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Downsized mutualisms: consequences of seed dispersers’ body-size reduction for early plant recruitment

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Summary

Extinction-driven, body-size reduction of seed dispersers (i.e. an ecological downsizing resulting from severe defaunation) can entail the loss of unique ecological functions, and impair plant regeneration. However, the manner in which the downsizing of mutualistic animals affects seed dispersal and plant recruitment remains understudied. Here, we took advantage of a natural experiment in the Canarian archipelago to document the consequences of lizards body-size reduction (*Gallotia*, Lacertidae) on the recruitment of *Neochamaelea pulverulenta* (Rutaceae), which relies exclusively on these frugivores for seed dispersal. Subsequent to the arrival of humans (ca. 2000-2500 yr BP), the extinction of large-bodied lizards generated a gradient of increasing defaunation on the three islands inhabited by this plant. We hypothesized a significant reduction, and eventually collapse, of early seedling recruitment mirroring the defaunation intensity of the frugivores. We sampled 42 populations spanning the whole geographic range of the plant to examine the quantitative (age structure pattern) and qualitative components (proportion of seedlings growing outside the canopy, number of seedlings established outside the canopy relative to the number of adults -effective recruitment rate-, and seedling vigour) of plant regeneration. Our results show that the age structure patterns did not differ among the three contrasted insular scenarios. However, we found significant reductions in seedling recruitment outside the canopy, effective recruitment rate, and delayed negative effects on seedling vigour in populations hosting small- to medium-sized lizard species. Thus, extirpation of large seed-dispersers did not cause substantial reductions in quantitative components of seed dispersal, but determined declines in qualitative aspects impairing dispersal effectiveness. Our study highlights the importance of examining all components of the dispersal and recruitment process to properly document the regeneration outcomes of plants in defaunated, downsized ecological scenarios.

Keywords: Canary Islands, defaunation, *Gallotia*, lizard extinction, *Neochamaelea pulverulenta*, seed dispersal.

Introduction
Extinction of vertebrate species has been a recurrent and taxonomically non-random pattern throughout the Earth’s history (Raup, 1986; Shodhi et al., 2009). Mass extinction events have reduced, in most cases, the number of large-bodied species (e.g. the disappearance of dinosaurs in the Cretaceous-Tertiary transition, Sheehan et al., 1991 or the demise of megafauna in the Late Pleistocene, Alroy, 2001) ending up with present-day defaunation scenarios in the anthropocene (Barnosky et al., 2011). This phenomenon often results in transitions from pristine communities, where large species are relatively abundant, to downsized communities dominated by small- to medium-bodied vertebrate species (Peres and Dolman, 2000), a pattern of ecological downgrading entailing the loss of unique ecological functions (Estes et al., 2011; Dirzo et al., 2014). Since the large species have are disproportionately important ecological roles in ecosystem dynamics (Cordeiro and Howe, 2001, 2003; Woodward et al., 2005; Wright et al., 2007; Johnson, 2009), the effect of their extinction is expected to cascade through the remainder of the biota and produce deep shifts in the composition, structure and function of downsized communities (Redford and Feinsinger, 2001; Rule et al., 2012; Harrison et al., 2013). A critical issue is thus to develop research frameworks potentially enabling a better forecasting of cascading effects and the potential for delayed consequences of extinction-driven body size reduction and the deterioration of their associated ecological functions (Dirzo et al., 2014).

Animal-mediated seed dispersal is a crucial process in the life cycle of many flowering plants. It allows seeds and seedlings to not only escape the higher mortality frequently associated with the adult neighbourhood (Janzen, 1970) but also colonize new sites (Howe, 1982), and it promotes gene flow within and among populations (Hamrick et al., 1993). Large frugivores have an important role in all of these components of seed dispersal because they can consume a larger amount of fleshy fruits, disperse larger seeds and move them further away than smaller species in mutualistic assemblages (Jordano et al., 2007; Muller-Landau, 2007; Wotton and Kelly, 2011). Thus, there are numerous ways in which natural regeneration, especially of large seeded plants, can be impaired by a body size reduction in frugivore assemblages. For example, if frugivores become
smaller, plants bearing large fruits can have strong seed dispersal limitations because frugivore gape width constrains the maximum fruit size animals can successfully handle and swallow (Wheelwright, 1985). Late-acting, post-dispersal effects may unfold, preventing or severely limiting seedling recruitment, and leaving defaunated ecosystems dominated by living-dead adult plants (Janzen, 1986) or with highly clumped regeneration within the neighbourhood of parent plants (Cordeiro and Howe, 2001). In addition, the extinction of large frugivores may trigger rapid evolutionary responses, given that extant small frugivores promote selection for reduced seed size (Galetti et al., 2013). Reduction of seed size may in turn negatively impact plant recruitment since it frequently correlates with reduced seed reserves and seedling size which result in reduced seedling survival under stress conditions (Howe and Richter, 1982; Moles and Westoby, 2004). Therefore, the downsizing of mutualistic frugivores can affect multiple scales of their interaction with plants, yet most of these cascading influences remain largely undocumented.

