water	0.76	-0.11	0	0.09	0.62
seed adhesion	0.37	< 0.01	0	0.03	0.11
seed viability after simulation of endozoochory	0.78	-0.10	0	0.11	0.31
plant height	0.33	0.06	0	0.08	0.13

Table 4: The results of Mantel test and D statistics for binary traits, D < 0 indicates a phylogenetically more conserved traits than under the Brownian model, D > 1 indicates phylogenetically overdispersion of traits, significant values of p at 0.05 level are in bold

Binary traits	**Mantel´s test**		**D statistics**		**Proportion of reversals**	**Proportion of a trait state**[*]
	p	r	p	Estimated D		
longevity	**0.01**	**0.48**	**0.02**	**0.07**	0.11	0.84
woodiness	**0.01**	**0.46**	**< 0.01**	**-0.57**	0.11	0.8
growth form	0.81	-0.03	0.15	0.57	0.30	0.56
pappus dimorphism	0.16	0.30	**0.02**	**-**0.12	0.11	0.84
vegetation zones	**0.03**	**0.12**	0.08	0.38	0.26	0.56
distribution among islands	**< 0.01**	**0.54**	**0**	**-0.99**	0.07	0.68
single island endemic	**0.05**	**0.04**	**0.03**	**0.18**	0.22	0.52
endemic to Macaronesian islands	**0.01**	**0.45**	**< 0.01**	**-0.50**	0.15	0.72
T50	**0.04**	**0.12**	**0.01**	**-0.11**	0.19	0.56

