Persisting in defaunated landscapes: reduced plant population connectivity after seed dispersal collapse

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Running title: Genetic impact of frugivore defaunation

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Summary

1. Defaunation of large-bodied frugivores could be causing severe losses of crucial ecosystem functions such as seed dispersal. The immediate ecological consequences may include alteration or even collapse of seed-mediated gene flow affecting plant population connectivity, with impacts on the regional scale distribution of genetic variation. Yet these far-reaching consequences of defaunation remain understudied.

2. Here we tested whether human-induced defaunation of the Canarian frugivorous lizards (*Gallotia*, Lacertidae) altered within-island population connectivity and the amount and large-scale distribution of genetic variation of *Neochamaelea pulverulenta* (Rutaceae), which relies exclusively on these lizards for seed dispersal. Our study system defines a lizard downsizing gradient with three contrasted ecological scenarios (islands) with relatively optimal (Gran Canaria; large-sized lizards), sub-optimal (Tenerife; medium) and collapsed seed dispersal processes (La Gomera; small). We extensively sampled individual plant genotypes from 80 populations spanning the full geographic range of the plant to examine their genetic diversity, population-genetic network topologies, and the patterns of isolation both by distance (IBD) and resistance (IBR) across these three ecological scenarios.

3. Plant genetic diversity appeared unaffected by defaunation-mediated downsizing of frugivorous lizards. However, we found a reduced overall plant population connectivity together with an increased isolation by distance within the most defaunated islands (La Gomera and, to a lesser extent, Tenerife) when compared with the scenario preserving the functionality of lizard-mediated seed dispersal (Gran Canaria). The results, with a significant effect of lizard downsizing, were robust when controlling for biotic/abiotic differences among the three islands by means of isolation by resistance models (IBR).

4. *Synthesis.* Our results provide valuable insights into the far-reaching consequences of the deterioration of mutualisms on plant population dynamics over very large spatial scales.

Conservation of large-bodied frugivores is thus essential because their irreplaceable mutualistic dispersal services maintain an extensive movement of seeds across the landscape, crucial for maintaining the genetic cohesiveness of metapopulations and the adaptive potential of plant species across their entire geographic range.

Key-words: Canary Islands, extinction, frugivorous lizards, *Gallotia*, genetic diversity, *Neochamaelea pulverulenta*, population connectivity, seed dispersal.

Introduction

Defaunation, the sustained loss of distinct groups of animals (e.g., top predators, megafauna), is causing added losses of crucial ecosystem functions (Dirzo *et al.* 2014; McCauley *et al.* 2015; Malhi *et al.* 2016; Young *et al.* 2016), such as dispersal of seeds for animal-dependent plants (Markl *et al.* 2012; Fontúrbel *et al.* 2015). Central ecological consequences of these cascading effects include changes in vegetation structure (Johnson 2009; Bakker *et al.* 2016), reductions of plant regeneration (Cordeiro & Howe 2003; Terborgh *et al.* 2008) and carbon storage potential (Bello *et al.* 2015; Peres *et al.* 2016), and altered evolutionary regimes (Valido 1999; Galetti *et al.* 2013). Additional consequences may follow defaunation, such as the alteration or even collapse of seed-mediated gene flow affecting connectivity among plant populations, with subsequent changes in the spatial distribution of genetic variation both at local and landscape scales. Recent studies have documented the fast-paced action of these changes and their consequences, ultimately leading to significant alterations of population-scale genetic diversity (i.e., the distribution of genetic variation within plant populations) (Pacheco & Simonetti 2000; Carvalho *et al.* 2016; Pérez-Méndez *et al.* 2016). Yet the consequences at larger, regional scales (i.e. among populations) remain understudied (Voigt *et al.* 2009; Calviño-Cancela *et al.* 2012).

Long-distance dispersal (LDD) assisted by frugivores (Nathan 2008) is a crucial ecological process affecting functional connectivity of fleshy-fruited plant populations at both local and regional scales. Yet this process is being severely altered by human-driven disturbances (Markl *et al.* 2012; Fontúrbel *et al.* 2015), such as anthropogenic defaunation selectively removing large-bodied vertebrates (Estes *et al.* 2011; Dirzo *et al.* 2014) which, in turn, are primarily responsible for LDD across landscapes (Jordano *et al.* 2007). Despite the fact that pollen flow may contribute to genetic exchange among isolated populations, seed dispersal is a crucial process because it moves both maternal and paternal gametic genomes and determines the final establishment of the genotypes, ultimately leading to realized gene-flow (e.g. Bacles *et al.* 2006; Grivet *et al.* 2009; Zhou & Chen 2010). The deterioration of the dispersal process (e.g., the reduction of seed dispersal distances) following defaunation-driven downsizing of frugivore assemblages (Pérez-Méndez *et al.* 2016), is then expected to impair and eventually collapse among-plant population connectivity, with potential effects on the regional distribution of genetic variation. Specifically, we should expect a loss of genetic diversity triggered by a reduced gene-flow and increased genetic drift. We also expect a loss of population connectivity and increased isolation by distance across the landscape that mirrors the extirpation of larger frugivores. These indirect effects of human-induced disturbances remain understudied despite their potential for triggering losses of ecological functionality (Western 2001; Valiente-Banuet *et al.* 2015).