The effects of large frugivore declines are expected to be much more pervasive in species-poor systems such as oceanic islands. Firstly, extinction or body-size reduction of frugivore species has been pronounced on islands (Hansen and Galetti, 2009) and quite often preceded by the loss of their functional roles associated with the reduced population size (McConkey and Drake, 2006; Boyer and Jetz, 2014). Secondly, insular environments frequently present low functional redundancy of dispersal agents (e.g. Woodward et al., 2005; Wotton and Kelly, 2011; González-Castro et al., 2014). Thus, seed dispersal may collapse in defaunated insular scenarios, causing substantial reductions of plant recruitment due to loss of efficient mutualistic dispersers. Previous studies have addressed the demographic consequences for plants when disruption of seed dispersal occurs (Meehan et al., 2002; Traveset and Riera, 2005; Rodríguez-Pérez and Traveset, 2009; Wotton and Kelly, 2011, 2012; Traveset et al., 2012). However, as far as we know, none of these investigations tracked the demographic consequences of impaired seed dispersal as a result of the downsizing of interacting animal species.
Lizard-mediated seed dispersal has been described as a widespread mutualism on oceanic
islands (Olesen and Valido, 2003; Valido and Olesen, 2007). In the Canary Islands, endemic
lacertid lizards (*Gallotia* spp.) are significant seed dispersers (Valido and Nogales, 1994; Valido,
1999; Valido and Nogales, 2003; Valido et al., 2003; Rodríguez et al., 2008). However, the arrival
of humans (ca. 2000-2500 yr BP) triggered a process of lizard species extinction and body size
reduction on these islands (e.g. Barahona et al., 2000). The pattern and magnitude of this extinction
has been markedly different on each island, related to differences in predation intensity by
introduced mammals, habitat disturbances, and life-history traits (Machado, 1985; see also
Appendix S1 for details). As a result, a gradient of defaunation-mediated lizard downsizing ranging
from subtle (Gran Canaria) to noticeable (Tenerife), to quite marked (La Gomera; see Fig.1 and Fig.
S1 for island-specific scenarios), exists in present-day environments of the archipelago.

Here, we document the effects of body size reduction of Canarian lizards on the early
recruitment of a plant species which relies exclusively on these reptiles for seed dispersal. We
selected *Neochamaelea pulverulenta* (Rutaceae), an endemic large-seeded treelet, as it is dispersed
exclusively by medium- to large-sized frugivorous lizards and, accordingly, it represents a
potentially useful model species to test downsizing effects (Valido, 1999). Our approach is a
comparative analysis among the unique three islands where *N. pulverulenta* is distributed (Gran
Canaria, Tenerife and La Gomera). These islands define a gradient of extinction-driven lizard body
size reduction: Gran Canaria preserves the largest extant lizard species, i.e. *G. stehlini*; Tenerife has
abundant medium-sized *G. galloti* lizards, whereas La Gomera hosts the smallest species *G.
caesaris* (Fig.1; see also Appendix S1 and Fig. S1 for further details). Since larger lizards consume
bigger and a greater amount of fruits (Valido, 1999), we hypothesize that the extinction-driven body
size reduction will negatively affect both quantitative and qualitative components of *N. pulverulenta*
recruitment. Among the former we considered the amount of seedlings established; among the latter
we analysed the proportion of those that effectively established outside adult plants and the
reduction in seedling vigour estimated resulting from reduced seed sizes being dispersed. We expect
the downsized scenarios will determine: i) differences among islands in overall recruitment patterns as indicated by differences in the age structure (i.e. the relative abundance of seedlings), ii) a decrease in the proportion of seedlings recruiting outside the canopy of adult plants, iii) a reduction of the effective recruitment rate of seedlings (per capita of adult plants), and iv) a reduced vigour of seedlings, resulting from a lack of consumption of large fruits (with large seeds; Howe and Ritcher, 1982; Valido, 1999).

