galloti

stehlini

defaunación

caesaris Neochamaelea pulverulenta

lagartos gigantes

dispersión de semillas

Islas Canarias

Gallotia

goliath

Estación Biológica de Doñana CSIC

UNIVERSIDAD PABLO DE OLAVIDE SEVILLA

Néstor Pérez Méndez

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LAS HUELLAS DE LA DEFAUNACIÓN EN EL ANTROPOCENO

El colapso de los mutualismos de la dispersión de semillas

TESIS DOCTORAL

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El colapso de los mutualismos de dispersión de semillas.

Memoria presentada por el Licenciado en Biología Néstor Pérez Méndez para optar al título de Doctor por la Universidad Pablo de Olavide, Sevilla.

Fdo: Néstor Pérez Méndez

Sevilla 2016

A mi familia
A Anna
A Taoro

Dr. Alfredo Valido, investigador postdoctoral de la Estación Biológica de Doñana (CSIC) y Dr. Pedro Jordano Barbudo, Profesor de Investigación de la Estación Biológica de Doñana (CSIC)

CERTIFICAN

Que los trabajos de investigación desarrollados en la Memoria de Tesis Doctoral, "*Las huellas de la defaunación en el Antropoceno. El colapso de los mutualismos de dispersión de semillas*", son aptos para ser presentados por el Ldo. Néstor Pérez Méndez ante el Tribunal que en su día se designe, para aspirar al Grado de Doctor por la Universidad Pablo de Olavide.
Y para que así conste, y en cumplimiento de las disposiciones legales vigentes, extendemos el presente certificado a 30 de Mayo de 2016.

Directores:

Fdo. Dr. Alfredo Valido Amador Fdo. Pr. Dr. Pedro Jordano Barbudo

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LISTA DE CONTENIDOS

LISTA DE ARTÍCULOS CIENTÍFICOS

Capítulo 2

Pérez-Méndez N., Jordano P., Valido A. (2015) Downsized mutualisms: consequences of seed dispersers' body size reduction for early plant recruitment. *Perspectives in Plant Ecology, Evolution and Systematics*, 17: 151-159. DOI: 10.1016/j.ppees.2014.12.001

Capítulo 3

Pérez-Méndez N., Jordano P., García C., Valido A. (2016) The signatures of Anthropocene defaunation: cascading effects of the seed dispersal collapse. *Scientific Reports*, 6: 24820. DOI: 10.1038/srep24820

Capítulo 4

Pérez-Méndez N., Jordano P., Valido A. (2016) Persisting in defaunated landscapes: reduced plant population connectivity after seed dispersal collapse. *En revisión*

RESUMEN

El ritmo de crecimiento de la población humana mundial en los últimos 500 años ha sido vertiginoso, pasando de menos de 500 millones de habitantes a casi 7.500 millones actualmente. Paralelamente, el ritmo de pérdida de biodiversidad asociado a este crecimiento, ha aumentado hasta niveles sólo comparables con los cinco grandes eventos de extinción masiva de la historia de La Tierra. Tal es la importancia de este fenómeno asociado a la actividad humana, que recientemente se ha definido una nueva era con el término "Antropoceno". A pesar de que este fenómeno ocurre en todos los grupos de vertebrados, hay ciertos rasgos, como un tamaño corporal grande, que hacen a algunas especies más vulnerables a la extinción que a otras. A menudo estas especies cumplen roles funcionales muy importantes y poco redundantes, por lo que las consecuencias de su pérdida pueden ser críticas para el funcionamiento de los ecosistemas. Por un lado por la pérdida directa de las funciones que desempeñan y por otro porque a menudo los efectos se propagan en cascada afectando a la estructura, la composición y a la dinámica de las comunidades alteradas. Es importante, por tanto, desarrollar nuevas vías de investigación que permitan entender y predecir cuáles son los efectos en cascada y las consecuencias potenciales de la defaunación mediada por la actividad antrópica y de la degradación de las funciones ecológicas asociadas.

La dispersión de semillas es un proceso clave en el ciclo de reclutamiento de las plantas, que en gran medida va a determinar las características demográficas y genéticas de sus poblaciones. En muchas especies este proceso se realiza a través de relaciones mutualistas con animales frugívoros, que reciben a cambio recursos tróficos. Sin embargo, la alteración antrópica del medio ha provocado la extinción de muchos de estos animales, especialmente los de mayor tamaño, provocando una reducción de tamaño en las comunidades de frugívoros. Estas especies de mayor tamaño normalmente ofrecen unos mejores servicios de dispersión a las plantas. Por un lado consumen un mayor número de frutos y de mayor tamaño (con un número o diámetro de semillas mayor) y por otro lado suelen dispersarlas a mayores distancias. A largo plazo sería esperable, por tanto, que la progresiva reducción de tamaño de los frugívoros provocara una disminución de los servicios de dispersión con consecuencias genéticas y demográficas para las plantas. Además se esperaría que estos efectos fuesen más acusados en aquellos ecosistemas donde existe una baja redundancia funcional, como las islas oceánicas.

El objetivo general de esta tesis doctoral es evaluar las consecuencias de la degradación progresiva y del colapso de los servicios de dispersión, asociados a una pérdida gradual de tamaño de los dispersores de semillas, en distintos aspectos funcionales (demográficos y genéticos) de las poblaciones de plantas insulares. Para ello se estudia la interacción mutualista entre la planta *Neochamaelea pulverulenta* (Rutaceae) y sus únicos dispersores de semillas, los lagartos frugívoros del género *Gallotia* (Lacertidae). *N. pulverulenta* es un arbusto endémico de las Islas Canarias que produce frutos carnosos (drupáceos) con semillas de gran tamaño. Éstos son consumidos exclusivamente por lagartos medianos y grandes del género *Gallotia*, también endémicos del archipiélago. Hasta la llegada de los humanos a las islas, hace aproximadamente 2500 años, los lagartos gigantes de este género eran abundantes en cada una de las tres islas donde se distribuye *N. pulverulenta* (Gran Canaria, Tenerife y La Gomera), con individuos que podían superar los 500 mm de longitud hocico-cloaca (LHC). Sin embargo, como consecuencia directa o indirecta de la actividad humana sólo en Gran Canaria se preservan individuos de tamaño relativamente grande en la actualidad (*G. stehlini*; LHC max= 280 mm), mientras que en Tenerife sólo son abundantes individuos de tamaño mediano (*G. galloti*, LHC max= 145 mm) y en La Gomera sólo se encuentran individuos pequeños (*G. caesaris*; LHC max= 111 mm). A través de una aproximación comparativa entre estos escenarios contrastados desde el punto de vista del tamaño de los dispersores de semillas, se abordan los objetivos específicos planteados en esta tesis doctoral.

En el **capítulo 2** se estudia cuál es el impacto de la reducción de tamaño de los lagartos frugívoros en el reclutamiento temprano (establecimiento de plántulas) de *N. pulverulenta*. Los resultados obtenidos muestran una estructura de edad similar en las poblaciones de plantas de las tres islas, con una proporción similar de plántulas establecidas. Sin embargo, la tasa de reclutamiento efectiva, es decir la cantidad de plántulas que se establecen fuera del vecindario materno respecto al número de adultos, fue extremadamente baja en la isla más defaunada (La Gomera). Inesperadamente, la tasa de reclutamiento efectivo en Tenerife fue significativamente mayor que en Gran Canaria, donde se conservan los lagartos de mayor tamaño. Esto puede ser explicado por una mayor abundancia (seis veces mayor) de lagartos medianos (*G. galloti)* en Tenerife que de lagartos grandes (*G. stehlini*) en Gran Canaria. Sin embargo, el vigor de las plántulas establecidas en Tenerife es mucho menor que en Gran Canaria, como consecuencia de la dispersión de un rango de semillas más pequeñas.

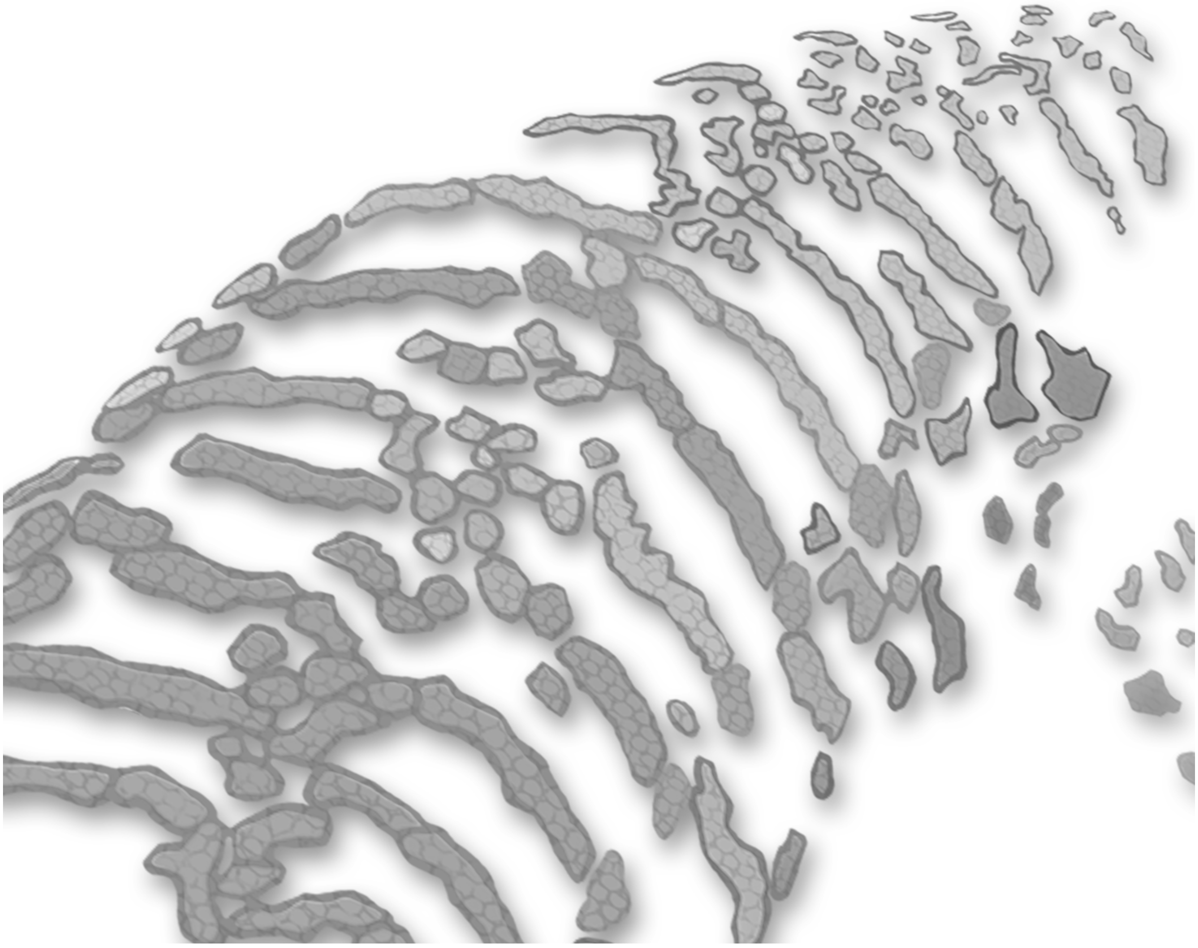
En el **capítulo 3** se evalúan las consecuencias de la reducción de talla de los lagartos en las distancias de dispersión de semillas. Para ello se asignó, mediante técnicas de asignación genética, cada semilla dispersada con su planta madre. Las distancias de dispersión disminuyen progresivamente y de forma paralela a la pérdida de tamaño de los lagartos. En Gran Canaria se detectan distancias de dispersión mucho mayores (distancia máxima= 94 m) que en Tenerife (distancia máxima = 46 m) y La Gomera (distancia máxima = 4 m). En La Gomera prácticamente todas las semillas se encuentran debajo de la planta madre, excepto una mínima proporción que se dispersa, de forma esporádica, fuera del vecindario materno más próximo. Igualmente se evalúa como afecta la reducción de estas distancias de dispersión de semillas en la diversidad genética y en la distribución espacial de esta diversidad a escala local, es decir, dentro de poblaciones. Los resultados muestran una diversidad genética similar en los tres escenarios (Gran Canaria, Tenerife y La Gomera), sin embargo difieren claramente en cómo se estructura en el espacio. Se observa un aumento progresivo del grado de estructuración genética espacial paralelo al gradiente de defaunación, y una reducción del tamaño efectivo poblacional en los dos escenarios más defaunados (Tenerife y La Gomera).

Por último, en el **capítulo 4** se evalúan las consecuencias de la reducción de las distancias de dispersión de semillas sobre la diversidad genética, en la conectividad genética entre poblaciones y en el aislamiento genético por distancia a escala regional, es decir, dentro de islas. Los resultados obtenidos muestran una mayor diversidad genética en el escenario con lagartos más pequeños (La Gomera). Sin embargo, se observa una pérdida de conectividad genética y un aislamiento genético por distancia mucho más acusado en esta isla, que en los dos escenarios (Gran Canaria y Tenerife) donde aún se conserva la interacción mutualista.