Here we use a comparative approach to address these questions on the Canary Islands by taking advantage of a gradient of human-driven frugivore downsizing across Gran Canaria, Tenerife and La Gomera islands. We focus on the mutualistic interaction occurring between *Neochamaelea pulverulenta* (Rutaceae), a fleshy-fruited long-lived shrub species only present in these islands, and their unique primary seed dispersers, the endemic frugivorous lizards of the genus *Gallotia* (Lacertidae) (Valido & Nogales 1994). Large-bodied lizards were abundant in these islands until the first arrival of Aborigines (~2500 years BP) (Onrubia-Pintado 1987) triggered a defaunation process that impacted the insular lizard fauna (Barahona *et al.* 2000; Gonzalez *et al.* 2014). Yet the magnitude of the impact was markedly different in each island, defining a lizard downsizing gradient with three contrasted ecological situations (Pérez-Méndez, Jordano & Valido 2015; Pérez-Méndez *et al.* 2016): 1) Gran Canaria, an island close to the pre-human situation; i.e., preserving large-sized lizards (*G. stehlini*) that facilitate relatively long-distance dispersal (up to 94.2 m), 2) Tenerife, hosting medium-sized lizards (*G. galloti*) dispersing seeds over shorter distances (up to 46.4 m), and 3) La Gomera, an island with collapsed seed dispersal because the extant lizards (*G. caesaris*) are not large enough to swallow whole fruits and efficiently disperse *N. pulverulenta* seeds, where the maximum dispersal distances recorded reach only 4.5 m.

Most previous analyses of the consequences of seed dispersers loss have focused at limited spatial scales, usually documenting local demographic effects or changes in within-population, fine-scale spatial distribution of genetic variation (Pacheco & Simonetti 2000; Pérez-Méndez *et al.* 2016). Specifically, in a previous study we evaluated the consequences of the defaunation-driven loss of large-bodied lizards on seed dispersal distances and the distribution of genetic variation over local scales (within populations) (Pérez-Méndez *et al.* 2016). Here, we examine how the loss of mutualistic species and interactions resulting from a defaunation process may extend beyond these local scales and imprint the large-scale structuring of genetic variation throughout the full geographic range of a plant species. We hypothesize a reduction of plant genetic diversity at landscape scales (i.e. average genetic diversity of populations within island) on islands where large-sized lizards have been extirpated because of the impact of both reduced gene flow and increased genetic drift. In addition, we expected a reduction of within-island population connectivity and an increase of genetic isolation by distance mirroring the reduction of lizard-mediated dispersal distances.

To test our hypothesis we first compare the amounts of genetic diversity across the three insular scenarios, with an extensive sampling of plant individual genotypes within populations spanning the species full geographic range. Then, we analyse the population connectivity within islands on each of these three ecological scenarios by using three complementary analytical approaches: population graphs to compare the topology of the genetic connectivity networks (Dyer & Nason 2004; Dyer, Nason & Garrick 2010; Dyer 2015), and isolation both by distance (IBD) and resistance (IBR) to effectively test for the simultaneous influence of biotic/abiotic environmental characteristics (e.g., topography, climate, vegetation) differences among islands (McRae 2006). Our results may provide valuable insights into the far-reaching consequences of the deterioration of mutualisms on plant population dynamics over very large spatial scales.

Material and methods

STUDY SYSTEM

Neochamaelea pulverulenta (Vent) Erdtman (Rutaceae) is an endemic shrub from the Canarian archipelago and only present in Gran Canaria, Tenerife and La Gomera (Fig. S1). Recent studies suggest that it is a neoendemism, which was originated during the Miocene in these islands (Appelhans *et al.* 2011, 2012). It was originated most likely from an African ancestor shared with *Cneorum tricoccon*, which is distributed in the Mediterranean Basin and in the Balearic Islands, and has lizards also acting as seed dispersers (Traveset, González-Varo & Valido 2012). *N. pulverulenta* is a relatively common species, distributed along xerophytic lowlands (< 400 m a.s.l.; Fig. S1) where mean temperature is 21ºC and mean annual precipitation < 300 mm (AEMET-IP 2012). The vegetation is dominated by *Euphorbia* spp. (Euphorbiaceae), *Lavandula* spp. (Labiatae), *Lycium intricatum* (Solanaceae), *Periploca laevigata* (Asclepiadaceae), *Plocama pendula* and *Rubia fruticosa* (Rubiaceae), among others. *N. pulverulenta* is a self-incompatible, insect-pollinated species. Although the identity of some pollinator species changes across islands, pollinator guilds of *N. pulverulenta* are very similar in the three studied scenarios and are composed mainly by flies (Fam. Bibionidae, Calliphoridae, Muscidae and Syrphidae), wasps (*Leptochilus cruentatus*), solitary bees (*Amegilla* spp., *Lasioglossum* spp., *Bombus canariensis*, *Colletes dimidiatus*) and ants (*Camponotus feae*, *Linephitema humile*) (Hohmann *et al.* 1993, Trojelsgaard *et al*. 2015, A. Valido & N. Pérez-Méndez, unpublished data). From spring to early summer *N. pulverulenta* bears fleshy fruits (1-4 cocci; functionally drupes) with hard-coated seeds (8-10 mm in diameter) (Valido 1999). Only medium- to large-bodied endemic lizards (g. *Gallotia*, Lacertidae) are legitimate seed dispersers of *N. pulverulenta* (Valido & Nogales 1994; Valido 1999; Valido, Nogales & Medina 2003). The percentage of seeds that germinate ranges between 29-37%. However, germination is much faster when seeds are consumed by *G. galloti* lizards (mean ± SD; 382.2 ± 583 days) than when they are not ingested by them (1177 ± 385 days). In addition, germination was even faster for those seeds consumed for the largest bodied *G. stehlini* species (189.8 ± 504 days) (Valido 1999). Seed germination also occurs beneath adult plants, where aggregated groups of seedlings are often found, but also in open spaces where lizards usually drop the consumed seeds (Pérez-Méndez *et al*. 2015). No seedling herbivory has been observed, thus seeds escaping from post-dispersal predation are likely to germinate and recruit beneath maternal plants. In addition, it is frequent to observe dispersed seeds and seedlings growing on open microhabitats, where *Gallotia* lizards usually defecate seeds while basking for thermoregulation.