Material and methods

Study species

*Neochamaelea pulverulenta* (Rutaceae) (Vent) Erdtman is an endemic treelet distributed in the dry lowlands (< 400 m a.s.l.) of Gran Canaria, Tenerife and La Gomera (Canary Islands). In these areas the average annual temperature and precipitation are around 21°C and 200 mm, respectively (AEMET-IP, 2012). The resulting lowland vegetation is dominated by *Euphorbia* spp. (Euphorbiaceae), *Lavandula* spp. (Labiatae), *Lycium intricatum* (Solanaceae), *Periploca laevigata* (Asclepiadaceae), *Plocama pendula*, and *Rubia fruticosa* (Rubiaceae), among others. Adult plants of *N. pulverulenta* average 1.1 ± 0.49 m in height but some individuals can reach 2.7 m (*n* = 2132 from all sampled populations). Plants can bloom almost all year round, with a peak in winter and spring. The main pollinators are ants, solitary bees and flies. Fruits include 1 to 4 ‘cocci’ (11.1 ± 1.6 mm in diameter each; Valido, 1999). Each coccus can be considered to be functionally a drupe composed of fleshy pulp containing invariably one hard-coated seed (8.6 ± 1.0 mm in diameter; Valido, 1999).

Only medium- and large-bodied lizards eat these fruits, adequately handling and swallowing individual cocci, acting as legitimate seed dispersers (Valido and Nogales, 1994; Valido, 1999; Valido et al., 2003). Secondary seed dispersal by raptor predators on lizards containing seeds of *N. pulverulenta* has also been documented (Padilla et al., 2012). Subsequent to human colonization (ca. 2000-2500 yr BP, Appendix S1), different extinction scenarios emerged on each island.
generating a gradient of lizard body-size reduction (Fig. 1). On Gran Canaria lizard downsizing has been relatively minor, from the large forms of *G. stehlini* sub-fossils (maximum snout-vent length, max SVL = 367 mm) to the extant *G. stehlini* (max SVL = 280 mm). In contrast, lizard size reduction has been intense on La Gomera, where the extant widespread species (*G. caesaris*, max SVL = 111 mm) is 4 times smaller than their extinct relatives (*G. goliath*, max SVL = 466 mm). In turn, on Tenerife *G. goliath* (max SVL = 502 mm) became extinct and currently only the medium-sized species *G. galloti* (max SVL = 145 mm) is widely distributed. Moreover, the large species *G. intermedia* (max SVL = 174 mm) and *G. bravoana* (max SVL = 212 mm) are present on Tenerife and La Gomera respectively, but they are critically endangered, surviving only in extremely reduced populations on highly localized, inaccessible cliffs (Fig. S1 and references therein for details).

**Plant demography**

To carry out a comparative study on plant recruitment under different seed dispersal scenarios, we sampled 42 *N. pulverulenta* populations from the three islands. We deliberately choose populations spanning the range of environmental conditions where the species occurs: Gran Canaria (*n* = 11), Tenerife (*n* = 19), and La Gomera (*n* = 12) (Table S1, Fig. S2). In each population we haphazardly set up 3-6 linear transects (25-100 m length; 5 m wide) depending on plant population size. Along these transects we counted and measured all individual plants, except for seedlings, for which only a subset were measured (*n* = 637 seedlings; sampling, on average, 29.3% of seedlings recorded in each population). The individual plant measurements included the maximum basal trunk/stem diameter at ground level (using a digital caliper), the maximum stretched height (except for adults; non-stretched height), and the two major diameters of the vertical canopy projection (using a measuring tape). Moreover, all individual plants were categorized according to their size-related age class (seedling, sapling, juvenile, adult). Seedlings were identified as plants with < 1 mm of basal diameter and with less than four leaves; individuals
not branched, with 1-7 mm basal diameter were recorded as saplings; plants with 7-15 mm basal
diameter and no evidence of reproduction (absence of floral buds and/or seeds beneath the plant)
were considered juveniles; otherwise they were recorded as adults. On average we collected data
from 228 plants per population (range: 102-571), with a total of 9402 plants sampled. We used
this dataset to describe the age structure of populations.