[*] the most abundant state of a trait

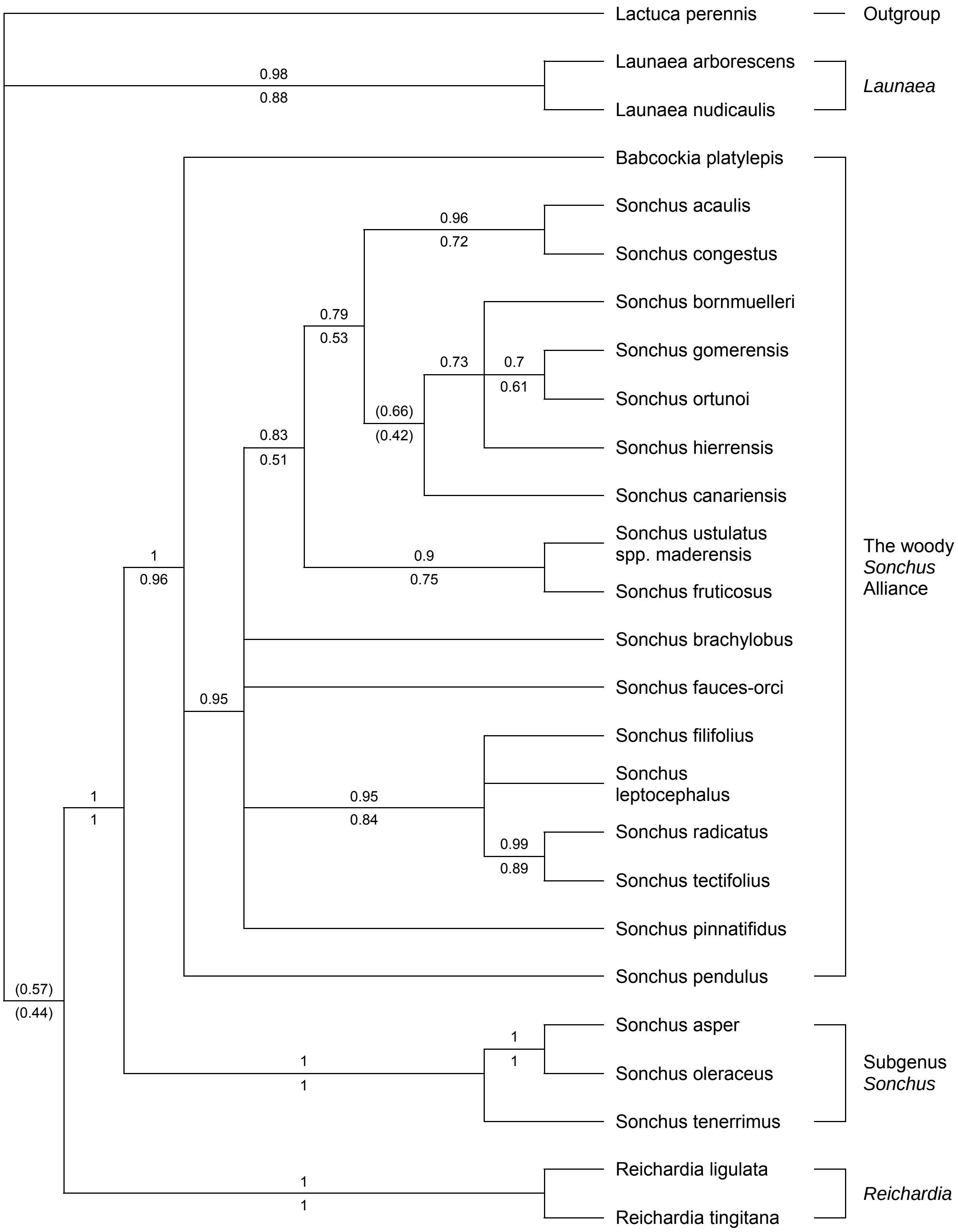

Figure 1: Bayesian majority- rule consesus tree of the subtribe Sonchinae based on Bayesian and maximum likelihood analyses of combined data set. Posterior probabilities are shown above branches, bootstrap supports are shown below branches (weakly supported branches are in brackets)

Conclusions

This thesis focused on evolutionary potential of dispersal traits of species in fragmented system represented by oceanic islands. I aimed to look on this topic on different levels. First, I compared traits between closely related endemic and non-endemic species to test for the existence of changes in their dispersal ability (**Paper 2**). Second, I searched for the traits predetermining species ability to colonize islands and to persist there (**Paper 3**). Third, I focused on the evolution of traits related to dispersal and persistence by inferring phylogenetic history of species group arising by adaptive radiation (**Paper 4**). All the objectives were explored on the species of Macaronesian islands (mostly Canary Islands).

Studies on changes of species dispersal traits usually use morphological parameters of seeds as a proxy of seed dispersal potential. However, such approach may not always be sufficient predictor of dispersal ability as the differences in dispersal ability in some species groups are given more likely in fine details of seed morphology which are not easily measurable. Moreover, species ability to disperse can be a result of combination of various seed parameters. This was shown for example for the relationship between direct dispersal by endozoochory and wide range of seed parameters in **Paper 1.**

In my PhD thesis I tested species direct dispersal abilities by performing dispersal experiments by anemo-, hydro-, exo- and endozoochory (**Papers 2, 3, 4**). To be able to test species ability by all these modes, first of all it was necessary to develop proper methodology for simulating bird internal dispersal (**Paper 1**). The approach of measuring direct dispersal abilities enabled me to estimate dispersal ability by multiple vectors reducing the risk of overlooking some important dispersal agent in each species. I obtained direct dispersal values, which are easily transferable into dispersal distances. However, by testing each species mode for all species, I potentially tested species for the dispersal mode which is not used by this species in reality. To eliminate this problem each species was also tested for the most likely dispersal mode, i.e. the value of a mode, which was found in literature to be most probably mode used by a species. Both approaches were applied to answer the questions raised in **Paper 2** and **3**, but the differences were found only in **Paper 2**.

Contrary to studies based on seed morphology, the use of direct dispersal values for testing the differences between endemic and non-endemic species on islands did not explicitly support the hypothesis about the reduction in dispersal potential of endemic species (**Paper 2**). Instead endemic species were detected to have better dispersal ability than their non-endemic congeners in many cases. I suggest that maintaining good dispersal ability is probably related to species subsequent dispersal among islands. This is confirmed by the fact that endemic species dispersing better than their non-endemic congeners occupy more islands within the archipelago. There are, however, other possible explanations such as the shift of the dispersal mode during species evolution.