Los resultados de esta tesis doctoral revelan los múltiples efectos en cadena asociados a la extinción de los grandes frugívoros y de las interacciones ecológicas en las que participan. En este caso, una degradación progresiva de los servicios de dispersión de semillas que deriva, a su vez, en la alteración de la dinámica demográfica y las características genéticas de las plantas con las que interaccionan. Se pone de manifiesto así la necesidad de conservar a los vertebrados de mayor tamaño para evitar no sólo la pérdida de estas especies, sino de todo el rango de procesos en los que intervienen. De otra forma, las consecuencias de la defaunación en el funcionamiento de los ecosistemas pueden aparecer mucho después de la extinción de estas especies, a veces de formas tan poco evidentes como las que se reportan en esta tesis doctoral.

CAPÍTULO 1

Introducción General



EL ANTROPOCENO: PATRONES DE DEFAUNACIÓN EN UNA NUEVA ERA DE EXTINCIÓN MASIVA

Cinco grandes eventos de extinción masiva ("*The big five*") han acontecido en la historia de la vida en la Tierra. Todos ellos caracterizados por una pérdida extrema de biodiversidad (> 75% de especies) en un período geológico corto (< 2 m.a.) y asociada a grandes catástrofes globales (sucesiones de glaciaciones y calentamientos, cambios en el nivel del mar, vulcanismo muy activo o impactos de asteroides) (Hallam & Wignall 1997). Evidencias actuales apuntan a que estamos inmersos en una nueva era de extinción masiva (Dirzo & Raven 2003; Ceballos *et al.* 2010, 2015; Barnosky *et al.* 2011; Ehrlich & Ehrlich 2013), con una magnitud y tasa de pérdida de especies similar o incluso superior a la de las cinco extinciones masivas previas (Barnosky *et al.* 2011; Ceballos *et al.* 2015). Las causas de este nuevo escenario se asocian igualmente a un proceso de degradación global, pero esta vez originado por la colonización humana del planeta y por el crecimiento exponencial de sus poblaciones en los últimos 500 años, que ha pasado de menos de 500 a 7500 millones de personas. Tal es la magnitud de este proceso de extinción asociada al impacto humano que recientemente se ha propuesto una nueva era geológica denominada "Antropoceno", del griego *Anthropos (*ser humano) y *kainos* (nuevo) (Crutzen 2002; Corlett 2015), aunque aún no hay concenso sobre la fecha exacta de su inicio (Lewis & Maslin 2015). La caza y la sobreexplotación de recursos (Redford 1992; Fa *et al.* 2002; Abernethy *et al.* 2013), la pérdida de hábitat (Brooks *et al.* 2002), la introducción de especies invasoras y enfermedades (Blackburn 2004; Clavero & García-Berthou 2005) y las extinciones en cadena (Colwell *et al.* 2012), denominadas en su conjunto como los cuatro jinetes del Apocalipsis (Wilson 1992; Diamond 1997; Delibes 2001), han sido y siguen siendo, junto con el cambio climático (Thomas *et al.* 2004; Harnik *et al.* 2012), los principales desencadenantes de esta pérdida tan acusada de biodiversidad. En este sentido, entre el 13 y el 41% de los vertebrados están amenazados (IUCN, http://www.iucnredlist.org), y al menos 338 especies se han extinguido en los últimos 500 años (Ceballos *et al.* 2015). Además, aunque muchas especies no están amenazadas globalmente, sus poblaciones se han visto gravemente diezmadas. Como analogía al término de "deforestación", este proceso de reducción drástica no sólo de especies animales sino también de abundancia de individuos y de poblaciones se ha denominado recientemente como "defaunación del Antropoceno" (Dirzo & Miranda 1991; Dirzo 2001; Dirzo *et al.* 2014; McCauley *et al.* 2015).

La defaunación no es un proceso taxonómicamente aleatorio. Tanto en el Antropoceno como en las extinciones masivas previas, los vertebrados de mayor tamaño corporal han sido, de forma desproporcionada, más vulnerables a los procesos de extinción (Raup 1986; Shodi *et al.* 2009). Dos ejemplos llamativos son la extinción de los grandes dinosaurios del Cretácico (Sheehan *et al.* 1991) o más recientemente, entre 50.000-10.000 años atrás, el declive de la megafauna del Pleistoceno (Martin & Klein 1984; Alroy 2001; Koch & Barnosky 2006; Malhi *et al.* 2016). Las causas de esta mayor vulnerabilidad son varias. Por un lado, el riesgo se agrava con tamaños poblacionales pequeños, con tasas reproductivas bajas, con rangos de distribución geográfica reducida y con hábitos ecológicos muy especializados (Pimm *et al.* 1988; Purvis *et al.* 2000). Todos estos rasgos demográficos suelen ser compartidos por estas especies de gran tamaño corporal, lo que les hace ser más vulnerables. Por otro lado, éstas tienden a ser preferidas tanto en la caza de subsistencia como en la caza con fines comerciales (Terborgh 1974; Redford 1992; Dirzo 2001; Peres 2001; Wright *et al.* 2007; Fa & Brown 2009), con lo cual experimentan una mayor sobreexplotación poblacional.

El fenómeno de la defaunación a menudo resulta en una transición desde comunidades prístinas, donde abundan especies de gran tamaño, hacia comunidades empobrecidas donde sólo se preservan especies de mediano y pequeño tamaño (Peres & Dolman 2000; Koch & Barnosky 2006). Teniendo esto en cuenta, la reducción de talla desde el Pleistoceno a la actualidad ha sido muy marcada en todos los continentes (Koch & Barnosky 2006), excepto en África y el Sureste Asiático, donde aún sobreviven grandes megaherbívoros. En el resto, se han perdido prácticamente todas las especies con un peso superior a 1.000 Kg y la reducción de tamaño en las comunidades de vertebrados en alguno de estos continentes ha sido de hasta dos órdenes de magnitud respecto a las comunidades del Pleistoceno (Hansen & Galetti 2009). El panorama en las islas oceánicas y continentales es incluso más preocupante con reducciones de talla generalizada que superan incluso los tres órdenes de magnitud como es el caso de las comunidades de vertebrados frugívoros en Nueva Caledonia o Fiji (Hansen & Galetti 2009) (véase cuadro 1.1 para el caso concreto de las comunidades de frugívoros en las Islas Canarias).

CUADRO 1.1 DEFAUNACIÓN Y REDUCCIÓN DE TALLA DE LOS VERTEBRADOS FRUGÍVOROS EN LAS ISLAS CANARIAS

Al igual que en un gran número de islas oceánicas y continentales (James 1995) la extinción de vertebrados en las Islas Canarias durante el Antropoceno ha sido muy marcada (Rando 2003). La fauna originaria de estas islas en épocas pre-humanas incluyó grandes tortugas terrestres del género *Geochelone* (Testudinidae; Hutterer *et al.* 1998), ratas gigantes del género *Canariomys* (Muridae; Crusafont-Pairo & Petter 1964; López-Martinez & López-Jurado 1987), lagartos gigantes (p. ej. *Gallotia goliath,* Lacertidae; Mertens 1942) y una gran variedad de aves (Rando 2003; Illera *et al.* 2016). El establecimiento de los primeros asentamientos humanos en las islas, hace aproximadamente 2500 años, marcó el inicio del declive de estas especies y su posterior extinción (García Cruz & Marrero Rodríguez 1978; Onrubia-Pintado 1987; Gonzalez *et al.* 2014). Este proceso se vio agravado por la llegada de los europeos, hace aproximadamente 600 años, y la introducción de especies de depredadores exóticos, principalmente gatos y ratas. La extinción de estas especies no fue aleatoria sino que, nuevamente, como ocurre a escala global, las especies de mayor tamaño sufrieron un impacto desproporcionadamente mayor. Como ejemplo ilustrativo, en la Fig. 1.1 se muestra cuál ha sido la magnitud de la reducción de tamaño de las comunidades de dispersores de semillas en las Islas Canarias desde el Pleistoceno hasta la actualidad.

Para ello comparamos el peso corporal de todas las especies extintas y actuales que potencialmente dispersaban semillas (véanse más información en la tabla 1.S1). Como podemos observar las especies extintas son significativamente mayores que las especies que han sobrevivido. De hecho la pérdida de tamaño ha sido de hasta dos órdenes de magnitud en algunas islas (Tenerife, Gran Canaria, Fuerteventura y Lanzarote), valores muy similares a los reportados para otras islas oceánicas, como las Islas Fiji y Mauricio, y continentales como Madagascar, Cuba o Nueva Caledonia (Hansen & Galetti 2009). Esta reducción drástica de tamaño en la comunidad de frugívoros es mayor incluso que en la mayoría de continentes y es esperable que los efectos en cascada que se desencadenen dentro de los ecosistemas ocurran de forma más rápida y acentuada. Esto se debe a que los ambientes insulares presentan una baja riqueza de especies y una baja redundancia funcional de manera que las especies que sobreviven, generalmente de mediano o pequeño tamaño, raramente podrán sustituir el rol funcional desempeñado por las especies extintas.

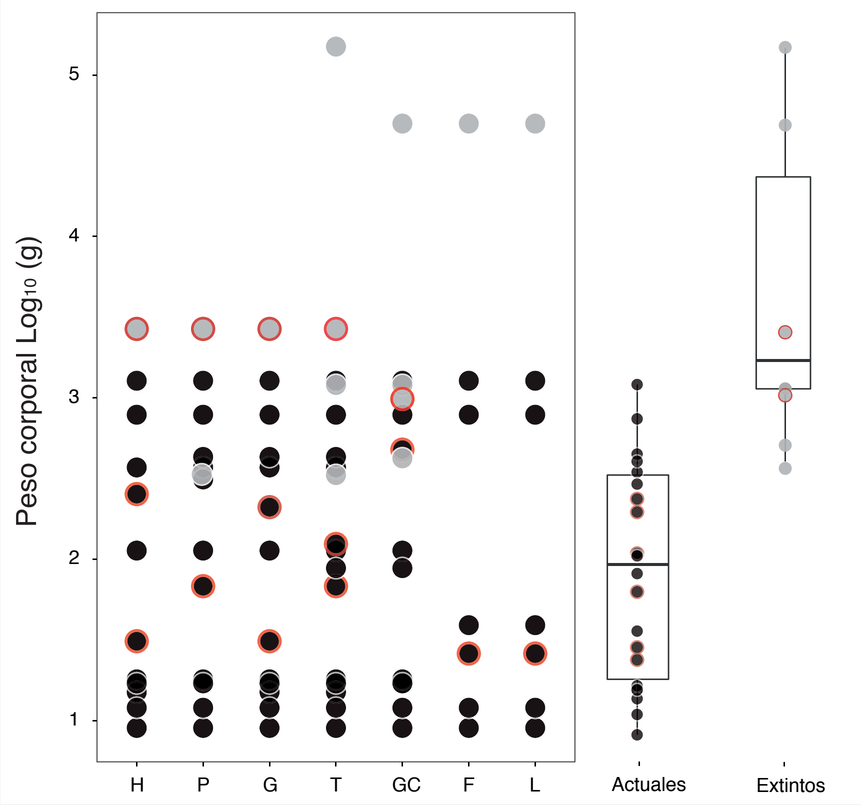


Fig. 1.1. Reducción de tamaño en la comunidad de frugívoros desde el Pleistoceno hasta la actualidad en las Islas Canarias. Los círculos grises representan especies de frugívoros extintas mientras que los puntos negros representan las especies actuales. Los puntos con contornos rojos representan las especies de lagartos frugívoros. En el panel de la izquierda se muestra la reducción de tamaño por isla: H (El Hierro), P (La Palma), G (La Gomera), T (Tenerife), GC (Gran Canaria), F (Fuerteventura), L (Lanzarote). Los boxplot del panel de la derecha muestran la diferencia de talla entre las especies de frugívoros extintas (peso corporal medio ± SD; 31972 ± 52552 g) y las actuales (223 ± 316 g) (t-test; $p < 0.01$). Véase tabla 1.S1 en Apéndices para una lista detallada de las especies, peso corporal, categoría de amenaza y distribución geográfica.

MUCHO MÁS QUE LA PÉRDIDA DE ESPECIES: EFECTOS EN CASCADA Y CONSECUENCIAS PARA EL FUNCIONAMIENTO ECOSISTÉMICO

Hasta hace unas décadas, la pérdida de biodiversidad se había interpretado casi exclusivamente como la pérdida de especies. Sin embargo, como anticipaba D. Janzen "*What escapes the eye…is a much more insidious kind of extinction: the extinction of ecological interactions*" (Janzen 1974), es decir que la pérdida de especies esconde otro tipo de extinción menos evidente, pero igualmente negativa, la extinción de las interacciones ecológicas (Janzen 1974; Valiente-Banuet *et al.* 2015). Las especies no están aisladas ecológicamente, sino que, por el contrario, conforman una red intrincada de interacciones con otros organismos que confieren estructura, funcionalidad y dinamismo a los ecosistemas (Bascompte 2009). En particular, las especies (e individuos) de gran tamaño suelen ejercer roles funcionales poco redundantes y muy importantes en la dinámica de los ecosistemas (Woodward *et al.* 2005; Vidal *et al.* 2013; Malhi *et al.* 2016). Esperaríamos así que la pérdida de estas especies (e individuos) conlleve una serie de efectos en cascada con consecuencias negativas para el funcionamiento ecosistémico.