For a subset of 32 populations (Table S1, Fig. S2), we also kept information on the
proportion of seedlings outside the canopy of *N. pulverulenta* plants and the effective recruitment
rate of seedlings along the transects. For the first variable, we recorded the number of seedlings
located within the transects and >1 m away from the canopy of the nearest adult plant relative to
the total seedlings recruited. The effective recruitment rate was calculated as the number of
seedlings outside the canopy relative to the number of adult plants. This demographic parameter
represents the per-adult number of seedlings successfully recruiting away from adults, i.e.
recruiting from effectively dispersed seeds.

Finally, for the analyses of seedling vigour, as reflected by stem diameter, we selected 22
populations with at least six seedlings measured (6-93 seedlings, depending on seedling
abundance; Table S1).

**Plant densities, climatic variation, and lizard abundance**

To obtain an estimate of plant density per population we set up two perpendicular 100 m x 4 m
transects, for which we counted all *N. pulverulenta* adult plants rooted within the transect. These
data were independent of the adult plants censused in the age structure sampling. In addition, we
gathered climatic data from meteorological stations located within a 7 km distance (1 to 4
stations), with long temporal data series available (range = 6–53 years). For each population we
recorded the average annual precipitation (*pp*), the mean maximum temperature of the hottest
month (*t*$_{\text{max}}$), and the mean minimum temperature of the coldest month (*t*$_{\text{min}}$). Then, we calculated
the Emberger index (Emberger, 1955) defined as $Q = (2000 \text{ pp})/(t_{\text{max}}^2 - t_{\text{min}}^2)$, which we log-
transformed (-LnQ; Tieleman et al., 2003) for statistical analyses.

We obtained an index of relative abundance of medium- to large-bodied lizards in the 32 populations selected for the detailed analyses of seedling recruitment. Given that lizard body-size and diameter of their droppings are positively correlated (Valido and Nogales, 2003), we recorded the number of medium- to large-sized droppings in 50 quadrats (0.5 x 0.5 m), regularly spaced 5 m apart along five linear transects (spaced 10 m apart) per population.

Statistical analyses

Quantitative plant recruitment

To examine differences in the age structure pattern (relative frequencies of each age class) among islands we fitted two generalized linear models (GLMs) with a binomial distribution of errors and a log link function. We included ‘island’ as a fixed factor using data from all sampled populations (n = 42). In the first model we tested for island differences in the proportion of subadults (pooled number of seedlings, saplings and juveniles) vs. the proportion of adult plants. In the second model, we tested for differences in the proportion of seedlings relative to the rest of the pooled age classes (saplings, juveniles, adults).

Qualitative plant recruitment

To assess the effect of the ecological scenarios (islands) on the proportion of seedlings outside the canopy we applied a GLM with a binomial distribution of errors and a logit link function. We used ‘island’ as the main fixed factor and both the density of N. pulverulenta adult plants and the Emberger index as covariates. Among-island differences in the effective recruitment rate of seedlings were tested by fitting a GLM with a Poisson distribution of errors and a log link function. The number of seedlings was used as a response variable, while ‘island’ was used as a fixed factor, the Emberger index as a covariate and the number of adults per population as an offset of the model. The stem diameter of sampled seedlings was used as an estimate of seedling
size and vigour. We tested variation in seedling vigour among islands by fitting a linear mixed
model (LMM) using ‘island’ as the main fixed factor with population identity as a random factor
nested within it and the Emberger index as a covariate.

In order to assess differences in all measured demographic parameters among pairs of
islands we used post-hoc contrasts (Tukey test). In addition, we checked for spatial autocorrelation
among model residuals by performing multivariate Mantel correlograms. Given that spatial
autocorrelation was not detected for any of the above demographic variables, we did not include
spatial information in the abovementioned models (details in Appendix S2, Fig. S3).

Finally, we tested for island effects on the density of large- to medium-sized droppings, as
a proxy of the relative density of large- to medium-sized lizards. We used an ANOVA with post-
hoc contrasts (Tukey test) to test for differences among islands. All statistical analyses were
carried out with R (R Development Core Team, 2014).

Results

Quantitative plant recruitment

The age structure pattern of *N. pulverulenta* was highly variable among populations within each
island, ranging from aged populations, where most individuals were adults (e.g. Montaña de
Tabaiba, Gran Canaria), to relatively younger stands where many censused plants were subadults
(e.g. Barranco de la Negra, La Gomera) (Table S1). When considering the frequencies of
subadults (pooling seedlings, saplings, and juveniles) relative to adult plants, differences were not
detected among the three insular scenarios (GLM, $p > 0.05$; Fig. 2), suggesting similar overall
amount of recruitment. In turn, the average percentage of seedlings was consistently similar
among islands, varying from $31.0 \pm 27.8\%$ in Tenerife to $25.1 \pm 20.7\%$ in Gran Canaria (GLM, $p
> 0.05$; Fig. 2).