The importance of dispersal traits for species dispersal among islands and colonization of new habitats was examined in **Paper 3**. This topic was studied by comparing traits of closely related species differing in their distribution among islands. The results revealed that no dispersal trait alone can explain the distribution of species among islands. Species presence on islands is the result of combination of both good dispersal ability and traits related to species ability to persist at the locality (e.g. seed size, longevity) as well as by abundance of species in the archipelago.

All these traits were examined for their long-term evolutionary potential in **Paper 4**. Within this study I asked which traits are conserved and did not change during evolution and which traits have a potential to evolve. This objective was studied on subtribe Sonchinae, which was previously reported to have experienced a great radiation in traits related to species persistence (e.g. growth habit, woodiness), in distribution as well as in pappus dimorphism. For this reason I also tested seed morphological parameters to compare whether similar evolutionary trends can be seen in both approaches of inferring dispersal ability. The results of testing for phylogenetic signal of traits revealed that traits related to persistence and distribution are more phylogenetically conserved than dispersal related traits. I also showed that the existence of phylogenetic signal can be constrained by rapid and easy evolutionary changes as it was demonstrated in the case of pappus length showing high variability in morphology. In contrast, the directly measured dispersal traits showed low variability indicating high level of niche conservatism.

Overall, according to above mentioned results and contrary to my expectation direct dispersal traits showed fewer patterns and had lower variation than morphological traits related to species dispersal. In addition, all the dispersal traits seem to be of lower importance than traits describing species persistence and distribution. This thus suggests that species in fragmented habitats may be more strongly limited by the amount of seeds available for dispersal, availability of suitable habitats and ability to establish than by seed dispersal ability. All this indicates that dispersal traits should be studied in the context of other traits related to species persistence and distribution as all the traits act in species colonization ability and its long-term survival on habitats. The conclusions of this thesis are potentially applicable to other fragmented systems including fragmented systems on the mainland.

Curriculum vitae

Personal data:

Name	Kristýna Vazačová
Born	1982, Strakonice
Email	vazacova@seznam.cz

Education:

2000 - 2005: Master degree, Department of Botany, Faculty of Science, Charles University of Prague, Czech Republic. Master thesis: The importance of spatial and temporal variability and plant individual history for the population biology of the species of *Polygonatum*. Supervisor: doc. RNDr. Zuzana Münzbergová, Ph.D.

Since 2005: Postgraduate study, Department of Botany, Faculty of Science, Charles University of Prague, Czech Republic. Thesis: The evolution of dispersal traits in plants. Supervisor: doc. RNDr. Zuzana Münzbergová, Ph.D.

Employment:

2006 - 2009: Department of Population Ecology, Institute of Botany of the Academy of Sciences. Scientific researcher.

2009 - 2012: Department of Mycorrhizal Symbioses, Institute of Botany of the Academy of Sciences. Scientific researcher. Project NPO – Development of strigolactone (STRGL) application technologies for their agrotechnical use (applicant M. Vosátka).

Since 2012: Department of Mycorrhizal Symbioses, Institute of Botany of the Academy of Sciences. Scientific researcher. Project GAČR – Correlation of changes in mycorrhizal community and diversity of rare species on abandoned fields (applicant M. Vosátka).

Research interests:

Plant ecology, population ecology, plant dispersal, island biogeography, seed traits.

Foreign stays:

2006 and 2007: Jardín Botánico Viera y Clavijo, Las Palmas, Gran Canaria, 5 months. Doctoral research.

Fellowships:

2006 and 2007: Fond mobility Univerzity Karlovy, fellowship supporting the stay in the Botanical Garden Jardín Botánico Viera y Clavijo, Las Palmas, Gran Canaria.

Grant projects:

2007-2009: GAUK 48807: The evolution of dispersal traits in plants.

Participation in other grants:

2005: FRVŠ 2380 G4: The importance of spatial and temporal variability and plant individual history for the population biology of the species of *Polygonatum.* (applicant J. Rothanzl).

2009: FRVŠ 415/2009: Experimental ecology of plants. (applicant M. Weiser).