A pesar de que sólo muy recientemente se ha comenzado a trabajar en estas líneas de investigación, existe un número creciente de estudios que evidencian la cantidad de formas diferentes en que la pérdida de especies de gran tamaño afecta al funcionamiento y estructura de los ecosistemas. Se ha comprobado, por ejemplo, que la extinción durante el Pleistoceno de la carismática megafauna (Malhi *et al.* 2016), cambió de forma drástica la estructura de la vegetación en diferentes biomas (Gill *et al.* 2009, 2012; Johnson 2009; Gill 2014; Bakker *et al.* 2015, 2016; Johnson *et al.* 2016), la productividad y almacenamiento de carbono (Bello *et al.* 2015; Peres *et al.* 2016), las frecuencias de incendios (Burney *et al.* 2003; Rule *et al.* 2012), el ciclo de los nutrientes (Gende *et al.* 2002; Maron *et al.* 2006; Zimov *et al.* 2009; Holdo *et al.* 2009; Wilmers *et al.* 2012; Doughty *et al.* 2013, 2016; Roman *et al.* 2014) o la estructura trófica de las comunidades (Estes *et al.* 2016; Pardi & Smith 2016). De forma similar, la defaunación del Antropoceno está impactando gravemente éstos y otros aspectos del funcionamiento ecosistémico (véase Dirzo *et al.* 2014 para una revisión). Por ejemplo, se ha observado una mayor incidencia de plagas en cultivos defaunados (Karp *et al.* 2013; Maas *et al.* 2013), una pérdida de la calidad del agua (Whiles *et al.* 2006, 2013) o un incremento de la prevalencia de enfermedades (Keesing *et al.* 2010; Johnson *et al.* 2013; Young *et al.* 2014). Además la defaunación desencadena la disrupción de las relaciones mutualistas que se establecen entre plantas y animales, provocando un empeoramiento notable de los servicios de polinización y dispersión de semillas que estos últimos proveen (Bond 1994). Dado que la inmensa mayoría de las plantas con flores interaccionan con polinizadores para llevar a cabo su ciclo reproductivo (Ollerton *et al.* 2011), al declive de los polinizadores (Potts *et al.* 2010) le ha seguido un declive paralelo de las poblaciones de plantas y de su abundancia (Biesmeijer *et al.* 2006; Anderson *et al.* 2011).

Sobre el efecto de la defaunación en los servicios de dispersión y sus consecuencias en las poblaciones de plantas de frutos carnosos se hablará a partir de aquí, puesto que sobre ello tratará esta tesis doctoral. Pero, ¿por qué es importante la dispersión de semillas y cuáles son las consecuencias potenciales de la degradación de este proceso?

DISRUPCIÓN DE LA INTERACCIÓN MUTUALISTA PLANTA-FRUGÍVORO

Una alta proporción de las especies de plantas existentes dependen de vertebrados frugívoros, con los que establecen relaciones mutualistas, para proveer servicios de dispersión de semillas (Jordano *et al.* 2011). En estos casos ambos interactuantes obtienen beneficios mutuos. Las plantas ofrecen recompensas en forma de recurso trófico nutritivo y los animales, a cambio, dispersan sus propágulos. La pulpa que rodea las semillas de los frutos carnosos supone un recurso habitual en muchas especies de vertebrados frugívoros, sobre todo de aves y mamíferos (Howe & Smallwood 1982; Howe 1986; Jordano 2014), pero también reptiles (Olesen & Valido 2003; Valido & Olesen 2007), peces (Gottsberger 1978; Howe & Smallwood 1982; Costa-Pereira & Galetti 2015) y anfibios (da Silva *et al.* 1989; da Silva & De Britto-Pereira 2006), incluidos renacuajos (Arribas 2015). Una vez que los animales han consumido los frutos, la semillas son defecadas o regurgitadas fuera del vecindario de la planta madre, donde las probabilidad de sobrevivir son mayores (Janzen 1970; Connell 1971). De esta forma los frugívoros configuran el patrón espacial de la distribución de semillas (lluvia de semillas) sobre el que posteriormente actuará otra serie de procesos demográficos post dispersivos (depredación de semillas, germinación y supervivencia de plántulas) que, en conjunto, determinarán el resultado final de reclutamiento (Nathan & Muller-Landau 2000; Wang & Smith 2002). Por otro lado, el comportamiento de forrajeo y los movimientos de los frugívoros condiciona la capacidad de colonización de nuevas áreas (Howe & Smallwood 1982) y además moldea los patrones de flujo de genes dentro y entre poblaciones de plantas (Jordano *et al.* 2007). Junto con la polinización y otros procesos como la deriva génica, los cuellos de botella o la adaptación local, determina las cantidad y la distribución espacial de la variabilidad genética de las poblaciones de plantas (Loveless & Hamrick 1984) .

Las especies de frugívoros de mayor tamaño contribuyen de manera desproporcionada a la efectividad de la dispersión (Jordano 2014); consumen un mayor número de frutos, dispersan un rango mayor de tamaños de semillas (Wheelwright 1985) y además presentan áreas de forrajeo más amplias, lo que les confiere la capacidad de mover semillas a grandes distancias (Jordano *et al.* 2007; Mueller *et al.* 2014). La reducción de talla (*downsizing*) en las comunidades de frugívoros va a tener, por tanto, importantes consecuencias demográficas y genéticas para las poblaciones de plantas con frutos carnosos debido a la pérdida de eficiencia del proceso de dispersión de semillas, que es un componente clave del flujo génico.

- *Consecuencias demográficas:* algunos estudios han demostrado que esta pérdida de tamaño en las comunidades de frugívoros puede provocar problemas graves de regeneración, reflejado en una baja tasa de reclutamiento de plántulas y un envejecimiento respecto a las poblaciones no defaunadas (Chapman *et al.* 1992; Chapman & Chapman 1995; Wright & Duber 2001; Cordeiro & Howe 2001, 2003; Traveset & Riera 2005; Rodríguez-Pérez & Traveset 2010; Wotton & Kelly 2011; Traveset *et al.* 2012). Además, el hecho de que la defaunación afecte en mayor medida a las especies con semillas más grandes, produciría transiciones hacia comunidades de plantas dominadas por especies de frutos carnosos con semillas pequeñas o con rasgos adaptados a la dispersión abiótica (Terborgh *et al.* 2008; Harrison *et al.* 2013). Esto se ha relacionado recientemente con cambios hacia comunidades forestales con menor capacidad de almacenaje de carbono (Bello *et al.* 2015; Peres *et al.* 2016). A pesar de que se ha hecho un enorme esfuerzo en documentar estos impactos, aún existen lagunas importantes en la literatura científica en torno a las posibles consecuencias negativas de la defaunación. Por una lado, la mayoría de estudios se han centrado en evaluar los efectos sobre la componente cuantitativa de la dispersión, es decir, en la cuestión puramente numérica (número de plantas reclutadas o semillas dispersadas), pero poniendo menos atención a la componente cualitativa. Por ejemplo, apenas se sabe nada sobre el efecto en el rango de tamaño de semillas dispersadas o sobre el vigor de las plántulas establecidas (Valido 1999; Galetti *et al.* 2013). Aún más escasos son los estudios que integran ambas componentes de la dispersión (Cordeiro & Howe 2003). Este tipo de trabajos en los que se examinen las dos componentes de la dispersión son de gran importancia para no infravalorar el efecto de la defaunación en el resultado final del reclutamiento.

- *Consecuencias genéticas:* Apenas se dispone de información acerca de los efectos genéticos de la extinción de animales frugívoros sobre las poblaciones de plantas (Pacheco & Simonetti 2000; Voigt *et al.* 2009; Calviño-Cancela *et al.* 2012). Se hipotetiza una reducción de las distancias de dispersión de semillas y del flujo de genes intra e interpoblacional a medida que se reduce la talla de los frugívoros (Hamrick *et al.* 1993). Esta disminución o eventual colapso del flujo de genes vía semillas tendría consecuencias medibles en la estructuración genética de las poblaciones de plantas a varias escalas espaciales. A escala local (dentro de poblaciones) se esperaría la generación de vecindarios de individuos muy emparentados genéticamente con consecuencias negativas a largo plazo como altos niveles de depresión por endogamia (Charlesworth & Charlesworth 1987) o una reducción del tamaño efectivo poblacional (Hedrick 2011). Además se esperaría una menor conectividad genética a escala de paisaje (metapoblación) (Urban & Keitt 2001) y, como efecto de la deriva y el aislamiento genético, una pérdida asociada de variabilidad genética y una mayor diferenciación de las poblaciones.

La gran mayoría de los trabajos publicados hasta la fecha utilizan una aproximación comparativa dicotómica entre escenarios muy contrastados: escenario defaunado *vs.* escenario no defaunado (p. ej. Chapman & Chapman 1995; Traveset & Riera 2005; Wotton & Kelly 2011; Rodríguez-Pérez & Traveset 2012). Sería ideal, sin embargo, disponer de un gradiente de defaunación y pérdida progresiva de tamaño de los dispersores de semillas, que permita evaluar el efecto no sólo de la disrupción de las interacciones mutualistas sino también de escenarios intermedios con servicios de dispersión sub-eficientes.

OBJETIVO GENERAL Y ESTRUCTURA DE LA TESIS

Teniendo en cuenta el contexto descrito anteriormente y las lagunas existentes en los estudios de defaunación, el objetivo último de esta memoria de tesis doctoral es comprender cómo afecta la progresiva degradación y colapso de los servicios de dispersión de semillas, asociada a una pérdida gradual de talla de los frugívoros, en distintos aspectos funcionales (demográficos y genéticos) de las poblaciones de plantas con frutos carnosos. Para abordar este objetivo nos centraremos en el estudio de la interacción mutualista altamente específica de la planta *Neochamaelea pulverulenta* (Rutaceae) y sus únicos dispersores de semillas, los lagartos frugívoros del género *Gallotia* (Lacertidae). *N. pulverulenta* es un arbusto endémico de las Islas Canarias que produce frutos carnosos (drupáceos) con semillas de gran tamaño. Éstos son consumidos exclusivamente por lagartos medianos y grandes del género *Gallotia* (Valido 1999), también endémicos del archipiélago. Hasta la llegada de los humanos a las islas (hace aprox. 2500 años; Onrubia-Pintado 1987), los lagartos gigantes de este género eran abundantes en cada una de las tres islas donde se distribuye *N. pulverulenta* (Gran Canaria, Tenerife y La Gomera), con individuos que podían superar los 500 mm de longitud hocico-cloaca (LHC) (Barahona *et al.* 2000). Sin embargo, como consecuencia directa o indirecta de la actividad humana sólo en Gran Canaria se preservan individuos de tamaño relativamente grande en la actualidad (*G. stehlini*; LHC max= 280 mm), mientras que en Tenerife sólo son abundantes individuos de tamaño mediano (*G. galloti*; LHC max= 145 mm) y en La Gomera individuos pequeños (*G. caesaris*; LHC max= 111 mm) (Fig. 1.2).

La idoneidad de nuestro sistema para el objetivo general de esta tesis reside en varios aspectos:

1. Los lagartos del género *Gallotia* han experimentado un proceso de extinción diferencial en las islas tras la llegada de los primeros humanos (Onrubia-Pintado 1987). Sin embargo, la magnitud de la reducción de talla resultante ha sido muy diferente en cada una de las islas donde se distribuyen (Fig. 1.1; Fig. 1.2), ofreciendo un marco comparativo idóneo para evaluar las consecuencias demogenéticas de este proceso.

2. N. pulverulenta se distribuye en 3 islas y cada una alberga a un dispersor exclusivo (lagarto) con una talla muy distinta respecto a las otras islas. Gran Canaria (*G. stehlini;* talla grande; dispersor óptimo), Tenerife (*G. galloti;* talla media; dispersor subóptimo) y La Gomera (*G. caesaris;* talla pequeña; dispersión ausente) (Fig. 1.1; Fig. 1.2)

3. Al ser una relación mutualista planta-animal altamente específica, permite caracterizar mejor los mecanismos subyacentes a los patrones demográficos y genéticos detectados que si consideramos redes de interacciones más complejas.