Qualitative plant recruitment
The proportion of seedlings outside the canopy of adult plants was significantly different among islands: Gran Canaria (12.7%), Tenerife (17.7%) and La Gomera (2.8%) (Table 1, Fig. 3). In the model, the effect of *N. pulverulenta* adult plant density was statistically significant ($Z = -3.19, p < 0.001$). However, variation in climatic conditions did not account for these differences.

Regarding the effective recruitment rate of seedlings, we detected significant differences for all the possible pairwise comparisons (Table 1, Fig. 4a). Populations on La Gomera recruited significantly fewer seedlings per adult (0.01 seedlings/adult) than those on Gran Canaria (0.06 seedlings/adult) and Tenerife (0.39 seedlings/adult). In this case, the Emberger index (i.e. aridity index) showed a significant effect ($Z = 6.05, p < 0.001$), indicating higher effective recruitment in more arid populations.

The observed differences among islands in the proportion of seedlings outside the canopy and the effective recruitment rate of seedlings match the variability detected in the abundance of medium-to-large lizard droppings. In the particular case of La Gomera, large droppings were totally absent in the sampled populations (Fig. 4b). Also, we recorded a significantly lower density of lizard droppings on Gran Canaria than on Tenerife ($t = 2.58, p < 0.05$).

Finally, we found seedlings with consistently smaller basal stem diameter in Tenerife, with no differences between Gran Canaria and La Gomera (Fig. 5, Table 1). Variation in stem diameter across populations was unrelated to the Emberger index.

**Discussion**

*Scenarios of downsized mutualisms*

By using a natural island-based field experiment from the Canary Islands we found support for the observation that defaunation-mediated downsizing of frugivorous lizards critically hampers recruitment of *N. pulverulenta*, an endemic shrub strictly dependent on these seed dispersers. Unexpectedly, our results suggest that a reduction of lizard body size has no effect on the quantitative component of seed dispersal (age structure pattern) in the different insular scenarios,
with similar overall amount of recruits (juveniles, saplings, and seedlings pooled) relative to the
number of adult plants. However, we detected critical effects on the qualitative components, such
as a significant reduction of seedling establishment away from adult plants, and delayed negative
effects on seedling vigour. First, there was a marked reduction in recruitment beyond the
neighbourhood of adult plants on La Gomera, where a drastic reduction of lizard body-size has
occurred. Second, even a relatively small decline of lizard body-size may result in less vigorous
seedlings as exemplified by the contrast between populations hosting medium-sized lizards
(Tenerife) and giant lizards (Gran Canaria) (see below for the specific case of La Gomera, with
extinct seed disperser). These differences are not attributable to variation in climatic factors or soil
characteristics, but appear closely associated with the downsizing pattern. Our results broadly
support the patterns reported in previous studies in which plant-frugivore mutualism disruption
affected the quantity and/or the quality of plant regeneration (e.g. Chapman and Chapman, 1995;
Cordeiro and Howe, 2003; Traveset and Riera, 2005; Galetti et al., 2013). Yet our study highlights
the fact that situations with reduced or collapsed dispersal services can remain undocumented if
not all the components of dispersal effectiveness are studied, as indicated by the significant
reductions in both effective dispersal and seedling vigour in the downsized scenarios.

Quantitative consequences for plant demography
We found a similar proportion of established seedlings despite the marked differences in lizard
body sizes among islands. In fact, we found high values (>25%) in most populations. These
populations do not differ in soil type (volcanic substrate) or climatic conditions (i.e. aridity index;
Table S1), so the similarity of the demographic pattern across islands cannot be attributed to
compensatory effects of abiotic conditions (e.g. favourable conditions for establishment in areas
with limited dispersal by lizards). Biotic interactions could also have associated compensatory
effects, for instance, if differences in competition, herbivory and/or post-dispersal seed predation
intensities counterbalance the effect of dispersers, yet we have no evidence supporting this, e.g.
we have no records of herbivory on seedlings. The lack of differences in overall recruitment
contrasts with previous studies indicating reductions of recruit density in systems hosting non-
effective seed dispersers (e.g. Cordeiro and Howe, 2003; Traveset and Riera, 2005, but see Bleher
and Böhning-Gaese, 2001 for similar results).