Scientific publications:

Vazačová, K., Münzbergová, Z., 2013. Simulation of seed digestion by birds: How does it reflect the real passage through a pigeon's gut? Folia Geobotanica 48, 257-269.

Presentations at conferences:

2005: 18th Annual Conference of the Section Plant Population Biology of the Ecological Society of Germany, Switzerland and Austria (GfÖ), Potsdam, Germany. Poster: The importance of spatial and temporal variability and plant individual history for the population biology of the species of *Polygonatum* (K. Vazačová, Z. Münzbergová).

2007: EMPSEB 13 (13th Annual Meeting of PhD. students in Evolutionary Biology) Lund, Sweden. Presentation: The evolution of dispersal traits in plants (K. Vazačová, Z. Münzbergová).

2008: 21th Annual Conference of the Section Plant Population Biology of the Ecological Society of Germany, Switzerland and Austria (GfÖ), Luxembourg. Poster: Endemic vs. non-endemic species: do they differ in their dispersal ability? (K. Vazačová, Z. Münzbergová).

2009: ESEB 12 (12th Congress of the European Society for Evolutionary Biology), Torino, Italy. Poster: The evolution of plant dispersal ability on islands: differences between endemic and non-endemic species (K. Vazačová, Z. Münzbergová).

2011: BioSystematics, Berlin, Germany. Poster: Do island non-endemic species really disperse better than their endemic congeners? (K. Vazačová, Z. Münzbergová).

Contribution of Kristýna Vazačová to the papers with co-authors included in the thesis:

Vazačová K., Münzbergová Z. (2013): Simulation of seed digestion by birds: How does it reflect the real passage through a pigeon's gut? Folia Geobotanica 48: 257-269.

theoretical background and idea – 70%
data collection and experiments – 100%
data analyses – 80%
writing – 90%

Vazačová K., Münzbergová Z.: Dispersal ability of island endemic plants: what can we learn using multiple dispersal traits? (accepted in Flora)

theoretical background and idea – 70%
data collection and experiments – 100%
data analyses – 80%
writing – 90%

Vazačová K., Münzbergová Z. (manuscript): The importance of species traits for species distribution on oceanic islands.

theoretical background and idea – 70%
data collection and experiments – 100%
data analyses – 80%
writing – 90%

Vazačová K., Šurinová, M. and Münzbergová Z. (manuscript): The evolutionary potential of different traits in subtribe Sonchinae.

theoretical background and idea – 70%
data collection and experiments – 100%

data analyses – 70%
writing – 70%

We hereby declare that we are aware that the work in above mentioned manuscripts of which we are co-authors, will form a part of the PhD thesis by Kristýna Vazačová, who made a major contribution to the work both in the research and in writing phase.

Zuzana Münzbergová

Mária Šurinová

May 27 2014

APPENDIX – PAPER 1

(SIMULATION OF SEED DIGESTION BY BIRDS: HOW DOES IT REFLECT THE REAL PASSAGE THROUGH A PIGEON’S GUT?)