There is a gradient of progressive reduction of both lizard body-sizes (Barahona *et al.* 2000), and seed dispersal distances (Pérez-Méndez *et al.* 2016) across these three islands. This is the result of a historical defaunation-mediated downsizing process starting ~ 2500 yr B.P. with the human colonization of the islands (Onrubia-Pintado 1987; Barahona *et al.* 2000;

Gonzalez *et al.* 2014) resulting in the markedly different body sizes of extant lizards on each of these islands (Barahona *et al.* 2000). Lizard downsizing in Gran Canaria was relatively subtle, from the large-sized individuals of *G. stehlini* sub-fossils (maximum snout- to vent-length; max. SVL= 367 mm) to the extant *G. stehlini* individuals (max. SVL= 280 mm) which still provide effective and long-distance dispersal of *N. pulverulenta* seeds, up to 94.2 m. Intermediate downsizing occurred in Tenerife, from the extinct *G. goliath* species (max. SVL= 502 mm) to the sub-efficient, medium-sized *G. galloti* (max. SVL= 144 mm) dispersing seeds at intermediate distances (maximum dispersal distance= 46.4 m). Finally, lizard size reduction was very intense in La Gomera, where the extinct *G. goliath* (max. SVL= 466 mm) was four times larger than the extant *G. caesaris* (max. SVL= 111 mm), which performs very inefficient seed dispersal of *N. pulverulenta* (maximum dispersal distance= 4.5 m). Additional giant lizard species inhabit Tenerife (*G. intermedia*) and La Gomera (*G. bravoana*), but only present in marginal remnant sites on very inaccessible cliffs (Hernández, Nogales & Martín 2000; Valido *et al.* 2000). Thus, in Gran Canaria the defaunation-mediated downsizing process included only one lizard species (*G. stehlini*), while in Tenerife and La Gomera included the already extinct *G. goliath* or near extinction of several large-bodied lizard species (*G. intermedia* in Tenerife, and *G. bravoana* in La Gomera) (for details see Barahona *et al.* 2000; Pérez-Méndez *et al.* 2015). Common kestrels (*Falco tinnunculus*, Falconidae), which prey upon *Gallotia* lizards, have been also cited as potential secondary seed dispersers of *N. pulverulenta* (Padilla, González-Castro & Nogales 2012).

SAMPLING DESIGN AND MICROSATELLITE GENOTYPING

We sampled leaves of adult individual plants from 30, 28 and 22 populations in Gran Canaria, Tenerife, and La Gomera, respectively, spanning the full distribution range of *N. pulverulenta* (Fig. S1). Within each population we followed 3-4 linear transects covering an area of ~1 ha, sampling 12-33 adult individual plants/population (total= 2358 individuals) spaced at least 5 m from any other (Fig. S1 and Table S1).

Unique multilocus genotypes from 12 microsatellite markers (Rigueiro *et al.* 2009) were obtained for all collected plants. Dried leaves were ground in a ball-mill (Mixer Mill MM301, Retsch, Germany) and DNA extraction was performed with a modified CTAB extraction protocol (Rigueiro *et al.* 2009). Amplified fragments were analysed on an ABI 3130xl, and the scoring was manually assessed using GeneMapper 4.0 (Applied Biosystems) and LIZ 500 size standard. A subset of the scoring was performed independently by two people and cross-checked to assess and reduce the frequency of genotyping errors.

PLANT GENETIC DIVERSITY

First, we built a diversity-accumulation curve to assess alleles/population richness with increasing sampling effort (Fig. S2). Population genetic diversity was estimated as the expected heterozygosity (*He*), observed heterozygosity (*Ho*), and average allelic richness (*AR*) by using HIERFSTAT package in R (Goudet 2005). To test for differences among islands, we applied post hoc contrasts (Tukey) after fitting linear models, with island as the main fixed factor. We also performed an analysis of molecular variance (AMOVA) with the genetic information of sampled populations. We used the R package *ade4* (Dray & Dufour 2007) and included three hierarchical levels in the analyses (among islands, among populations within islands, and among individuals within populations).

POPULATION CONNECTIVITY

To understand how defaunation of large-bodied lizard species affects among-population connectivity we used genetic information to create a network of populations within each island (Dyer 2007). We used an approach based on population graph theory (Dyer & Nason

2004; Dyer *et al.* 2010; Dyer 2015), which uses the concept of conditional dependence to obtain a network with the minimal edge (links) set that sufficiently describes the total among-population genetic covariance structure. Links denoted the presence of significant genetic covariance among populations after accounting for overall genetic covariation. We started from a full-connected network in each island where all populations were connected with each other by an edge with a variable weight (proportional to genetic distance). Then, redundant edges that did not sufficiently contribute to explaining the overall genetic covariance structure of the network were pruned. Thus, populations v_i and v_j will share an edge if and only if there is significant genetic covariance between the populations after removing the covariation each population has with all the remaining populations in the data set. A significance level of 0.05 was established as a threshold value for edge retention (see Dyer and Nason 2004, Dyer 2007 for details). We used the R package *popgraph* (Dyer 2014). We compared the resulting networks for each island using several parameters: 1) *Degree*: the number of edges a population has to other populations; thus we estimated the average degree across populations within island. Larger values indicate higher overall connectivity at the island level. 2) *Edge length*: a proxy of conditional genetic dependence among pairs of populations; the shorter the link between two populations, the smaller is their conditional genetic distance (cGD; i.e. higher connectivity) after controlling for the differences with the rest of populations. 3) *Closeness*: a centrality measure of networks, which is defined as the number of steps required to connect every node of the network from a given node. As genetic population connectivity increases, the average value of node closeness increases. We used the R package *igraph* for these analyses (Csárdi & Nepusz 2006). In addition, the standard deviation plots (mean ± SD *vs.* sample size) were assessed for each parameter and network to check the accuracy of parameter estimations and sampling robustness (Fig. S3). We applied a set of generalized linear models (GLMs) with islands as a fixed factor and post-hoc Tukey comparisons to evaluate among island differences for each network parameter.