Los objetivos específicos son los siguientes y quedan estructurados en tres capítulos que ya han sido publicados o están en vías de publicación:

Objetivo 1. Evaluar las consecuencias de la reducción de talla de los lagartos frugívoros en el reclutamiento temprano (componente cuantitativa y cualitativa) de *N. pul-*

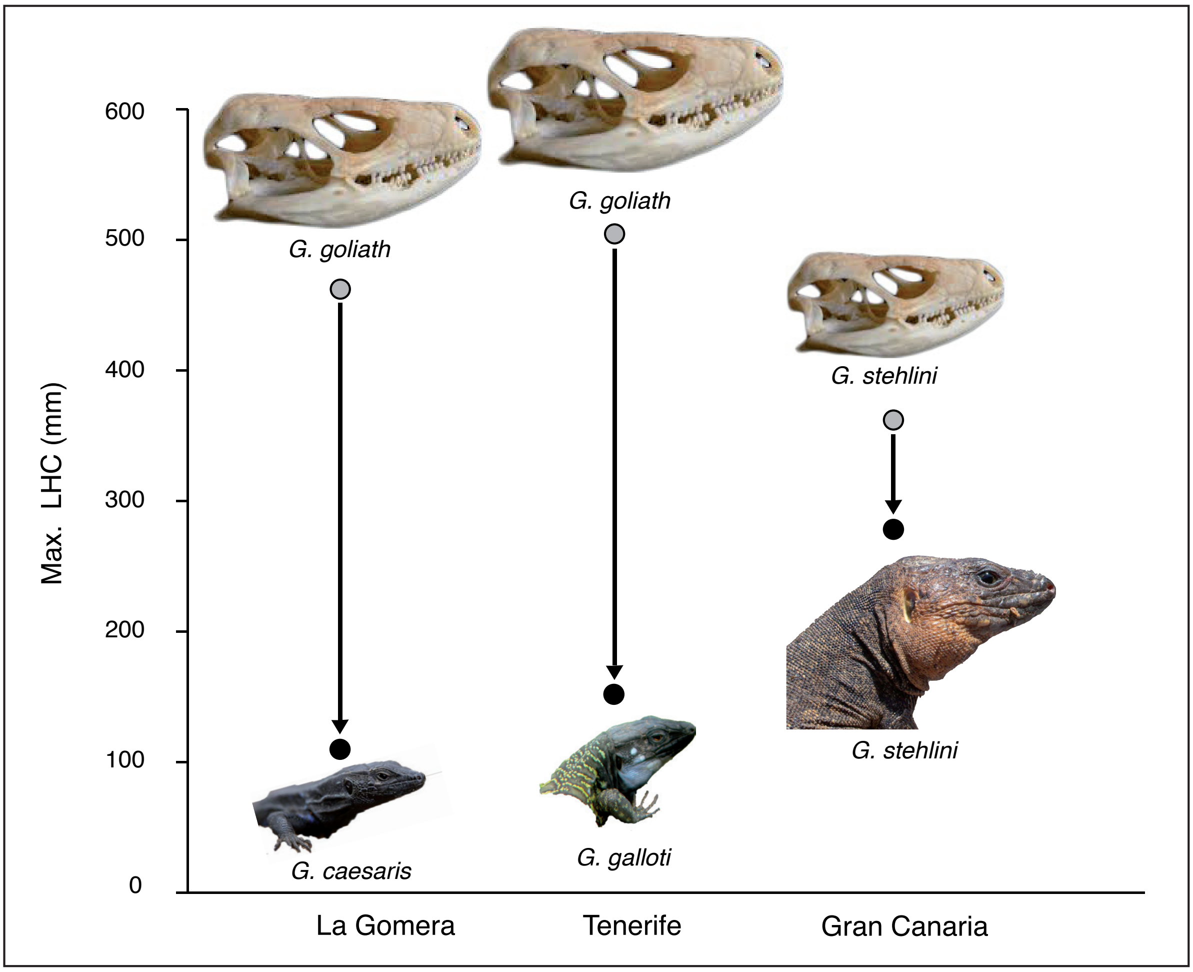


Fig. 1.2. Esquema de la reducción de la longitud máxima hocico-cloaca (Max. LHC) de los lagartos frugívoros de las Islas Canarias (*Gallotia* sp.) desde el pasado hasta el presente. En esta figura sólo se muestra la reducción de talla para las tres islas donde se distribuye *Neochamaelea pulverulenta* (Rutaceae). Para ilustrar este fenómeno en el resto de islas véase Fig. 1.1 y Fig. 2.S1. Fotos: *G. caesaris* (B. Rodríguez); *G. galloti* (C. Camacho); *G. stehlini* (A. Valido)

verulenta. **Capítulo 2**

Objetivo 2. Evaluar si la reducción gradual de tamaño de los lagartos frugívoros conlleva una reducción paralela de las distancias de dispersión de semillas. **Capítulo 3**

Objetivo 3. Evaluar si la reducción de las distancias de dispersión de semillas (si existe) tiene consecuencias en la cantidad y en la distribución espacial de la variación genética a escala local (dentro de poblaciones). **Capítulo 3**

Objetivo 4. Evaluar si la reducción de las distancias de dispersión de semillas (si existe) tiene consecuencias en la cantidad de diversidad genética, el grado de conectividad genética y el aislamiento por distancia entre poblaciones a escala regional (dentro de islas). **Capítulo 4**

Para alcanzar estos objetivos específicos se utilizará una aproximación comparativa a distintas escalas espaciales contrastando los tres escenarios ecológicos descritos anteriormente. La comparación de estos escenarios ecológicos constituye una aproximación experimental observacional, al tratarse de una aproximación realizada de forma replicada en cada una de las áreas contrastantes en defaunación. Además se combinará trabajo de campo intensivo, técnicas moleculares aplicadas a la genética de poblaciones y teoría de grafos y redes complejas. Todo ello en un contexto espacialmente explícito. Con la consecución de los objetivos marcados pretendemos aportar nuevas evidencias que, por un lado, revelen la importancia de las interacciones planta-animal en la dinámica y funcionamiento ecosistémico y que, por otro lado, permitan diseñar estrategias efectivas para la conservación no sólo de la biodiversidad en sentido estricto (especies) sino de todo el rango de procesos ecológicos en los que intervienen.

SISTEMA DE ESTUDIO

Neochamelea pulverulenta

*Neochamaelea pulverulenta (*Rutaceae) (Vent) Erdtman, conocida de forma vernácula como Orijama, Leña buena o Leña Santa (Cáceres & Salas 1995), es una especie endémica de las Islas Canarias. Se incluye en un género monoespecífico, estando muy emparentada con otro género monoespecífico de la misma familia cuya única especie es *Cneorum tricocoon*, una especie nativa del oeste del Mediterráneo europeo. Algunos autores sugerían un carácter relicto de *N. pulverulenta*, con un origen en los bosques mediterráneos del Terciario, clasificándola por tanto como un paleoendemismo (Bramwell 1976; Borgen 1979). Sin embargo, filogenias recientes apuntan a un neoendemismo de las Islas Canarias, probablemente derivado de un ancestro de origen norteafricano (Appelhans *et al.* 2012).

N. pulverulenta se distribuye actualmente en tres de las islas del archipiélago (Gran Canaria, Tenerife y La Gomera; Fig. 1.3), aunque no se descarta que también pudiera haber estado presente en El Hierro (Ceballos & Ortuño 1951; Hansen & Sunding 1993; Arechavaleta *et al.* 2010). Esta especie es característica del matorral xerofítico (Bramwell & Bramwell 2001) distribuido a lo largo de un cinturón costero (< 400 m s.n.m.) donde la temperatura media ronda los 25ºC y la precipitación anual está por debajo de los 200 mm (AEMET-IP 2012). Aparte de esta especie, también son frecuentes varias especies de *Euphorbia* spp. (Euphorbiaceae), *Lycium intricatum* (Solanaceae), *Periploca laevigata* (Asclepiadaceae), *Plocama pendula*, *Rubia fruticosa* (Rubiaceae) o *Schyzogine sericea* (Asteraceae), entre otras.

N. pulverulenta es una especie de porte arbustivo que puede alcanzar una altura de 2.7 m (*media*= 1.1 m; *d.t.*= 0.5; *n*= 2139) y un diámetro de la copa máximo de 3.9 m (*media*= 1.1 m; *d.t.*= 0.6; *n*= 2139). Presenta un sistema reproductivo androdioico con individuos con flores masculinas e individuos bisexuales. Es una especie autoincompatible y es polinizada por dos grupos funcionales de insectos. Por un lado hormigas, principalmente *Camponotus feai* (Formicidae), pero también la hormiga exótica *Linepithema humile* (Formicidae). Por otro lado insectos voladores, entre los que destacan varias especies de abejas solitarias como *Lasioglossum* spp. (Halictidae), *Amegilla quadrifasciata* (Anthophoridae), *Colletes dimidiatus* (Colletidae), *Bombus canariensis* y *Apis mellifera*

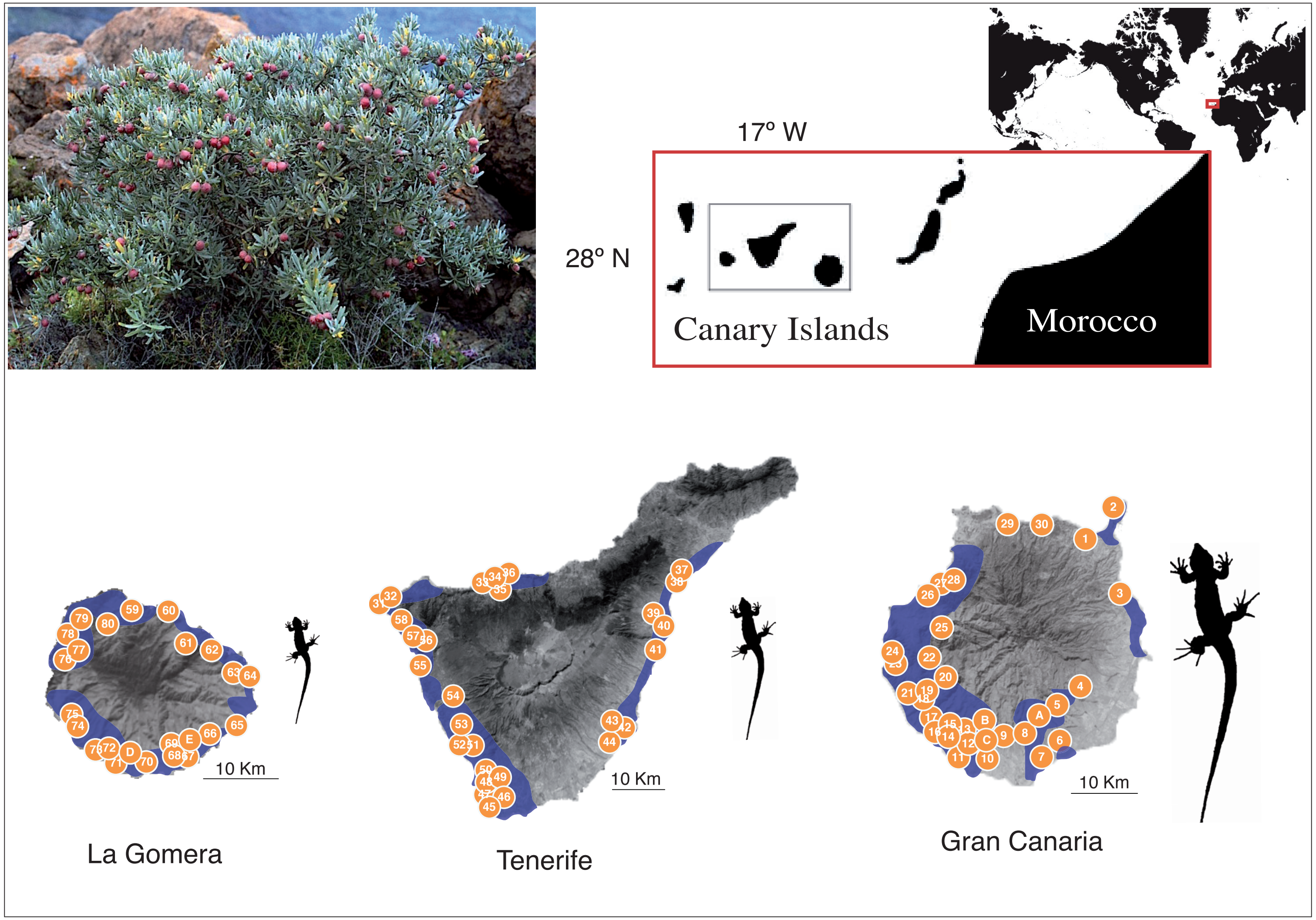


Fig. 1.3. Localización de Las Islas Canarias (arriba a la derecha). Individuo reproductivo de *Neochamaelea pulverulenta* con frutos maduros (arriba a la izquierda). Área de distribución potencial de *N. pulverulenta* (en violeta) modificado de ATLANTIS 3.1 (http://www.biodiversidadcanarias.es/atlantis/). En naranja la localización de las poblaciones muestreadas. Los códigos coinciden con los de la tabla 1.S2 donde se muestran los detalles de cada población y los capítulos de la tesis para los que han sido muestreadas

(Apidae) y varias especies de dípteros como *Myathropa florea* y *Syritta pippiens* (Syrphidae) y *Lucilia sericata* y *Stomorhina lunata* (Calliphoridae) entre otras. El período de floración es bastante lábil y puede florecer a lo largo de todo el año dependiendo de las lluvias, aunque suele ser entre diciembre y mayo. Los frutos están formados por 1-4 cocos. Cada uno de estos cocos puede ser considerado funcionalmente una drupa (diámetro medio= 11.1 mm; *d.t.*= 1.6) que contiene una semilla con endocarpo muy duro (diámetro medio= 8.6 mm; *d.t.*= 1.0) (Valido 1999). La pulpa que rodea la semilla es rica en azúcares y adquiere un color rojizo o blanquecino (según la isla) al madurar, normalmente a mitad o final de primavera. Los frutos son consumidos exclusivamente por lagartos de tamaño grande y tamaño mediano, ya que presentan claras restricciones morfológicas para el consumo por lagartos pequeños (Valido 1999). Dada la distribución de la especie, relegada a Gran Canaria, Tenerife y La Gomera, y las restricciones morfológicas que impiden a los lagartos de pequeño tamaño consumir sus frutos, las semillas de esta especie es sólo dispersada legítimamente por *Gallotia stehlini* en Gran Canaria (lagarto de talla grande) y *G. galloti* en Tenerife (lagarto de talla mediana), mientras que en La Gomera *G. caesaris* no dispersa las semillas de forma eficiente.