Electronic Supplementary Material

Table S1 Measured values of seed parameters (Mean±S.E.) of 20 species

Species	**Seed coat thickness (mm)**	**Water permeability (%)**	**Seed weight (g)**	**Seed volume (mm^3)**
Andryala pinnatifida	0.027±0.004	3.2±0.1	0.001±0.000	0.067 ±0.003
Brachypodium arbuscula	0.102±0.003	6.1±0.5	0.023±0.001	4.940±0.338
Carex canariensis	0.084±0.002	6.1±0.4	0.006±0.000	1.838±0.110
Cistus monspelliensis	0.056±0.003	0.7±0.2	0.005±0.000	0.211±0.016
Crambe strigosa	0.192±0.002	7.6±0.4	0.012±0.000	1.987±0.122
Descurainia artemisioides	0.038±0.001	7.9±0.2	0.004±0.000	0.167±0.014
Echium plantagineum	0.091±0.003	3.7±0.9	0.014±0.002	1.244±0.098
Hypericum canariense	0.093±0.004	4.5±0.7	0.001±0.000	0.501±0.034
Lavatera cretica	0.198±0.004	9.3±0.8	0.035±0.001	2.062±0.045
Limonium pectinatum	0.098±0.004	3.0±0.8	0.001±0.000	0.233±0.014
Lotus arinagensis	0.049±0.006	0.9±0.3	0.005±0.000	0.759±0.017
Melica minuta ssp.*latifolia*	0.014±0.001	4.2±0.1	0.006±0.000	1.868±0.118
Plantago arborescens	0.034±0.002	16.2±0.2	0.014±0.000	0.436±0.026
Plantago lagopus	0.019±0.008	13.4±0.1	0.003±0.000	0.151±0.006
Reichardia ligularis	0.222±0.004	10.5±1.3	0.003±0.000	2.222±0.125
Reseda luteola	0.031±0.002	2.1±0.2	0.002±0.000	0.232±0.011
Rumex vesicarius	0.023±0.001	7.9±0.2	0.019±0.000	1.702±0.089
Salvia canariensis	0.112±0.000	19.0±0.7	0.010±0.001	0.752±0.036

Sideritis discolor	0.086±0.004	5.3±0.5	0.004±0.000	0.438±0.018
Sonchus regis-jubae	0.019±0.001	4.4±0.1	0.002±0.000	0.061±0.004

Table S2 Differences in seed viability of 20 species among 7 types of simulation, digestion and control viability, in the first column of digestion are numbers of seeds retrieved from pigeon feces (all retrieved seeds)

Species	**Type of simulation**							**Digestion**		**Control**
	2/5	**2/30**	**2/120**	**12/5**	**12/30**	**12/120**	**24/240**			
Andryala pinnatifida	0.2	0.27	0.27	0.42	0.2	0.1	0	0	0	0.67
Brachypodium arbuscula	0.17	0.3	0.1	0.32	0.1	0	0	0	0	0.91
Carex canariensis	0	0	0	0	0.07	0	0	15	0.17	0.59
Cistus monspelliensis	0.93	1	0.93	0.9	0.87	0.90	0.77	5	0.13	0.94
Crambe strigosa	0	0	0	0	0	0	0	0	0	0.544
Descurainia artemisioides	0.03	0	0	0	0	0	0	0	0	0.90
Echium plantagineum	0	0	0	0.23	0.13	0	0	0	0	0.91
Hypericum canariense	0	0	0	0	0	0	0	0	0	0.56
Lavatera cretica	0.74	0.43	0.63	0.57	0.5	0.43	0.4	4	0.14	1
Limonium pectinatum	0	0	0	0	0	0	0	0	0	1
Lotus arinagensis	0.8	0.93	0.93	0.77	0.83	0.83	0.87	0	0	0.91

Melica minuta ssp. latifolia	0.97	0.63	0.8	0	0	0	0	1	0	0.99
Plantago arborescens	0.07	0	0.04	0	0	0	0	6	0.11	0.16
Plantago lagopus	0	0	0	0	0	0	0	0	0	1
Reichardia ligularis	0.3	0.27	0.23	0.37	0.37	0.2	0.13	0	0	0.73
Reseda luteola	0.53	0.3	0.73	0.7	0.7	0.33	0.43	5	0.07	0.67
Rumex vesicarius	0.9	0.9	0.5	0.83	0.73	0.4	0.33	0	0	0.88
Salvia canariensis	0	0	0	0	0	0	0	2	0.03	0.7
Sideritis discolor	0.13	0	0	0.13	0	0	0	0	0	0
Sonchus regis-jubae	0.2	0.07	0.13	0	0	0	0	0	0	0.68

Type of simulation – duration of shaking in hours/duration of immersion in acid in minutes.

Table S3 Correlation coefficients of seed parameters

Seed parameters	**Water permeability**	**Seed coat thickness**	**Seed weight**
Seed coat Thickness	0.22		
Seed weight	0.29	0.39	
Seed volume	0.07	**0.46**	**0.62**

Values in bold are significant at 0.05 *P*-level.