ISOLATION BY RESISTANCE (IBR)

The classical 'isolation by distance' models (IBD hereafter) (Wright 1943) predicts, as the outcome of both dispersal limitation and genetic drift, an increase of genetic differentiation between populations with the Euclidean geographic distance. The IBD models assume that gene flow is symmetric and homogeneous across space, however it is known that landscape complexity also shapes patterns of gene flow (McRae & Beier 2007). At this respect, some discrete or continuous landscape characteristics can impose "resistance" to dispersal, i.e. isolation by resistance (IBR hereafter; McRae 2006). Generally, the IBR approach improves IBD models by using the resistance distance, a metric based on circuit theory (McRae 2006, McRae *et al*. 2008). In our comparative inter-insular context, an added advantage of the IBR model is that it takes into account different biotic/abiotic variables (landscape characteristics) that may modify the genetic connectivity among plant populations in parallel to the variation in lizard downsizing. Thus, according to our hypothesis we would expect an increased IBR (i.e. higher slope for the regression "genetic distance ~ resistance distance") in the scenarios showing impaired seed dispersal (i.e. we would expect a higher genetic differentiation at a given resistance distance in those scenarios showing reduced gene flow). For genetic distances, we incorporate two estimates, the classical Euclidean genetic distance (GD), and the conditioned genetic distance obtained from *popgraph* (cGD). To test this hypothesis, we modelled resistance distances as a function of five landscape variables, each of them with different potential effects on gene flow (Fig. S4): topographic complexity, climate-related variables, potential and current vegetation, and the range shape of *N. pulverulenta*.

The topographic complexity was measured by using the surface ratio index for each cell from the global digital elevation model (ASTER GDEM 2011) using the 'DEM SURFACE TOOLS' script package (Jennes 2004) in ARCGIS 10.1 (ESRI, Redlands, CA, USA). We create raster layers with a spatial resolution standardized to 30-m grid cell size. To be consistent with the rest of resistance maps, the final layer of this continuous, topographic complexity variable was transformed to 500 m resolution. Values of resistance close to one indicate flat areas (lesser resistance to lizards movement) and higher values indicate abrupt reliefs with deep slopes (Jennes 2004). For the climate-related resistance map, we used the Köppen-Geiger climate classification for the Canary Islands which defines different types of climate using average monthly precipitation and temperature (AEMET-IP 2012). We reclassified climatic areas into three basic categories that may impose increasing resistances to lizard-mediated seed dispersal because of increasing limitation of lower temperatures for lizard activity: dry (Type B in Köppen-Geiger), temperate (Type C), and cold (Type D) climates. Resistance maps of both potential and current vegetation were characterized using the cartographic information provided by del Arco *et al.* (2006). The vegetation categories were reclassified into three broad groups: *i*) evergreen or wet forests (including laurel and 'fayal-brezal' woodlands) imposing a high resistance for lizards, *ii*) pine forests (intermediate resistance), and *iii*) the remaining vegetation grouped as 'scrublands' (low resistance). This latter category includes xerophytic lowlands (e.g. *Euphorbia*, *Kleinia*), termophylous forests (e.g. *Olea*, *Rhamnus*), and sub-alpine vegetation (e.g. *Spartocytisus*, *Descurainia*). We considered these scrubland areas as 'minimal barriers' for dispersal as lizards are extremely abundant in this type of vegetation in comparison with evergreen and pine forests. Urban and rural areas were also included as maximum barriers on the current vegetation map. Besides, lizard abundances are much lower on evergreen than on pine forests as the result of the less insolation and higher humidity of the evergreen forests that mismatch the thermoregulation requirements of ectothermic animals. Under this context, we assume that dispersal of seeds by lizards in this habitat is impaired when compared with warmer habitats (xerophytic scrublands or pine forests). Finally, the range shape of *N. pulverulenta* was included as a simple binary habitat/nonhabitat resistance map, assuming that the habitat occupancy of the plant is bounded (McRae & Beier 2007). For this we used the *N. pulverulenta* distribution map available in the ATLANTIS project from the Canarian government. (http://www.biodiversidadcanarias.es/atlantis) (see Figs. S4 for a detailed account of all used resistance maps).

We used CIRCUITSCAPE v.4.0.5 (McRae 2006) to calculate pairwise resistance distance among populations for each landscape resistance scenario. Populations were included as focal points of a single cell in the raster, and adjacent cells were connected to eight neighbours by average resistances. In addition, we calculated pairwise distances among populations under a non-resistance scenario (i.e. an uniform raster with all cells equaling to 1; non-resistance; IBD) as they are more appropriate than euclidean geographic distances for comparisons with IBR models (McRae & Beier 2007; Noguerales *et al.* 2016).

For each island, IBR models were tested using a set of Mantel tests on the different resistance distances against the genetic distances (GD, cGD) between all pairs of populations. For this, we used the IBDWS web service version 3.23 (Jensen, Bohonak & Kelley 2005). The significance of Mantel's Z test statistics and the estimation of the Mantel *r* coefficient were based on 10,000 permutations. For this, we applied a reduced major axis regression. We calculated the 95% confidence intervals for slopes of the regression for each resistance variable in the three islands after 10,000 permutations (Jensen *et al.* 2005) and tested for differences among islands by applying a set of t-tests.