Además, algunos trabajos recientes (Rodríguez *et al.* 2007; Padilla & Nogales 2009; Padilla *et al.* 2012; López-Darias & Nogales 2016) han puesto de manifiesto el potencial de algunas especies de aves, principalmente rapaces (Padilla & Nogales 2009; Padilla *et al.* 2012; López-Darias & Nogales 2016), alcaudones (Padilla *et al.* 2012) y garzas (Rodríguez *et al.* 2007), como dispersores secundarios de semillas. En este sentido, *Padilla et al.* (2012) encontraron semillas viables de *N. puleverulenta* en egagrópilas de cernícalo vulgar (*Falco tinnunculus)*, evidenciando el probable rol de esta rapaz como dispersor a larga distancia de semillas de esta especie.

Los lagartos gigantes de Canarias y su extinción

Los lagartos de las Islas Canarias están incluidos dentro del género endémico *Gallotia* (Arnold 1973), de la familia Lacertidae. Actualmente, este género está compuesto de siete especies (Arechavaleta et al., 2010; Cox et al., 2010): *G. atlantica* (Peters and Doria, 1882) en Lanzarote y Fuerteventura, *G. stehlini* (Schenkel, 1901) en Gran Canaria, *G. galloti* (Oudart, 1839) en Tenerife y La Palma, *G. intermedia* (Hernández *et al.* 2000) en Tenerife, *G. caesaris* (Lehrs, 1914) en La Gomera y El Hierro, *G. bravoana* (Hutterer 1985) en La Gomera, y *G. simonyi* (Steindachner, 1889) en El Hierro (Fig. 1.4). Sin embargo, en las islas occidentales existieron también otros taxones de lagartos gigantes en el pasado, pero que se consideran extintos actualmente: *Gallotia simonyi* (Steindachner, 1889; Tenerife, La Gomera y La Palma), *G. goliath* (Mertens 1942; Tenerife, La Gomera, La Palma y El Hierro) y *G. auaritae* (Mateo *et al.* 2001); La Palma) (Fig.1.4). El *status* taxonómico de algunas de estas especies se encuentra aún bajo debate. Algunos autores consideran que fue una sola especie la que habitó cada una de las islas occidentales (Mateo *et al.* 2011; pero ver Maca-Meyer *et al.* 2003) y que, por tanto, todas estas especies descubiertas recientemente en las islas occidentales sobreviviendo en poblaciones muy aisladas en acantilados poco accesibles (*G. simonyi* en 1975 en El Hierro, Böhme & Bings 1975; *G. intermedia* en 1995 en Tenerife, Hernández *et al.* 1997, 2000; *G. bravoana* en 1999 en La Gomera, Valido *et al.* 2000; Nogales *et al.* 2001) son individuos de tamaño reducido de estas especies (Rando *et al.* 1997; Bischoff 1998; Barahona *et al.* 2000; Mateo *et al.* 2001, 2011). Por tanto, hasta que la posición taxonómica de estos lagartos extintos se resuelva mediante análisis genéticos con ADN antiguo, podemos tan solo decir que cada una de las islas occidentales estuvo habitada por lagartos gigantes en el pasado. Las especies descubiertas recientemente (*G. simonyi, G. bravoana* y *G. intermedia*) estarían muy emparentadas con estos taxones extintos, pero actualmente están restringidos a poblaciones muy pequeñas y aisladas. Por su parte, las especies de mediano (*G. galloti*) y pequeño tamaño (*G. caesaris*) son abundantes y están bien distribuidas en cada una de las islas donde están presentes.

El origen de este grupo monofilético se remonta al Mioceno (hace ~17-20 m.a.), cuando un ancestro común de *Psammodromus* y *Gallotia* colonizó las islas más orientales y geológicamente más antiguas (Lanzarote/Fuerteventura) desde el Sur de Europa o el Noroeste de África (Cox *et al.* 2010). Posteriormente se produjo una serie de colonizaciones en *stepping-stone* y procesos de diversificación desde las islas orientales hacia las occidentales. De esta forma, durante el Mioceno (~13-10 m.a.) ya se había formado tres linajes genéticos distintos: *G. atlantica* en Lanzarote/Fuerteventura, *G. stehlini* en Gran Canaria y un linaje diferente en Tenerife. Este último linaje evolucionó a su vez en dos linajes distintos durante el Plioceno y el Pleistoceno (6 - 0.6 m.a.): un linaje de lagartos de talla media-pequeña y un linaje de lagartos gigantes. Estos lagartos colonizaron el resto de islas occidentales y se originaron nuevas especies y subespecies en cada isla. Por lo tanto, en el Pleistoceno Medio, las islas de Tenerife, La Gomera, La Palma y el Hierro albergaban cada una a una especie de lagarto gigante, coexistiendo con otra especie de lagarto mediano o pequeño. Por su parte, Gran Canaria ya albergaba una especie gigante de lagarto (*G. stehlini*) desde el Mioceno (Barahona *et al.* 2000; Cox *et al.* 2010) (Fig. 1.1).

El gigantismo en islas oceánicas es un patrón recurrente entre los vertebrados (Lomolino 2005), incluyendo lagartos (Pregill 1986; Meiri 2008). El tamaño corporal de los lagartos gigantes de Canarias es el mayor (Longitud hocico cloaca; LHC = 502 mm) dentro de la familia Lacertidae y son considerados un ejemplo paradigmático de gigantismo en islas (Barahona *et al.* 2000; Lomolino 2005; Whittaker & Fernández-Palacios 2007; Meiri 2008). Muy probablemente la ausencia de depredadores y la expansión del nicho ecológico hacia dietas más herbívoras permitió a los lagartos alcanzar estas tallas tan grandes y divergir de las especies pequeñas y medianas (Maca-Meyer *et al.* 2003; Cox *et al.* 2010). De acuerdo con el registro paleontológico, los lagartos gigantes se distribuían ampliamente dentro de cada isla hasta la llegada de los primeros habitantes a las islas, hace aproximadamente 2500 años (Onrubia-Pintado 1987). El descubrimiento de huesos de lagarto gigante en concheros (Alberto 1999) junto con algunos resultados obtenidos de análisis de isótopos estables en huesos de aborígenes (Arnay-de-la-Rosa *et al.* 2010, 2011) y recientes estudios genéticos (Gonzalez *et al.* 2014) sugieren que esta primera colonización humanos marcó el inicio del declive de las poblaciones de lagarto gigante

Fig. 1.4. Lagartos frugívoros (*Gallotia* spp., Lacertidae) de las Islas Canarias ordenados según su talla corporal decreciente. Arriba: *G. goliath* (foto: Angelica & Siegfried Troidl), Izquierda y de arriba abajo: *G. stehlini* (foto: Beneharo Rodríguez), *G. simonyi* (foto: Jose Juan Hernández), *G. bravoana* (foto: Jose Juan Hernández), *G. intermedia* (foto: Beneharo Rodríguez). Derecha y de arriba abajo: *G. galloti* (foto: Beneharo Rodríguez), *G. caesaris* (foto: Beneharo Rodríguez), *G. atlantica* (foto: Beneharo Rodríguez)

en estas islas. La colonización posterior de los europeos hace unos 600 años supuso la introducción de depredadores y competidores exóticos (gatos), así como la destrucción de gran parte de su hábitat. Estos factores junto con algunas características biológicas de estas especies, como una tasa reproductiva baja, provocó finalmente la extinción o la reducción drástica de tamaño de las especies de lagarto gigante en las islas (Mateo & López-Jurado 1992). Aunque el proceso de extinción y/o de reducción de tamaño de los lagartos ocurrió en todas las islas occidentales y centrales, la intensidad del fenómeno no fue homogénea en todas ellas (Fig. 1.1; Fig.1.2). Por ejemplo, en Gran Canaria sólo se produjo una reducción sutil (de 350 mm de LHC máxima a 280 mm) en los individuos de *G. stehlini.* En Tenerife la reducción fue intermedia ya que hoy en día aún preserva lagartos de talla media (LHC= 145 mm; *G. galloti*) después de la extinción de *G. goliath* (502 mm). Por su parte, La Gomera sufrió un reducción más drástica desde individuos de 466 mm de LHC (*G. goliath)* hasta ejemplares de 111 mm (*G. caesaris)*. Esto nos permite definir tres escenarios eco-evolutivos muy contrastados, proporcionándonos un experimento natural muy interesante para evaluar el papel de la reducción progresiva del tamaño de los dispersores de semillas en distintos aspectos demográficos y genéticos de las poblaciones de *Neochamaelea pulverulenta.* Todos las especies de lagarto del género *Gallotia* presentan una dieta omnívora, consumiendo frutos carnosos a lo largo de todo el año. De esta forma, dispersa semillas de muchas especies de plantas con frutos carnosos en una gran variedad de hábitats e islas (Valido & Nogales 1994; Olesen & Valido 2003; Valido *et al.* 2003; Rodríguez *et al.* 2008). La importancia cuantitativa de la componente vegetariana en la dieta, sin embargo, covaría ampliamente con el tamaño corporal de los lagartos. Así, los lagartos de mayor tamaño son más vegetarianos y/o frugívoros que las especies más pequeñas (Mateo & López-Jurado 1992; Valido *et al.* 2003; Carretero *et al.* 2006). Estos resultados, y un estudio de paleodieta de *G. goliath* en Tenerife (Bocherens *et al.* 2003) sugieren que los lagartos gigantes extintos fueron incluso más vegetarianos y/o frugívoros que las especies más grandes actuales. Por tanto asumimos que estos lagartos gigantes fueron también importantes dispersores de semillas no sólo de *N. pulverulenta* (Valido 1999) sino también de otras muchas especies de frutos carnosos. Nuestros resultados por tanto podrían ser aplicables no sólo al caso concreto de *N. pulverulenta* sino a escala de comunidad.

Apéndices

CAPÍTULO 1: Introducción general

Clase	Especie de vertebrado	Peso corporal (g)	Status	H	P	G	T	GC	F	L
Reptil	*Geochelone vulcanica*	50000	EX					*		
Reptil	*Geochelone buchardi*	150000	EX				*			
Reptil	*Geochelone* sp	50000*	EX						*	*
Reptil	*Chalcides simonyi*	39	EN						*	*
Reptil	*Chalcides sexlineatus*	17	LC					*		
Reptil	*Chalcides viridanus*	15	LC	*		*	*			
Reptil	*Gallotia atlantica*	26	LC						*	*
Reptil	*Gallotia caesaris*	31	LC	*		*				
Reptil	*Gallotia galloti*	68	LC		*		*			
Reptil	*Gallotia stehlini*	477	LC					*		
Reptil	*Gallotia stehlini*^	1060	EX					*		
Reptil	*Gallotia simonyi*	253	CE	*						
Reptil	*Gallotia intermedia*	117	CE				*			
Reptil	*Gallotia bravoana*	210	CE			*				
Reptil	*Gallotia auaritae*	NA	EX		*					
Reptil	*Gallotia goliath*	2671	EX	*	*	*	*			
Ave	*Larus michahellis*	1275	LC	*	*	*	*	*	*	*
Ave	*Columba bollii*	380	LC	*	*	*	*			
Ave	*Columba junoniae*	430	NT		*	*	*			
Ave	*Columba* sp	430	EX					*		
Ave	*Erithacus rubecula*	18	LC	*	*	*	*	*		
Ave	*Turdus merula*	113	LC	*	*	*	*	*		

Clase	Especie de vertebrado	Peso corporal (g)	Status	H	P	G	T	GC	F	L
Ave	*Sylvia conspicillata*	9	LC	*	*	*	*	*	*	*
Ave	*Sylvia melanocephala*	12	LC	*	*	*	*	*	*	*
Ave	*Sylvia atricapilla*	17	LC	*	*	*	*	*		
Ave	*Pyrrhocorax pyrrhocorax*	312	LC		*	†	†			
Ave	*Pyrrhocorax graculus*	277	LC		†		†			
Ave	*Corvus corax*	785	LC	*	*	*	*	*	*	*
Ave	*Sturnus vulgaris*	88	LC				*	*		
Mamífero	*Canariomys tamarani*	1200	EX					*		
Mamífero	*Canariomys bravoi*	1200	EX				*			