APPENDIX – PAPER 3

(THE IMPORTANCE OF SPECIES TRAITS FOR SPECIES DISTRIBUTION ON OCEANIC ISLANDS)

Appendix - Paper 3: Species traits influence its distribution on islands

Table S4: Values of dispersal traits of 18 species pairs used in the study (the first mentioned is species absent from El Hierro)

	ANEMOCHORY		HYDROCHORY			EXOZOOCHORY	ENDOZOOCHORY	
species	**terminal velocity (m/s)**	**dispersal distance (m)**	**buoyancy[1]**	**seed survival in salt water[2]**	**T50 (min)[3]**	**seed adhesion[4]**	**seed survival after simulation[5]**	**most likely dispersal mode[6]**
Aeonium sedifolium	1.04	0.27	0.90	0.90	10080	0.20	0	0.27
Aeonium spathulatum	0.76	0.54	0.31	0.31	10080	0.05	0.10	0.54
Carex perraudieriana	1.35	1.69	0.35	0.35	10080	0.10	0	1.69
Carex canariensis	2.20	0.50	1	1	10080	0.32	0.43	0.50
Cistus symphytifolius	2.50	0.53	0.81	0.81	10080	0.15	0.78	0.78
Cistus monspeliensis	3.19	0.21	0.25	0.57	5281	0.13	0.85	0.85
Euphorbia segetalis	2.92	0.24	0.69	1	7920	0.28	0.63	0.69
Euphorbia lamarckii	3.28	0.36	0.41	0.78	6720	0.02	0.56	0.33
Hypericum glandulosum	0.89	1.31	0.77	0.77	10080	0.18	0	1.31
Hypericum grandifolium	1.12	0.96	1	1	10080	0.14	0.49	0.96
Limonium imbricatum	1.58	0.13	0.05	1	5760	0.30	0	0.30
Limonium pectinatum	1.20	0.17	0	0	5760	0.47	0	0.47
Plantago ovata	2.26	0.06	0.16	1	3195	0.30	0.04	0.30
Plantago lagopus	2.48	0.09	0.52	0.97	7620	0.05	0.01	0.05

Polycarpaea aristata	1.35	0.05	0.20	0.20	10080	0.05	0	0.05
Polycarpaea nivea	1.39	0.15	0.86	0.86	10080	0.08	0	0.15
Reichardia tingitana	0.24	0.85	0	1	900	0	0.91	0.85
Reichardia ligulata	0.49	0.87	0	0.97	410	0.15	0.52	0.87
Reseda scoparia	1.54	0.35	0.38	0.42	10080	0.12	0.62	0.35
Reseda luteola	1.82	0.49	0.56	0.60	10080	0.03	0.46	0.49
Salvia aegyptiaca	2.40	0.09	0.29	1	3382	0.53	0.01	0.53
Salvia canariensis	2.63	0.68	0.30	0.30	10080	0.47	0	0.47
Scrophularia glabrata	1.54	0.46	0.52	0.90	10080	0.12	0.32	0.46
Scrophularia arguta	1.55	0.25	0	1	45	0.10	0	0.25
Senecio leucanthemifolius	1.57	0.27	1	1	10080	0.20	0.57	0.27
Senecio glaucus	0.75	0.48	0	1	76	0.18	0	0.48
Tolpis lagopoda	1.70	0.21	0	0.73	63	0.25	0	0.21
Tolpis barbata	1.09	0.19	0	0.79	3330	0.15	0.02	0.19
Trifolium stellatum	1.74	0.09	0	0.66	900	0.10	0.97	0.10
Trifolium arvense	1.11	0.29	0.65	0.69	10080	0.12	0.59	0.12
Emex spinosa	3.02	0.11	0.16	1	90	0.20	0	0.20
Rumex bucephalophorus	1.72	0.15	0.25	0.80	5760	0.45	0.74	0.45
Monanthes laxiflora	0.47	0.17	0.35	0.35	10080	0.13	0	0.17
Aichryson laxum	0.50	0.66	0.12	0.18	10080	0.14	0.09	0.66
Descurainia millefolia	1.86	0.27	0.30	0.97	3382	0.45	0.27	0.45
Arabis caucasica	1.07	0.17	0.98	0.98	10080	0.50	0.21	0.50

[1] proportion of viable seeds which kept floating until the end of the experiment/seed viability before the experiment

[2] seed survival in salt water - the proportion of viable seeds after the experiment (both floating and sunk)/seed viability before the experiment.