Finally, for testing differences due to defaunation effects when controlling for the effects of among-islands variation in IBR we specified a saturated linear model with genetic distance (GD, cGD) as a response variable and defaunation status (fixed factor), and IBR variables as predictors in the model (see Wang 2013). Defaunation status was coded as "Large-sized lizards" (level 1); "Medium-sized lizards" (level 2) and "Small-sized lizards" (level 3), according to an increasing level of loss of dispersers effectiveness with increasing defauntaion intensity across the three islands. In order to avoid strong collinearity among the six IBR variables we applied a stepwise selection of non-collinear variables based on their variance inflated factors (package VIF, R Development Core Team 2015), with threshold parameter of 10. The significance of the multiple regression models for genetic distance on both defaunation and the IBR predictors was tested with the *lmPerm* R package, using 10,000 iterations. We used a combined AIC weights analysis on candidate models including and excluding the defaunation effect to assess the importance value of the defaunation parameter (aictab and importance functions in R library *AICcmodavg*). For this we defined a set of models including both defaunation and the IBR variables retained with the VIF criterium on one hand, and another set just excluding defaunation. The importance function calculates the relative importance (w+) of the defaunation effect based on the sum of Akaike weights (model probabilities) of the models that include the effect compared to the importance (w-) of models excluding it and including just the IBR predictors. To account for the potential effects of differences among islands in abiotic and biotic variables masking the defaunation effect, we estimated the partial regression for just the effect of defaunation level on GD (and cGD) when the other predictors are held constant. We used the *visreg* package in R (Breheny & Burchett 2017).

GENETIC STRUCTURE

A Bayesian clustering method implemented in the STRUCTURE software v.2.3 (Pritchard, Stephens & Donnelly 2000) was used to estimate the genetic structure of populations. We used the admixture model and correlated allele frequency among populations. We ran 100,000 MCMC repetitions after a burn-in of 10,000 for a range of K between 1 and the maximum number of populations in each island. The optimal number of K was estimated following the method of Evanno, implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012). We ran this model 5 times, and we averaged to correct for label switching by applying CLUMPP (Jakobsson & Rosenberg 2007). The DISTRUCT software (Rosenberg 2003) was used to graphically display the outputs of the models.

Finally, to assess the genetic separation of populations at the archipelago scale, we calculated a UPGMA cluster analysis of pairwise Euclidean genetic distances (Nei's distances) with bootstrap support (n= 1000 replications), and displayed it as a phylogram. We used the R package *poppr* for analyses (Kamvar, Brooks & Grünwald 2015).

All statistical analyses were conducted in R, version 3.2.4 (R Development Core Team 2015).

Results

GENETIC DIVERSITY

Genetic diversity (*He*, *Ho*, and *AR*) within populations was highly variable, ranging from populations exhibiting relatively low values (e.g. populations 29, 30, 33) to others with much higher values (e.g. populations 16, 67, 68) (Table S1). Unexpectedly, the most defaunated La Gomera showed the highest genetic diversity at the island level (i.e. average genetic diversity of populations within island), with all parameters (except *He*) being significantly higher than Gran Canaria and Tenerife (p< 0.05 for all pairwise contrasts; Table 1). In turn, *He* was similar between La Gomera and Gran Canaria, and both islands showed higher values than Tenerife (Table 1). The AMOVA indicates that among the spatial hierarchical levels examined, most variation is explained by among-island differences (17.6%), with lower, but significant, values accounted for by differences among populations within island (13.9%), and among individuals within populations (3.2%), yet with a high fraction of overall genetic variation being attributed to residual variation (65.3%) (Table S2).

POPULATION CONNECTIVITY

The network topology parameters differ markedly among islands (Table 1, Fig. 1). First, *degree* decreases from Gran Canaria to Tenerife and to La Gomera, although no significant statistical differences were found ($p > 0.05$; Tukey a-posteriori test). Secondly, the average *edge length* increases from Gran Canaria to Tenerife, and to La Gomera, with significant statistical differences between La Gomera and the other two islands ($p < 0.05$). Finally, *closeness* decreased from Gran Canaria to La Gomera, and to Tenerife, being significantly lower in Tenerife ($p < 0.01$). Taken together these results suggest the highest among-population connectivity in Gran Canaria than the other, more defaunated, scenarios, especially La Gomera.

ISOLATION BY RESISTANCE (IBR)

All the univariate IBR models fit better to the pattern of genetic differentiation than the non-resistance models for each island (i.e. resistance distances based on a completely ‘flat’ landscape) (Fig. S5). Both the Mantel tests and the reduced major axis regressions indicate a significant correlation between Euclidean genetic distances (GD) and all the resistance distances (Fig. 2; Table S3), with slopes being significantly higher in both La Gomera and Tenerife than in Gran Canaria for almost all IBR variables (t-test; $p < 0.01$) (Fig. 2) (See also figures S5 for results including conditional genetic distances; cGD).

We fitted a final linear model including current vegetation (VIF= 7.21), topography complexity (VIF= 7.20), and defaunation status (VIF= 1.01) as predictors, as these were the variables retained after applying the collinearity criterium. Euclidean genetic distances (GD) among plant populations were significantly correlated with the combined defaunation and the retained IBR variables (F= 370.0, d.f.= 3 and 1040, R^2_{adj}= 0.516, P< 0.0001; Fig. 3) (see also Fig. S7 for results including conditional genetic distance; cGD). Moreover, the importance analysis of models excluding the defaunation effect (just with topographic complexity and/or current vegetation) yielded extremely lower weights (w-= 6.57E-08) than the models set including this effect (defaunation and topographic complexity and/or current vegetation; w+>> 0.999) (χ^2= -17.3, P< 0.0001). Moreover, accounting for the two retained IBR variables, the conditional plots for just the effect of defaunation (insets in Fig. 3 and Fig. S7) revealed a significant effect.