Tabla 1.S1. Lista de vertebrados extintos y actuales que incluyen frutos en su dieta y su distribución en las Islas Canarias. Los datos de peso corporal de las tortugas del género *Geochelone* (Testudinidae) fue estimado a través de una comparación entre el tamaño de caparazón de los fósiles canarios (Hutterer *et al.* 1998) con el de la tortuga de Aldabra actual (Rhodin et al. 2015). Para Lanzarote y Fuerteventura sólo se describen huevos de talla muy similar a los de *Geochelone vulcanica* en Gran Canaria. Asumimos por tanto una talla muy similar. Los datos de peso corporal para los géneros *Gallotia* (Lacertidae) y *Chalcides* (Scincidae) fueron estimados a partir de su logitud hocico-cloaca (LHC) máxima. Se usó la recta de regresión log10(peso corporal) = -4.543 + 2.951*log10(LHC) para los lacértidos y log10(peso corporal) = -4.821 + 3.029*log10(LHC) para los escíncidos (Meiri 2010). Los datos de peso para las aves fueron obtenidos del Handbook of Avian Body Masses (Dunning Jr. 2008). Las tallas estimadas de *Canariomys tamarani* y *Canariomys bravoi* son muy similares, por tanto el peso estimado es compartido (Lopez-Martínez & López-Jurado 1987). El status de conservación de las especies proviene de la clasificación propuesta por la IUCN (http://www.iucnredlist.org): EX(Extinto), CE (Amenazado críticamente), EN (Amenazado), NT (Casi amenazado), LC (Preocupación menor). H (El Hierro); P (La Palma); G (La Gomera); T (Tenerife); GC (Gran Canaria); F (Fuerteventura); L (Lanzarote). * A pesar de que no existen datos de talla de caparazón para esta especie de *Geochelone*, el tamaño de los huevos fósiles, un carácter usado para estimar talla, es muy similar al de *G. vulcanica* (Hutterer et al. 1998). ^ Según las estimas de Barahona *et al* (2000) los individuos de *G. stehlini* en el Pleistoceno podían alcanzar tallas muy superiores a las actuales. Se han encontrado restos de una paloma (*Columba* sp.) en Gran Canaria, con una morfología similar a *Columba junoniae* aunque aún se encuentra bajo debate sus status taxonómico. Asumimos un peso corporal igual al de *Columba junoniae.* Además, recientemente se ha introducido *C. junoniae* en Gran Canaria y se han detectado también individuos de *C. bollii*, probablemente como resultado de una colonización muy reciente. Otras especies de aves no incluidas en esta lista (p. ej. *Cyanistes* spp., *Fringilla* spp.), pueden incluir esporádicamente frutos en su dieta, pero no ejercen una función importante como dispersores de semillas de plantas con frutos carnosos.† Extinto a nivel insular.

Población	Código	Isla	Latitud	Longitud	Capítulos
Los Giles	1	Gran Canaria	28.125347	-15.460028	2, 4
La Isleta	2	Gran Canaria	28.175283	-15.421117	4
Jinámar	3	Gran Canaria	28.037350	-15.408592	4
Barranco de Tirajana	4	Gran Canaria	27.888915	-15.480562	4
Los Gallegos	5	Gran Canaria	27.859346	-15.522248	4
Montaña de Tabaiba	6	Gran Canaria	27.802300	-15.517867	2, 4
Barranco de las Burras	7	Gran Canaria	27.776124	-15.550269	4
Montañeta Redonda	8	Gran Canaria	27.814059	-15.580894	4
Barranco de Ayagaures	9	Gran Canaria	27.810056	-15.619050	2, 4
Cuartería de los Indígenas	10	Gran Canaria	27.77325	-15.648168	4
Barranco de la Verga I	11	Gran Canaria	27.780511	-15.695091	2, 4
Barranco de la Verga II	12	Gran Canaria	27.797568	-15.682778	4
Barranco de La Perra	13	Gran Canaria	27.814776	-15.695991	4
Barranco de Tauro I	14	Gran Canaria	27.818639	-15.718887	2, 4
Barranco de Tauro II	15	Gran Canaria	27.828422	-15.716317	4
Barranco de Tiritaña	16	Gran Canaria	27.816082	-15.743210	2, 4
Barranco de Mogán I	17	Gran Canaria	27.839225	-15.750136	4
Barranco de Veneguera I	18	Gran Canaria	27.869183	-15.764745	4
Barranco de Veneguera II	19	Gran Canaria	27.882428	-15.757628	2, 3, 4
Barranco de Veneguera III	20	Gran Canaria	27.903610	-15.723602	4
Barranco de Tasarte I	21	Gran Canaria	27.878133	-15.792349	4
Barranco de Tasarte II	22	Gran Canaria	27.935047	-15.753334	2, 4
Barranco de Tasartico	23	Gran Canaria	27.925150	-15.814221	4
Barranco de Güi-Güi	24	Gran Canaria	27.933504	-15.820674	2, 4
Barranco de la Aldea	25	Gran Canaria	27.983044	-15.731114	4
Tirma	26	Gran Canaria	28.034736	-15.756321	2, 4
El Risco	27	Gran Canaria	28.053333	-15.732778	2, 4
Guayedra	28	Gran Canaria	28.059221	-15.729010	4
El Salado	29	Gran Canaria	28.145995	-15.603889	4
Lomo de La Guancha	30	Gran Canaria	28.141794	-15.554902	4
Barranco de Fataga	A	Gran Canaria	27.838889	-15.569483	3
Barranco de Arguineguín I	B	Gran Canaria	27.829339	-15.679503	3
Barranco de Arguineguín II	C	Gran Canaria	27.803656	-15.673231	3
Punta de Teno Bajo I	31	Tenerife	28.347297	-16.913317	2, 4
Punta de Teno Bajo II	32	Tenerife	28.359722	-16.900833	2, 3, 4
Cueva del Rey	33	Tenerife	28.383056	-16.711667	2, 4
Punta de Juan Centella	34	Tenerife	28.392222	-16.693889	2, 4
Barranco de las Ánimas	35	Tenerife	28.390033	-16.683754	4
Punta Charco del Viento	36	Tenerife	28.399167	-16.673333	2, 4
Tabaiba Alta	37	Tenerife	28.403889	-16.339167	2, 4
Barranco Hondo	38	Tenerife	28.389167	-16.348611	4
La Hidalga	39	Tenerife	28.331491	-16.392895	2, 4
Malpaís de Güímar	40	Tenerife	28.310278	-16.372778	2, 4
Punta Prieta	41	Tenerife	28.270331	-16.388206	4
Abades	42	Tenerife	28.139758	-16.456732	2, 4

Villa de Arico	43	Tenerife	28.151277	-16.471664	2, 4
Barranco de Tajao	44	Tenerife	28.115475	-16.476023	4
Malpaís Punta de Rasca	45	Tenerife	28.011664	-16.698341	2, 3, 4
El Palm-Mar	46	Tenerife	28.023170	-16.691881	2, 4
Montaña de Guaza I	47	Tenerife	28.033075	-16.707498	2, 4
Montaña de Guaza II	48	Tenerife	28.048539	-16.700953	2, 4
Montaña de Guaza III	49	Tenerife	28.056667	-16.690000	2, 4
Arona	50	Tenerife	28.068611	-16.710833	4
Caleta de Adeje I	51	Tenerife	28.110278	-16.744444	3, 4
Caleta de Adeje II	52	Tenerife	28.110833	-16.761111	4
Los Menores	53	Tenerife	28.145222	-16.757279	2, 4
Barranco de Guía	54	Tenerife	28.192996	-16.772860	2, 4
Los Gigantes	55	Tenerife	28.243778	-16.835992	4
Barranco del Natero	56	Tenerife	28.288611	-16.833746	4
Barranco de Masca	57	Tenerife	28.293082	-16.848972	2, 4
Barranco de Los Carrizales	58	Tenerife	28.320202	-16.871472	2, 4
Lomo de La Sepultura	59	La Gomera	28.198683	-17.247400	4
Cerro del Cepo	60	La Gomera	28.197750	-17.203267	2, 4
El Palmar	61	La Gomera	28.163056	-17.181944	4
Barranco de Juel	62	La Gomera	28.155833	-17.150833	4
Aluse	63	La Gomera	28.131716	-17.124567	2, 4
Punta Llana	64	La Gomera	28.128056	-17.104722	2, 3, 4
Punta de Juan Daza	65	La Gomera	28.075333	-17.120450	4
Roque de la Roja	66	La Gomera	28.066399	-17.152998	2, 4
Barranco de Chinguarime	67	La Gomera	28.042102	-17.179768	2, 4
Barranco de Tapahuga I	68	La Gomera	28.046389	-17.190556	4
Barranco de Tapahuga II	69	La Gomera	28.055833	-17.199722	4
Antoncojo	70	La Gomera	28.036309	-17.229809	2, 4
Barranco de Quise	71	La Gomera	28.035083	-17.266825	2, 4
Las Negras	72	La Gomera	28.051050	-17.280967	2, 4
Barranco de Arguayoda	73	La Gomera	28.049144	-17.291173	4
Lomo Gerián	74	La Gomera	28.074495	-17.313254	2, 4
Argaga	75	La Gomera	28.083808	-17.320161	4
Riscos de Heredia	76	La Gomera	28.145833	-17.328333	4
Taguluche	77	La Gomera	28.155750	-17.311867	2, 3, 4
Barranco de Los Monos	78	La Gomera	28.172783	-17.325317	4
Tazo	79	La Gomera	28.189233	-17.308483	4
La Era Nueva	80	La Gomera	28.183617	-17.276733	2, 4
Barranco del Medio	D	La Gomera	28.034092	-17.256494	3
Barranco de Biguillo	E	La Gomera	28.043644	-17.184272	2

Tabla 1. S2. Lista de poblaciones de *Neochamaelea pulverulenta* (Rutaceae) muestreadas en esta tesis. Los códigos de las poblaciones corresponden con los de la Fig. 1.3.

CAPÍTULO 2

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

Pérez-Méndez N., Jordano P., Valido A. (2015)
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Abstract

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; Shodi *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 & 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 & 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 & 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 & Smallwood 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 & 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 & 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 & Richter 1982; Moles & 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 & Galetti 2009) and quite often preceded by the loss of their functional roles associated with the reduced population size (McConkey & Drake 2006; Boyer & Jetz 2014). Secondly, insular environments frequently present low functional redundancy of dispersal agents (e.g. Woodward *et al.* 2005; Wotton & Kelly 2011; González-Castro *et al.* 2015). 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 & Riera 2005; Rodríguez-Pérez & Traveset 2010; Wotton & Kelly 2011; 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 & Valido 2003; Valido & Olesen 2007). In the Canary Islands, endemic lacertid lizards (*Gallotia* spp.) are significant seed dispersers (Valido & Nogales 1994, 2003; Valido 1999; 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 2.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. 2.1 and Fig. 2.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. 2.1; *see also* Appendix 2.S1 and Fig. 2.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

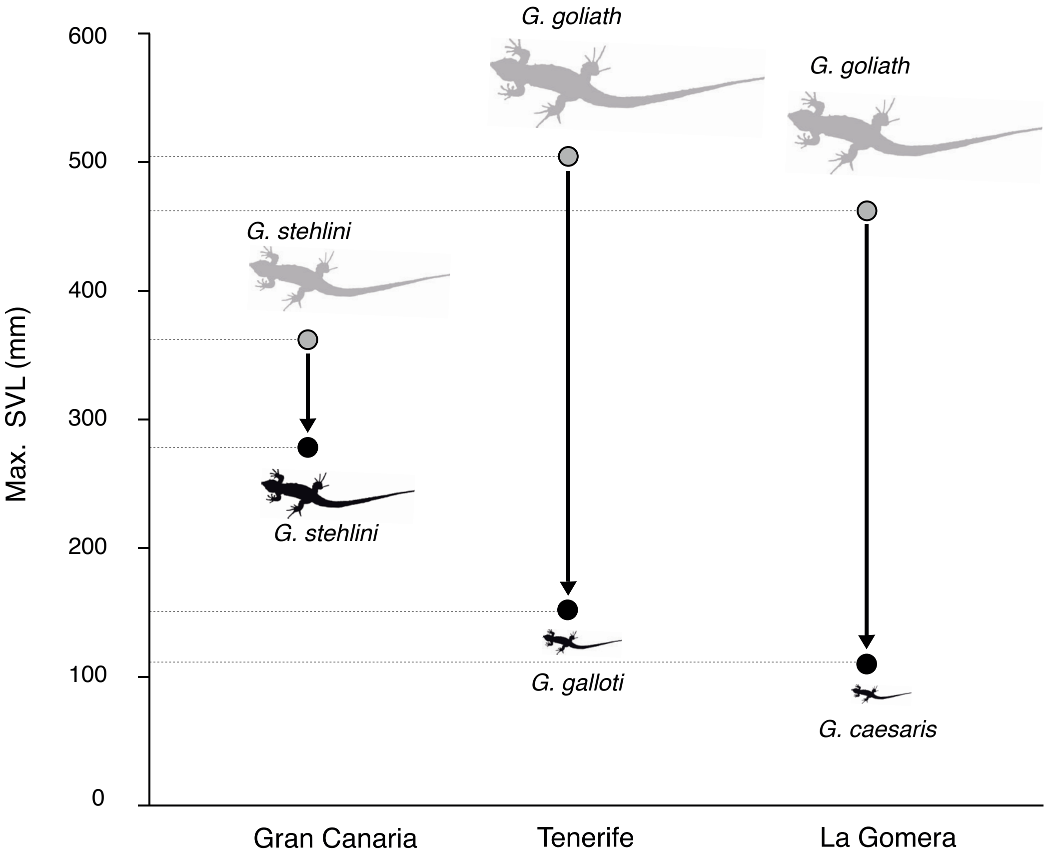

Fig. 2.1. Schematic representation of the maximum snout-vent length (max SVL) reduction of Canarian giant lizards (g. *Gallotia*, Lacertidae) from the past (light-grey silhouettes) to the present day (black silhouettes). Silhouettes are scaled to the max SVL. Only the islands hosting *Neochamaelea pulverulenta* populations are shown (see Fig. 2.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*).