In our study system, several factors might explain the large proportion of seedlings of *N.
pulverulenta* observed on the three islands. A very large fraction of the fully-developed fruit crop
falls beneath parents, usually during early summer. Thus, it is common to find a large amount of
*N. pulverulenta* seeds without pulp beneath conspecific plants. Small lizards can bite and tear off
the pulp without removing the fruit, thus not acting as legitimate dispersers but potentially
enabling seed germination (Fig. S4). In addition, we have evidence that rodents consume the pulp
and leave seeds accumulating beneath adult plants, but most of them are also predated (pers. obs.).
Besides, both lizards and rodents can move a minor proportion of these seeds away from mother
plants. Thus, seed movement by runoff, or haphazard dispersal by seed predators and/or small
lizards is most likely contributing to early establishment even in situations with limited or absent
legitimate dispersal (La Gomera).

**Qualitative consequences for plant demography**

Despite the absence of differences in the age structure pattern among islands, a clear inter-insular
trend emerges when considering several qualitative components of seed dispersal effectiveness
(Schupp et al., 2010), i.e. proportion of seedlings outside the canopy of adult plants, effective
recruitment rate of seedlings, and seedling vigour.

At one extreme of the defaunation and downsizing gradient at La Gomera, we found the
lowest values for both the proportion of seedlings outside the canopy and the effective recruitment
rate of seedlings. These results, together with an absolute absence of seeds of *N. pulverulenta* in
Gomeran lizard droppings, indicate the collapse of the lizard-mediated dispersal interactions on
this island. This contrasts with data recorded from islands hosting medium- and large-bodied
lizards (Tenerife and Gran Canaria, respectively). The human-driven extinction of the largest
known species on La Gomera (G. goliath) and the marginal presence of the extant giant lizard G.
bravoana only in an isolated remnant population (Valle Gran Rey; Valido et al., 2000), have
actually deprived N. pulverulenta of effective seed dispersers throughout the island. The
remaining abundant species G. caesaris is unable to effectively handle fruits and seeds due to
marked morphological restrictions, i.e. fruit size considerably exceeds gape width of the lizard
(Valido, 1999), a factor potentially impairing fruit removal and effective seed dispersal.

Significant reductions of seeds dispersed away from adult parents have been reported in other
defaunated scenarios (Chapman and Chapman, 1995; Cordeiro and Howe, 2003) where the lack of
efficient dispersal agents leads to seed accumulations beneath the mother plants.

The plant populations from La Gomera, however, still preserve a marginal effective
recruitment. We recorded approximately 3% of seedlings recruiting beyond the vicinity of adult
plants and a very low (but non-zero) effective recruitment rate. Small-sized G. caesaris, which
frequently take the fleshy pulp from fruits of the undispersed crop, can sporadically move some
fruits and remove the pulp away from adult plants where a minor fraction of seeds may likely
germinate. In addition, N. pulverulenta populations are usually distributed on ravine slopes, where
these seeds without pulp can be dispersed by rain or gravity. Lastly, although rodents mostly act as
seed predators they can also disperse some seeds infrequently. Similar results have been reported
for other plants (Traveset and Riera, 2005; Guimarães et al., 2008) where vertebrate-mediated
seed dispersal has been disrupted and plants rely solely on haphazard, marginal dispersal. In fact, a
recent study tracking seed fates (Jansen et al., 2012) showed that scatter-hoarding rodents provide
effective seed dispersal to widowed plants, acting as substitutes of an extinct megafauna.

We would expect the largest fraction of seeds being dispersed away from maternal plants
on Gran Canaria, whose populations currently host the largest-sized lizards (G. stehlini). However,
contrary to this expectation, the probability of finding seedlings recruiting beyond the parent
plants and the effective recruitment rate of seedlings was higher on the island (Tenerife) hosting
medium-sized lizards (*G. galloti*). Neither abiotic factors nor differential enemy-mediated mortality of seeds or seedlings, as discussed above, help explain this difference between the Gran Canaria and Tenerife scenarios. A more plausible explanation is related to the variation in abundance of Canarian lizards on both islands. It is known that larger lizards are relatively less abundant than small ones (Buckley et al., 2008). In this respect, our estimates (density of lizard droppings) indicate that *G. galloti* on Tenerife is 6-fold more abundant than *G. stehlini* on Gran Canaria. This result suggests that increased abundance of the less-effective, medium-sized lizards on Tenerife may explain the large number of seedlings found beyond maternal plants, i.e. a type of compensatory mass effect directly favouring higher fruit removal rates and dispersal. This supports the idea that the contribution of less-effective animal mutualists to the reproductive success of plants may frequently be overcompensated by their abundance (Vázquez et al., 2005).