[3] number of minutes, after which 50 percent of diaspores was still floating

[4] proportion of diaspores which kept attached to feathers after 1 hour

[5] proportion of viable seeds which survived the simulation/seed viability before the experiment

[6] values of the most likely dispersal mode estimated from literature

Table S5: Values of persistence traits, traits related to distribution and other traits of 18 species pairs used in the study (the first mentioned is species absent from El Hierro)

	other traits			persistence traits		distribution	
species	**seed mass (g)**	**seed viability**	**plant height (m)**	**longevity**	**woodiness**	**no. of vegetation zones**	**no. of islands**
Aeonium sedifolium	0.00003	0.56	0.28	perennial	woody	2	3
Aeonium spathulatum	0.00001	0.40	0.40	perennial	woody	1	5
Carex perraudieriana	0.00297	0.25	1	perennial	non-woody	1	4
Carex canariensis	0.00094	0.68	1.05	perennial	non-woody	1	5
Cistus symphytifolius	0.00052	0.99	1.30	perennial	woody	2	2
Cistus monspeliensis	0.00087	0.98	0.65	perennial	woody	1	5
Euphorbia segetalis	0.00234	0.88	0.70	perennial	woody	2	3
Euphorbia lamarckii	0.00857	0.89	1.15	perennial	woody	1	4
Hypericum glandulosum	0.00009	0.72	1.15	perennial	woody	1	5
Hypericum grandifolium	0.00004	0.90	1.05	perennial	woody	4	7
Limonium imbricatum	0.00121	1	0.20	perennial	non-woody	1	2
Limonium pectinatum	0.00013	0.61	0.20	perennial	woody	1	5
Plantago ovata	0.00311	0.97	0.13	annual	non-woody	1	6
Plantago lagopus	0.00061	0.87	0.20	annual	non-woody	2	7
Polycarpaea aristata	0.00003	0.57	0.07	perennial	woody	1	3
Polycarpaea nivea	0.00004	0.47	0.20	perennial	woody	1	6

Reichardia tingitana	0.00036	0.72	0.20	perennial	non-woody	3	6
Reichardia ligulata	0.00062	0.75	0.30	perennial	woody	3	6
Reseda scoparia	0.00016	0.77	0.50	perennial	woody	1	4
Reseda luteola	0.00024	0.80	0.85	annual	non-woody	2	6
Salvia aegyptiaca	0.00046	0.91	0.20	perennial	woody	1	5
Salvia canariensis	0.00119	0.50	1.75	perennial	woody	3	7
Scrophularia glabrata	0.00009	0.32	0.70	perennial	woody	2	2
Scrophularia arguta	0.00008	0.91	0.38	annual	non-woody	3	7
Senecio leucanthemifolius	0.00020	0.76	0.40	annual	non-woody	1	5
Senecio glaucus	0.00016	0.28	0.30	annual	non-woody	1	6
Tolpis lagopoda	0.00031	0.32	0.35	perennial	woody	1	3
Tolpis barbata	0.00007	0.72	0.20	annual	non-woody	2	7
Trifolium stellatum	0.00223	0.72	0.15	annual	non-woody	2	6
Trifolium arvense	0.00025	0.88	0.30	annual	non-woody	2	7
Emex spinosa	0.01872	0.94	0.33	annual	non-woody	1	7
Rumex bucephalophorus	0.00020	1	0.25	annual	non-woody	1	7
Monanthes laxiflora	0.00001	0.80	0.08	perennial	woody	3	6
Aichryson laxum	0.00001	0.56	0.30	annual	non-woody	3	6
Descurainia millefolia	0.00024	0.97	0.50	perennial	woody	3	3
Arabis caucasica	0.00015	0.97	0.18	perennial	non-woody	2	5