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 & Richter 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 & 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 2.S1), different extinction scenarios emerged on each island generating a gradient of lizard body-size reduction (Fig. 2.1). 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. 2.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 2.S1, Fig. 2.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 2.S1, Fig. 2.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 2.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_{max}), and the mean minimum temperature of the coldest month (t_{min}). Then, we calculated the Emberger index (Emberger 1955) defined as $Q = (2000\,pp)/(t_{max}^2 - t_{min}^2)$, 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 & 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).

Demographic parameters	**Gran Canaria**	**Tenerife**	**La Gomera**
Proportion of seedlings outside canopy[1]	12.70 ± 6.79 ^{a} (9)	17.71 ± 6.58 ^{b} (13)	2.76 ± 2.12 ^{c} (10)
Effective recruitment rate of seedlings[2]	0.06 ± 0.03 ^{a} (9)	0.39 ± 0.22 ^{b} (13)	0.01 ± 0.00 ^{c} (10)
Seedling stem diameter[3] (mm)	0.74 ± 0.06 ^{a} (7)	0.47 ± 0.05 ^{b} (7)	0.81 ± 0.05 ^{a} (8)

Table 2.1. 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 binomial distribution and logit link, GLM[2] with a Poisson distribution, and log link function and LMM[3]. Non-shared, superscript letters indicate significant differences among island. Data are mean ± S.E.

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 2.S2, Fig. 2.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 2015).

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 2.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. 2.2), suggesting similar overall amount of recruitment. In turn, the average percentage of seedlings was consistently similar among islands, varying from 31.0 ± 27.8% in Tenerife to 25.1 ± 20.7% in Gran Canaria (GLM, $p > 0.05$; Fig. 2.2).

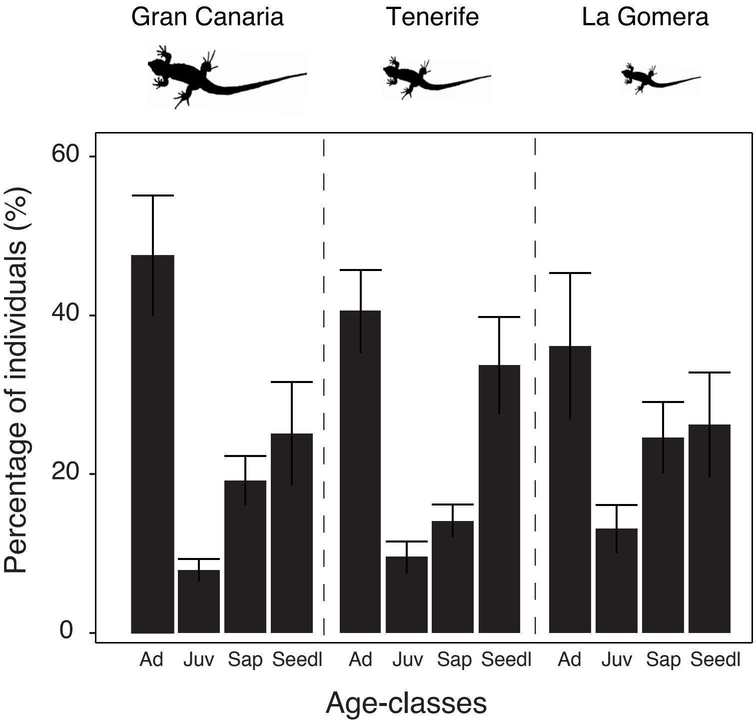

Fig. 2.2. Age-structure patterns (distribution of age classes) for the 42 analysed populations of *Neochamaelea pulverulenta* in Gran Canaria (11 populations), Tenerife, (19), and La Gomera (12). Data were pooled at the island level. Age classes: Ad (Adults), Juv (Juveniles), Sap (Saplings), Seedl (Seedlings). Bars indicate mean ± SE.

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 2.1, Fig. 2.3). 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.

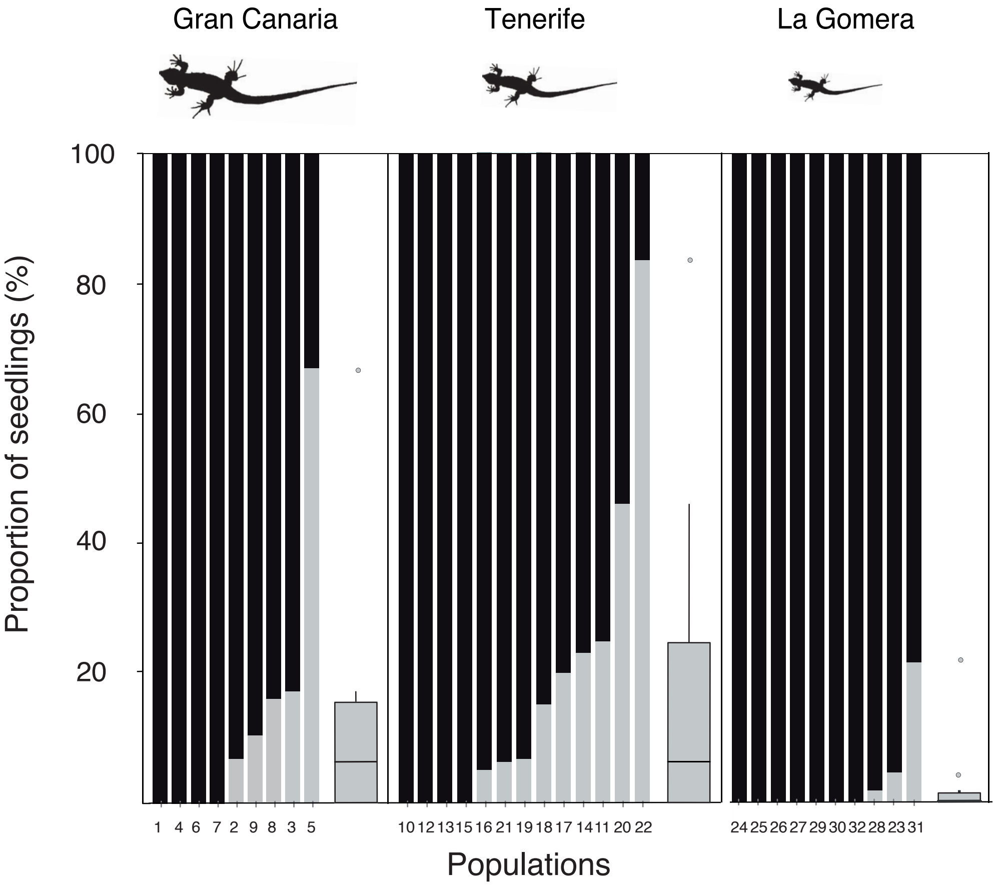

Fig. 2.3. Proportion of *Neochamaelea pulverulenta* seedlings growing outside (grey bars) and beneath the canopy (black bars). Each bar represents a sampled population, sorted within islands in decreasing order of the proportion of seedlings recruiting beneath the canopy (see Table 2.S1, Fig. 2.S2 for 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.

Regarding the effective recruitment rate of seedlings, we detected significant differences for all the possible pairwise comparisons (Table 2.1, Fig. 2.4a). 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. 2.4b). Also, we recorded a significantly lower density of lizard droppings on Gran Canaria than on Tenerife ($t = 2.58$, $p < 0.05$).

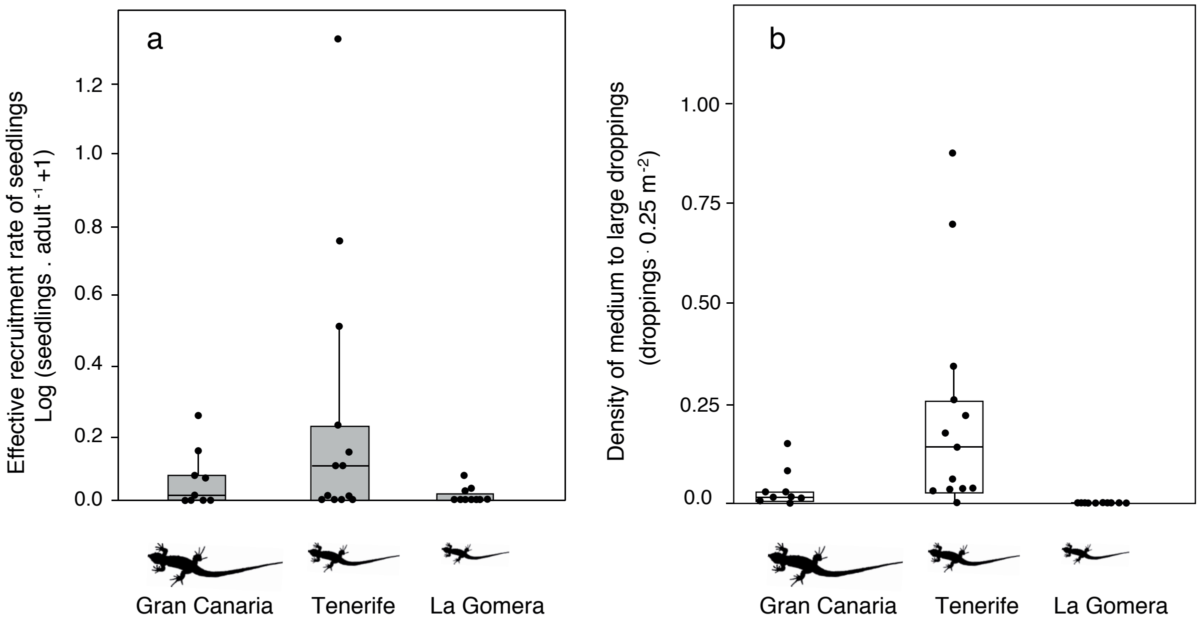

Fig. 2.4. (a) Effective recruitment rate of seedlings on each island (no. of seedlings outside the canopy . no. of adult plants^{-1}). Data are shown on the log (x+1) scale. (b) Density of medium to large droppings of lizards on each island (no. of lizard droppings . 0.25 m^{-2}). In both panels, population 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.

Finally, we found seedlings with consistently smaller basal stem diameter in Tenerife, with no differences between Gran Canaria and La Gomera (Fig. 2.5, Table 2.1). 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

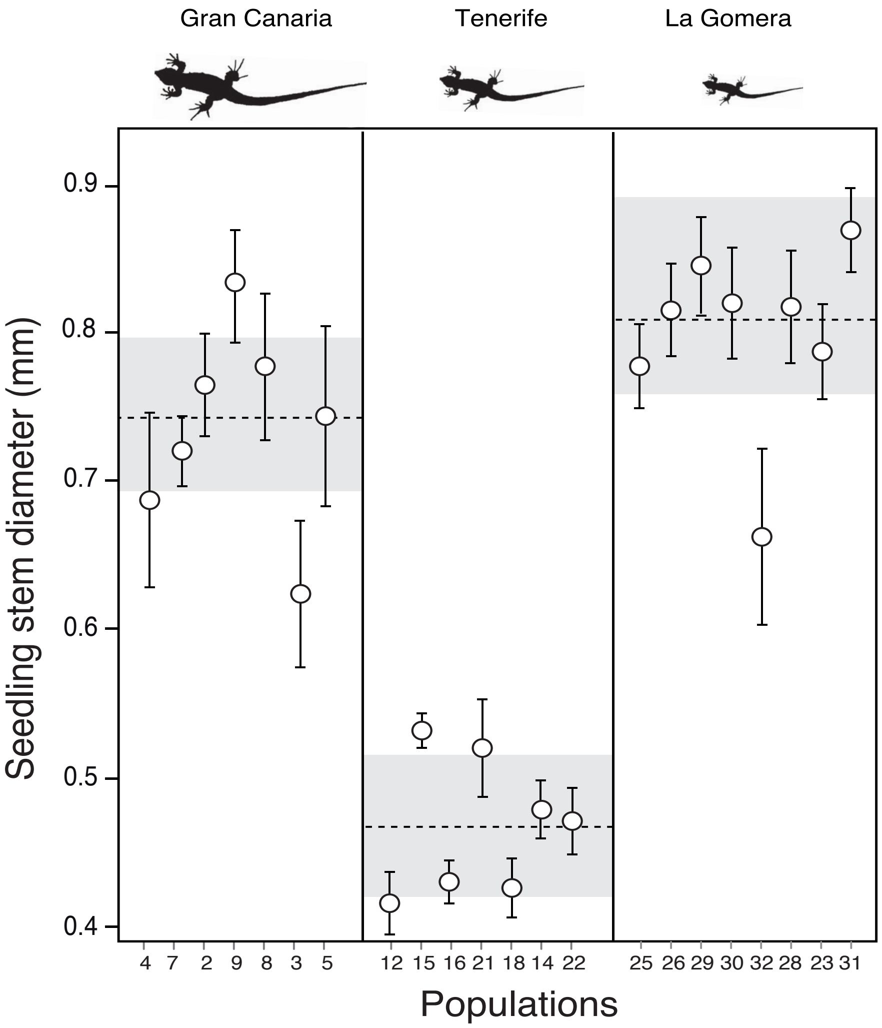

Fig. 2.5. Variation in seedling stem diameter within and among islands. Data are population mean ± SE (unfilled circles). At the island level, dotted lines and grey shadows indicate mean and SE respectively. Population codes along the abscissa are as in Table 2.S1 and Fig. 2.S2. Populations are ordered as in Fig. 2.3.