A key variable driving the outcome of mutualistic interactions with gape-limited frugivores is fruit size (Wheelwright, 1985). Small-bodied frugivores do not adequately handle and process large fruits or seeds. Large-bodied frugivores usually disperse larger seeds and a wider range of seed sizes, thus potentially favouring large seeds (Valido, 1999; Galetti et al., 2013) which results in larger seedlings (Howe and Richter, 1982; Moles and Westoby, 2004). We hypothesized that body-size reduction of mutualistic lizards could entail a late-acting reduction of seedling vigour of *N. pulverulenta* due to consistent size reductions of successfully removed seeds. Our results partially support this hypothesis. On the one hand, we found a consistent and significant reduction of seedling stem diameter in populations hosting medium-sized lizards (Tenerife) compared to populations with large-sized lizards (Gran Canaria). On the other hand, seedling stems on La Gomera were unexpectedly thicker than those on Tenerife, and similar to those on Gran Canaria. As previously discussed, these differences are not related to climatic conditions or soil type differences. Thus, the large seed sizes and vigorous seedlings currently observed on La Gomera may reflect the phenotypic selection pattern on fruit size exerted by giant lizards in the recent past (Valido, 1999) and the more recent extinction events.
In summary, our comparative approach included three contrasting ecological scenarios along a gradient of progressive reduction of frugivore body size due to extinction-driven downsizing. After controlling for variation in abiotic conditions, differences in the early recruitment of a plant species mirrored this defaunation-mediated downsizing gradient. At one extreme, Gran Canaria populations illustrate a scenario of preserved interactions; whereas Tenerife represents an intermediate suboptimal scenario, and La Gomera exemplifies a scenario in which both the seed dispersal process and the regeneration away from maternal plants have collapsed. The example of La Gomera is paradigmatic since plant populations have persisted for a long period without their effective seed disperser partners, as reported for other widowed megafaunal-dispersed plant species surviving more than 10,000 years (Janzen and Martin, 1982; Guimarães et al., 2008). Reliance on secondary dispersal has been proposed as a key mechanism underlying this persistence of widowed plant species (Guimarães et al., 2008; Jansen et al., 2012). Accordingly, our results suggest that in the case of *N. pulverulenta* the very limited secondary dispersal mediated by abiotic and biotic vectors in combination with apparently low seedling mortality under parent plants may be allowing the long-term local persistence of the plant populations on La Gomera.

**Conclusions**

Anthropogenic impact is causing a very fast decline of frugivore size on islands worldwide, where the projected downsizing in the future is up to three orders of magnitude above mainland ecosystems (Hansen and Galetti, 2009). Despite the limitations (number of insular replicates) associated with this natural-based experiment, our results highlight a number of effects that such downsizing may entail in relation to plant demography and population recruitment. Extirpation of large-bodied frugivores may not cause a marked decline in some quantitative components of dispersal (Markl et al., 2012), but it will certainly determine a reduction in qualitative aspects critical for ensuring dispersal effectiveness. It remains unknown if this downsizing pattern also
drives reduced gene flow via seed dispersal within and among populations, with a lasting signal on
the genetic structure both at local and regional scales. Meanwhile, our results highlight the
importance of conserving the full range of functional processes (qualitative and quantitative
components) involved in mutualistic interactions crucial for the persistence of local regeneration
and plant population dynamics in a changing world.

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Appendix A. Supplementary data
The following are the supplementary data to this article:

Appendix S1. Natural history of Canarian lizards (g. Gallotia, Lacertidae).
Appendix S2. Additional information for the statistical analyses.
Table S1. Information about the sampled N. pulverulenta populations.
Figure S1. Distribution of Gallotia species in the Canary Islands.
Figure S2. Distribution map of sampled N. pulverulenta populations.
Figure S3. Autocorrelograms of the model residuals.
Figure S4. Photo-collage illustrating the study system.