Genetic structure at large scales revealed by both the STRUCTURE (Fig. S8 and S9) and the UPGMA analyses (Fig. S10) is consistent with IBR patterns, suggesting a progressive genetic differentiation among populations with distance.

Discussion

Defaunation-mediated downsizing of frugivorous vertebrates may trigger cascading effects with a myriad of consequences for demography (Cordeiro & Howe 2003; Traveset & Riera 2005; Traveset *et al.* 2012; Pérez-Méndez *et al.* 2015) and genetics of plant populations (Pacheco & Simonetti 2000, Voigt *et al*. 2009, Calviño-Cancela *et al*. 2012, Pérez-Méndez *et al*. 2016). Here, we demonstrate that when seed dispersal interactions are disrupted (La Gomera) or functionally impaired (Tenerife), the genetic consequences may spread well beyond the local population scale. Although plant genetic diversity does not appear to be related to reduced or even collapsed seed dispersal, the contrasting topology of the population networks and the IBR patterns revealed an overall reduction of genetic connectivity among plant populations mirroring the downsizing gradient of frugivorous lizards. Our previous study (Pérez-Méndez *et al*. 2016) showed a progressive reduction of seed dispersal distances of *N. pulverulenta* according to lizard sizes, with marked implications for the fine-scale spatial genetic structure within populations. Here, we show that these effects extend beyond local population limits to have consequences for the spatial distribution of genetic diversity at much broader scales.

PLANT GENETIC DIVERSITY

Genetic variation in plants results from both the demographic history and the extent of gene flow among populations acting together with selection, drift, and mutation. The reduction, and eventual collapse, of gene flow among plant populations in islands hosting small- to medium-sized lizard species is expected to result in an increase of genetic isolation and genetic drift, with added reductions of genetic variation across populations. Contrary to our predictions, genetic plant diversity was higher in the island hosting the smallest lizards (La Gomera) and no major differences were found between the other two islands. This result is also consistent with our previous study (Pérez-Méndez *et al*. 2016), in which we found similar within-population genetic diversity across the three studied insular scenarios. Overall, our results suggest that current levels of overall genetic diversity in *N. pulverulenta* may be more related to the past biogeographic and/or demographic history of the species than the effect of reduced contemporary gene flow by seeds. Although no specific information is available on the biogeographic history of *N. pulverulenta*, one possible explanation relates to the idea that La Gomera could have acted as a center of high genetic diversity and source of propagules for the other islands, as reported for other Canarian plant species such as *Olea europaea* (García-Verdugo *et al.* 2010), and *Ruta* spp. (Salvo *et al.* 2010). In addition, genetic diversity differences may arise as the result of a reduced human pressure in La Gomera (60 inhabitants/km^2) than in the most populated Gran Canaria (546 inhab./km^2) and Tenerife (442 inhab./km^2) (http://www.gobiernodecanarias.org/istac). This explanation seems to be supported by the relatively consistent, ring-arranged spatial structure of the populations in La Gomera when compared with the other two islands. It suggests a collection of populations that are relatively robustly connected in a way that maintains island-wide diversity without the spatial gaps that are so prominent in the other two islands. This pattern appears prominent despite the growing distance between populations and the relatively low number of connections for each population. Accordingly, Miraldo *et al*. (2016) recently reported a global decline of intraspecific genetic diversity in terrestrial mammals and amphibians in the most human-disturbed habitats. Whatever the explanation, the high levels of genetic diversity found in La Gomera suggest a delayed effect of defaunation relative to other anthropogenic pressures such as fragmentation, which is usually accompanied by a rapid impoverishment of genetic pools (e.g. Young, Boyle & Brown 1996).

POPULATION CONNECTIVITY

Our results of topological variation of the population graphs suggest a limited gene flow among populations and that defaunation of frugivorous lizards impacts the overall landscape connectivity. We detected that gene flow of *N. pulverulenta* was mostly restricted among populations separated by no more than 10 km (Fig. 1; Fig. S1), a very short distance when compared with other animal-dispersed plant species (e.g. Dyer 2015). Thus, the low population connectivity detected in the three islands indicates overall highly restricted gene flow. This is most likely the result of the specific interaction with their unique primary seed dispersers (Valido & Nogales 1994; Valido 1999; Pérez-Méndez *et al.* 2015) and a small set of pollinator species. Canarian lizards have very limited home ranges (Molina-Borja 1985; Valido *et al*., unpublisehd data) with reported seed dispersal distances below 100 m (Pérez-Méndez *et al.* 2016). Likewise, the *N. pulverulenta* assemblage of pollinators is species-poor, composed by several species of ants, small bees, and flies, which seem to exhibit restricted foraging patterns according with pollination distances (median: 40.2 m) estimated by using parentage analysis of embryos (Pérez-Méndez *et al.* 2016). In turn, the role of secondary seed dispersers (e.g. common kestrels) (Padilla *et al.* 2012) as potential long distance seed dispersers remains unstudied.

Despite this general trend, the topologies of the genetic networks also indicate that the population connectivity varies markedly according with the defaunation status of the islands. Gran Canaria, which still preserves large-size lizards, showed the highest connectivity or degree, the shortest links, the largest closeness and a slight decrease of population connectivity with distance. In contrast, the same parameters indicate a much more marked isolation and severe loss of among population connectivity in the most defaunated scenario (La Gomera). Tenerife should represent an intermediate scenario according with our hypothesis, its graph parameters being similar to those of Gran Canaria, except for closeness. Besides, the obtained results of La Gomera are conservative as this island is much smaller (370 km^2) than Gran Canaria (1560 km^2) and Tenerife (2034 km^2), and mean geographic distances among sampled populations are also shorter in La Gomera (13 ± 6 km; mean ± 1 SD) than in Gran Canaria (22 ± 12 km) and Tenerife (29 ± 15 km). The observed differences in genetic connectivity among populations could not be interpreted solely in terms of variation in biotic or abiotic landscape conditions among islands, with defaunation having a highly significant effect on the variation across islands in landscape characteristics (see below).

Other ecological and historical processes related with abiotic and biotic characteristics of the islands may also explain the observed differences of the spatial distribution of genetic variation. We have taken into account this abiotic/biotic variation in our analytical approach to try to isolate these confounding factors (see resistance models below). In addition, differences may be attributed to a compensatory effect of pollen-mediated gene flow in La

Gomera (e.g. dispersing pollen from more distant sources and enriching the population genetic pools). Yet, although species identity can change, pollinator guilds in *N. pulverulenta* are quite similar among these islands (Hohmann *et al.* 1993, Trøjelsgaard *et al*. 2015, A. Valido & N. Pérez-Méndez, unpublished data). Thus, we have no evidences supporting this. It would be, however, an important issue to consider in future research. Overall, assuming the limitations of this study, we lack evidences to relate the observed reduction of genetic connectivity with habitat factors or contrasted pollen-mediated gene flow across populations. We think that a more parsimonious explanation for the observed patterns relates with a reduction of gene flow via seeds associated to the extinction of the largest seed dispersers.

These genetic patterns agree with our previous study of seed dispersal distances (Pérez-Méndez *et al.* 2016). In the particular case of La Gomera, given the collapse of both primary and, in consequence, the secondary seed-mediated gene flow, the question remains of how *N. pulverulenta* preserves residual connectivity among their populations. First, despite pollen movement not being very extensive, several species such as honeybees or native bees may eventually move over large distances, providing opportunities for gene exchange among relatively distant populations (Steffan-Dewenter & Kuhn 2003, Zurbuchen *et al*. 2010, Danner *et al*. 2016). However, similar pollinator assemblage is found in these islands (Trojelsgaard *et al.* 2015). Secondly, current patterns of genetic connectivity may reflect a historical inertia of a past efficient gene flow among populations mediated by the now extinct giant lizards (decline date ~ 2344 BP) (Gonzalez *et al.* 2014).

Network parameters (*degree*, *edge length* and *closeness*) might vary with the spatial distribution of sampled populations within islands. However, if the genetic connectivity were similar within these three islands, we would expect a higher *degree* and *closeness* and shorter *edge lengths* in the islands where the geographic distances among sampled populations are shorter (i.e. La Gomera). However, the pattern found was exactly the opposite, i.e. we found the lowest *degree*, *closeness* and the largest links in the island sampled at shorter geographic distances among populations (the most defaunated La Gomera). Therefore, our results are conservative and strongly support our hypothesis; we would expect even more marked differences (e.g. lower *degree* or longer *edge lengths*) if geographic distances among populations in La Gomera were similar to those of the other two islands.

ISOLATION BY RESISTANCE (IBR)

Genetic isolation by distance (IBD) is a common pattern among plant species, which theoretically arises as the outcome of a limited exchange of genes among nearby populations, (Sexton, Hangartner & Hoffmann 2014). Given the short dispersal distances previously reported for our study system and directly related to lizard sizes (Pérez-Méndez *et al.* 2016), we predict a marked pattern of IBD in the three islands, mirroring the lizard downsizing gradient. Accordingly, geographical distance (i.e. non-resistance model) explained a large proportion of the spatial distribution of genetic variation of *N. pulverulenta*, and the detected differences among islands in their regression slopes match the differences in the seed dispersal process occurring in each island. The different IBR models also support this result which suggest that a reduction of seed dispersal distances resulting from the impaired or disrupted mutualistic relationships may alter profoundly the distribution of genetic variation at broad spatial scales independent of different resistances landscapes (e.g., topography complexity, vegetation physiognomy). The magnitude of IBR (i.e., the slope of the Euclidean genetic distance-IBR regressions) was much larger in the more defaunated scenario (Tenerife and La Gomera) than in the island hosting the large-sized lizards (Gran Canaria) for almost all resistances variables. The significant result for the defaunation effect remained consistent after controlling for the effects of the two other predictors retained in the model. This result is not surprising, when considering the absence of long distance dispersal events in the former islands (Pérez-Méndez *et al.* 2016). Thus, in our study system, defaunation fundamentally caused a strong and lasting limitation of dispersal in distance. In addition, our results suggest that this phenomenon entails important changes in the structure of genetic diversity of the plant species across its geographic range.

To the best of our knowledge no previous study has specifically assessed the impact of frugivore downsizing on plant genetic connectivity over large spatial scales. Our findings suggest that downsizing of mutualistic vertebrate frugivores hinders seed-mediated gene flow among plant populations, most likely through effects imposing substantial limitation of seed dispersal in distance. This entailed subsequent, negative cascading effects on the interacting plant populations at the landscape scale, such as the reduction of genetic connectivity and the increase of genetic differentiation among populations. Despite the specificity of the plant-frugivore interaction analyzed here, we would expect similar genetic outcomes in both insular and mainland ecosystems characterized by low functional redundancy on seed dispersers, where key large frugivores have become extinct (Bueno *et al.* 2013; McConkey & Drake 2015). Functional extinction of these species is expected to collapse seed dispersal process beyond local patches, triggering a parallel reduction of overall landscape genetic connectivity Thus, long before to have the possibility to identify some negative consequences of frugivores defaunation on plant genetic diversity at both population and landscape scales, our results provide valuable insights to detect early signals of Anthropogenic frugivore defaunation by analysing the genetic cohesiveness of plant metapopulations dynamics.