References


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Table 1. Demographic parameters and results of multiple comparisons (Tukey test) among islands. The number of sampled populations is indicated within brackets. Post-hoc analyses were conducted after application of GLM\(^1\) with a binomial distribution and a logit link function, GLM\(^2\) with a Poisson distribution and a log link function, and LMM\(^3\). Non-shared, superscript letters indicate significant differences among islands. Data are mean ± SE.

<table>
<thead>
<tr>
<th>Demographic parameters</th>
<th>Gran Canaria</th>
<th>Tenerife</th>
<th>La Gomera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of seedlings outside canopy(^1)</td>
<td>12.70 ± 6.79 (^a) ((9))</td>
<td>17.71 ± 6.58 (^b) ((13))</td>
<td>2.76 ± 2.12 (^c) ((10))</td>
</tr>
<tr>
<td>Effective recruitment rate of seedlings(^2)</td>
<td>0.06 ± 0.03 (^a) ((9))</td>
<td>0.39 ± 0.22 (^b) ((13))</td>
<td>0.01 ± 0.00 (^c) ((10))</td>
</tr>
<tr>
<td>Seedling stem diameter(^3) (mm)</td>
<td>0.74 ± 0.06 (^a) ((7))</td>
<td>0.47 ± 0.05 (^b) ((7))</td>
<td>0.81 ± 0.05 (^a) ((8))</td>
</tr>
</tbody>
</table>
Fig. 1. Schematic representation of the maximum snout-vent length (max SVL) reduction of Canarian giant lizards (g. Gallotia, Lacertidae) from the past (light-grey silhouettes) to the present day (black silhouettes). Silhouettes are scaled to the max SVL. Only the islands hosting *Neochamaelea pulverulenta* populations are shown (see Fig. S1 for details of the other islands): Gran Canaria (from *G. stehlini* sub-fossils to extant *G. stehlini*), Tenerife (from *G. goliath* to *G. galloti*) and La Gomera (from *G. goliath* to *G. caesaris*).

Fig. 2. Age-structure patterns (distribution of age classes) for the 42 analysed populations of *Neochamaelea pulverulenta* in Gran Canaria (11 populations), Tenerife, (19), and La Gomera (12). Data were pooled at the island level. Age classes: Ad (Adults), Juv (Juveniles), Sap (Saplings), Seedl (Seedlings). Bars indicate mean ± SE.

Fig. 3. Proportion of *Neochamaelea pulverulenta* seedlings growing outside (grey bars) and beneath the canopy (black bars). Each bar represents a sampled population, sorted within islands in decreasing order of the proportion of seedlings recruiting beneath the canopy (see Table S1, Fig. S2 for population codes and locations). Grey boxplots show the median as well as the upper and the lower quartile of the proportion of seedlings outside the canopy on each island (the whiskers are 1.5 times the interquartile range of the box). Dots outside of the whiskers are considered outliers.

Fig. 4. (a) Effective recruitment rate of seedlings on each island (no. of seedlings outside the canopy / no. of adult plants). Data are shown on the $\log(x+1)$ scale. (b) Density of medium to large
droppings of lizards on each island (no. of lizard droppings \( \cdot 0.25 \, \text{m}^2 \)). In both panels, population parameters are represented with points. Boxplots show the median as well as the upper and the lower quartile, the whiskers are 1.5 times the interquartile range of the box. Dots outside of the whiskers are considered outliers. Gran Canaria, \( n = 9 \) populations; Tenerife, \( n = 13 \) populations; La Gomera, \( n = 10 \) populations.

**Fig. 5.** Variation in seedling stem diameter within and among islands. Data are population mean ± SE (unfilled circles). At the island level, dotted lines and grey shadows indicate mean and SE respectively. Population codes along the abscissa are as in Table S1 and Fig. S2. Populations are ordered as in Fig. 3.
Figure

Gran Canaria  Tenerife  La Gomera

Percentage of individuals (%) vs Age-classes
Figure 1: Proportion of seedlings (%)

Gran Canaria | Tenerife | La Gomera

Proportion of seedlings (%)

Populations

1 4 6 7 2 9 8 3 5 10 12 13 15 16 21 19 18 17 14 11 20 22 24 25 26 27 29 30 32 28 23 31
Figure

Effective recruitment rate of seedlings
Log (seedlings . adult⁻¹ + 1)

Density of medium to large droppings
(droppings . 0.25 m⁻²)

Gran Canaria  Tenerife  La Gomera
Gran Canaria  Tenerife  La Gomera