Author's contributions

A.V. and P.J. conceived the ideas and designed methodology; N.P.-M. and A.V. conducted fieldwork. N.P.-M. performed labwork with the assistance of one technician. N.P.-M.

analysed the data with the contribution of P.J. and A.V.; and N.P.-M., P.J., and A.V. wrote the paper. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.pr284 (Pérez-Méndez *et al.* 2017).

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of plant populations, sample sizes, and genetic diversity indices (see Fig. S1 for their geographic distribution).

Table S2. Results of the analysis of molecular variance (AMOVA) of *Neochamaelea pulverulenta* (Rutaceae).

Table S3. Results from univariate IBR models by using both Euclidean genetic distance (GD) and conditional genetic distance (cGD) of *Neochamaelea pulverulenta* (Rutaceae) at island level.

Figure S1. Geographic distribution of sampled populations of *Neochamaelea pulverulenta* (Rutaceae).

Figure S2. Sample-based accumulation curves of allelic diversity for each plant population.

Figure S3. Values for population network parameters on each island.

Figure S4. Raster layers showing information of the different landscape variables used for IBR analysis: climate classification, range shape of *Neochamaelea pulverulenta* (Rutaceae), sampled populations, topographic complexity, and potential and current vegetation in Gran Canaria, Tenerife and La Gomera.

Figure S5. Isolation by resistance models derived from each landscape variables at island level by using both GD (Euclidean genetic distance) and cGD (conditional genetic distance).

Figure S6. Slopes of the isolation by resistance models (IBR) (conditional genetic distances ~ Resistance distances).

Figure S7. Relationship resulting from the multiple matrix regression using conditional genetic distances (cGD) as response variable.

Figure S8. Genetic structure within island (STRUCTURE).

Figure S9. Values of *Delta K* as a function of the number of clusters (STRUCTURE).

Figure S10. Phylogram of the UPGMA based on Euclidean genetic distances.

Table 1. Summary of genetic diversity indices (*He*: expected heterozygosity; *Ho:* observed heterozygosity; *AR:* average allelic richness), and network parameters (*degree*, *edge length*, *closeness*) of *Neochamaelea pulverulenta* (Rutaceae) in Gran Canaria, Tenerife and La Gomera. For the genetic diversity indices, data are mean ± 1 SD. For the network parameters data are mean ± 1 SE. Different letters in superscripts indicate significant differences among islands (Tukey a-posteriori test). The numbers of sampled populations and individual plants are: Gran Canaria (30, 883), Tenerife (28, 823), and La Gomera (22, 652). See Fig. S1 and Table S1 for details.

	Gran Canaria	*Tenerife*	*La Gomera*
A) Genetic diversity indices			
He	0.61 ± 0.08 $^{a\,b}$	0.58 ± 0.10 ^{a}	0.64 ± 0.05 ^{b}
Ho	0.57 ± 0.07 ^{a}	0.54 ± 0.11 ^{a}	0.64 ± 0.04 ^{b}
AR	4.64 ± 0.86 ^{a}	4.32 ± 0.89 ^{a}	5.23 ± 0.62 ^{b}
B) Network parameters			
Degree	4.9 ± 0.3 ^{a}	4.4 ± 0.3 ^{a}	4.3 ± 0.2 ^{a}
Edge length	6.7 ± 0.3 ^{a}	7.2 ± 0.3 ^{a}	8.5 ± 0.2 ^{b}
Closeness	0.053 ± 0.002 ^{a}	0.039 ± 0.002 ^{b}	0.048 ± 0.001 ^{a}

Fig. 1. Population graphs showing genetic connectivity for *Neochamaelea pulverulenta* (Rutaceae) populations in Gran Canaria, Tenerife and La Gomera. Nodes represent populations with node sizes indicating relative differences in within population genetic variation. Edge lengths show the among population conditional genetic distances (cGD; see Fig. S1 and Table S1 for details). Grey silhouettes illustrate extinct lizard taxa; black silhouettes represent the three extant, widely distributed species (photos in Fig. S1). Lizard silhouettes are scaled to indicate relative body sizes (largest silhouette= 502 mm SVL, *Gallotia goliath* from Tenerife). The spatial extent of this map encompasses the entire species geographic distribution of *N. pulverulenta* in the Canary Islands (see Fig. S1).

Fig. 2. Slopes of the isolation by resistance models (IBR) (Euclidean genetic distances ~ Resistance distances) of *Neochamaelea pulverulenta* (Rutaceae) estimated by applying reduced major axis regressions. Points indicate mean slope and bars the bootstrapped 95 % CI (1000 resamplings). Different letters in superscripts indicate significant differences among islands: Gran Canaria (GC), Tenerife (TF), and La Gomera (Go). See Fig. S5 and table S3 for a detailed account of IBR plots. See also Fig. S6 for results including cGD (conditional genetic distance).

Fig. 3. Relationship resulting from the multiple matrix regression with randomization analysis for the combined effects of topography complexity (Topo. compl.) and current vegetation (Veg. act) resistance distances, and defaunation status (Defnum) on pairwise (within island) genetic distances among a total of 80 sampled plant populations of *Neochamaelea pulverulenta* (Rutaceae) in Gran Canaria (GC), Tenerife (TF), and La Gomera (Go). Separate regression lines for each island are shown. The fitted model is: Euclidean genetic distance = 0.167 (defaunation) + 0.522 (topographic complexity distance) + 0.195 (current vegetation distance). The inset corresponds to the conditional plot for just the effects of defaunation level when controlling for the effects of two other covariates, estimated with the R package *visreg*.

Horizontal, blue, lines show mean GD values (with 95% confidence intervals in grey) for each island after controlling for differences in topographic complexity and current vegetation. See also Fig. S7 for results including cGD (conditional genetic distance).