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 & Chapman 1995; Cordeiro & Howe 2003; Traveset & 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 2.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 & Howe 2003; Traveset & Riera 2005, but see Bleher & 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. 2.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 & Chapman 1995; Cordeiro & 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 & 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 & Richter 1982; Moles & 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 10000 years (Janzen & 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 & 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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Apéndices

CAPÍTULO 2: Downsized mutualisms: consequences of seed dispersers' body size reduction for early plant recruitment

APPENDIX 2.S1. NATURAL HISTORY OF THE CANARIAN LIZARDS (g. *Gallotia*).

The Canarian lacertid lizards are included into the endemic genus *Gallotia* (Arnold, 1973). Currently, it comprises seven extant species (Arechavaleta *et al*. 2010; Cox *et al*. 2010): *G. atlantica* (Peters & Doria, 1882) from Lanzarote and Fuerteventura, *G. stehlini* (Schenkel, 1901) from Gran Canaria, *G. galloti* (Oudart, 1839) from Tenerife and La Palma, *G. intermedia* (Hernández *et al*. 2000) from Tenerife, *G. caesaris* (Lehrs, 1914) from La Gomera and El Hierro, *G. bravoana* Hutterer, 1985 from La Gomera, and *G. simonyi* (Steindachner, 1889) from El Hierro (Fig. 2.S1). However, the central and western islands were inhabited in the past by other giant taxa, considered extinct nowadays: large specimens of *G. stehlini* (López-Jurado, 1985; Gran Canaria), *Gallotia simonyi* (Steindachner, 1889; Tenerife, La Gomera and La Palma), *G. goliath* (Mertens, 1942; Tenerife, La Gomera, La Palma and El Hierro), and *G. auaritae* (Mateo *et al*. 2001); La Palma) (Fig. 2.S1). The taxonomic status of these extinct taxa is certainly controversial. Some authors consider that just one giant species inhabited each of the western islands in the past (Maca-Meyer *et al*. 2003; but see Mateo *et al*. 2011). According to this, some of the recently discovered species in these islands surviving in very restricted and isolated populations i.e., *G. simonyi* (1975 in El Hierro; Böhme & Bings, 1975), *G. intermedia* (1996 in Tenerife; Hernández *et al*. 1997, 2000) and *G. bravoana* (1999 in La Gomera; Valido *et al*. 2000; Nogales *et al*. 2001) are considered as dwarf individuals of the giant taxa already described from fossil/subfossil specimens assigned sometimes as subspecies (Rando *et al*. 1997; Bischoff 1998; Barahona *et al*. 2000; Mateo *et al*. 2001, 2011). Therefore, until the taxonomic position of these extinct lizards is resolved by sequencing ancient DNA, we can say that each of these islands was inhabited in the past by giant lizards. The large-sized, recently discovered, extant species (*G. simonyi*, *G. intermedia* and *G. bravoana*) are closely related to these giant taxa, but restricted to small and isolated populations. In turn, the medium- to smaller-bodied species (*G. galloti* and *G. caesaris* respectively) are abundant and widespread within each of the western islands. The resulting downsize gradient caused by the extinction process is summarized in Fig. 2.1 and Fig. 2.S1.

The origin of this monophyletic group of lizards goes back to the Miocene (ca. 17 - 20 mya), when a common ancestor of *Psammodromus* and *Gallotia* colonized the older, easternmost islands (Lanzarote/Fuerteventura) from Southern Europe or Northwest Africa (Cox *et al*. 2010). Subsequent stepping-stone colonization and diversification processes were occurring from eastern to western islands. Therefore, in the Middle Miocene (ca. 13 - 10 mya) three genetic lineages had been already formed: *G. atlantica* in Lanzarote/Fuerteventura, *G. stehlini* in Gran Canaria and a different genetic lineage in the westernmost proto-islands. Later, during the Pliocene and Pleistocene (6 - 0.6 mya), the western lineage evolved in two subgroups in some of these proto-islands: one subgroup evolved to small- to medium-bodied lizards and another genetic group tended to giant forms. Both lineages were colonizing the rest of western islands as they were emerging and formed a new species/subspecies in each island. Thus, by the mid-Pleistocene, Tenerife, La Gomera, La Palma and El Hierro each hosted a giant lizard coexisting with a small- or medium-sized species (Cox *et al*. 2010).

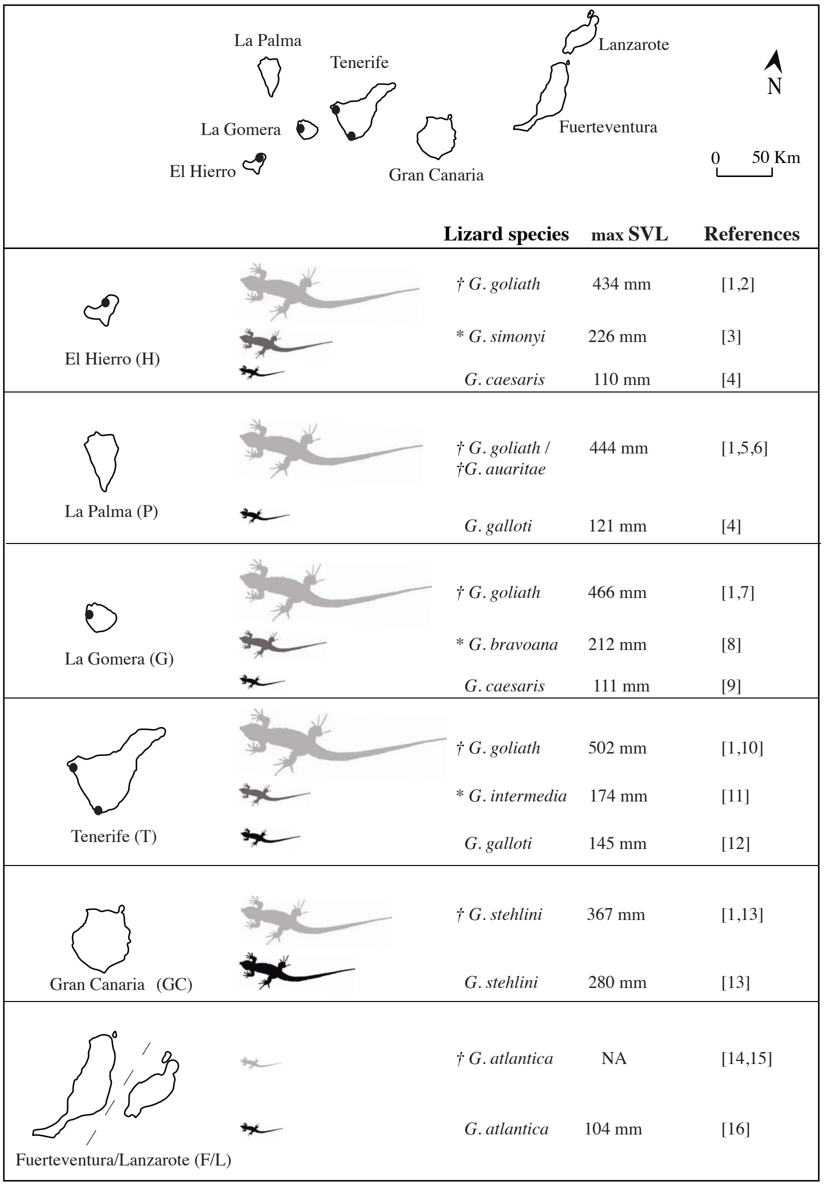
Gigantism on oceanic islands is a recurrent evolutionary pattern among vertebrates (Lomolino 2005), including lizards (Pregill 1986; Meiri 2008). Body-sizes of the *Gallotia* giant lizards are the largest (max. SVL = 502 mm) within the Larcertidae family, and they are considered a paradigmatic example of gigantism on islands (Barahona *et al*. 2000; Lomolino 2005; Whittaker & Fernández-Palacios 2007; Meiri 2008). It is likely that the absence of large predators on the Canaries and the expansion of ecological niches (e.g. to herbivorous diets) enabled lizards to reach these giant sizes and diverge from smaller species (Maca-Meyer *et al*. 2003; Cox *et al*. 2010). Giant *Gallotia* lizards were widely distributed until the arrival of the first settlers to the Canary Islands, approximately 2000-2500 yr BP (Mateo & López-Jurado 1992). The discovery of giant lizard bones on shell deposits (Alberto 1999), together with results obtained from stable isotopes analysis of aboriginal bones (Arnay-de-la-Rosa *et al*. 2010, 2011) suggest that the first human colonization marked the beginning of the decline of giant lizard populations in these islands. Therefore, subsequent to Europeans colonization ca. 500-600 yr BP, introduced predators and competitors (e.g. rats, cats, dogs and goats), the habitat destruction, and some life-history traits (e.g. low reproductive rate), finally resulted in the extinction and the body size reduction of the giant lizard species in these islands (Mateo & López-Jurado 1992).

Despite the extinction process and/or the resulting downsize of lizards occurred in all central and western islands (Fig. 2.S1), its intensity was not homogeneous among them (Fig. 2.1, Fig. 2.S1). For example, a subtle size reduction (according to max SVL recorded) took place in Gran Canaria, from 350 mm (*G. stehlini*) of extinct largest individuals to 280 mm (*G. stehlini*) of the smaller extant individuals. Tenerife suffered an intermediate reduction of lizard body sizes because it still preserves the medium-sized *G. galloti* (145 mm) after the extinction of *G. goliath* (502 mm). In contrast, the extinction process resulted in a drastic downsize of lizards in La Gomera, from 466 mm (*G. goliath*) to 111 mm (*G. caesaris*). This allowed us defining three well-contrasted eco-evolutionary scenarios, providing an interesting natural experiment to evaluate how the progressive reduction of lizard body-sizes affected the early recruitment of the large-seeded treelet *Neochamaelea pulverulenta* (Rutaceae), an endemic species whose seeds are dispersed exclusively by the large-to medium Canarian lizards (Valido 1999).

All *Gallotia* lizard species present a marked omnivorous diet, with fleshy fruits being an important component year round. Thus, they legitimately disperse seeds of many fleshy-fruited plant species in different habitats and islands (Valido & Nogales 1994; Olesen & Valido 2003; Valido *et al*. 2003; Rodríguez *et al*. 2008). Nevertheless, the importance of the vegetarian components in the diet positively covary with lizard body sizes both within- and among species. Larger lizards are more vegetarian and/or frugivores than the smaller ones (Mateo & López-Jurado 1992; Valido *et al*. 2003; Carretero *et al*. 2006). These results, and a recent paleodiet study of *G. goliath* in Tenerife (Bocherens *et al*. 2003) suggest that extinct giant species might be even more vegetarians than the extant largest species. Thus, it is assumed that these extinct lizards were also important seed dispersers of *N. pulverulenta* (Valido 1999).

FIG. 2.S1. DISTRIBUTION OF *Gallotia* (LACERTIDAE) SPECIES IN THE CANARY ISLANDS AND THEIR MAXIMUM SNOUT-VENT LENGTH (MAX. SVL).

Silhouettes are scaled to the max. SVL. Light-grey silhouettes (†) represent extinct lizards. Dark-grey silhouettes (*) represent critically-endangered species with very restricted and isolated populations (dots on the maps). Black silhouettes represent extant, widely distributed species. Max. SVL of fossil G. atlantica has not been estimated, although López-Jurado & Mateo (1992) suggest a similar length to extant conspecifics according to data reported by Michaux et al. (1991). Some authors consider the presence of other extinct taxa in these islands (see text for details).



	Lizard species	max SVL	References
El Hierro (H)	† *G. goliath*	434 mm	[1,2]
	* *G. simonyi*	226 mm	[3]
	G. caesaris	110 mm	[4]
La Palma (P)	† *G. goliath* / †*G. auaritae*	444 mm	[1,5,6]
	G. galloti	121 mm	[4]
La Gomera (G)	† *G. goliath*	466 mm	[1,7]
	* *G. bravoana*	212 mm	[8]
	G. caesaris	111 mm	[9]
Tenerife (T)	† *G. goliath*	502 mm	[1,10]
	* *G. intermedia*	174 mm	[11]
	G. galloti	145 mm	[12]
Gran Canaria (GC)	† *G. stehlini*	367 mm	[1,13]
	G. stehlini	280 mm	[13]
Fuerteventura/Lanzarote (F/L)	† *G. atlantica*	NA	[14,15]
	G. atlantica	104 mm	[16]

[1] Barahona et al. 2000; [2] Izquierdo et al. 1989; [3] Rodriguez-Dominguez et al. 1998; [4] Valido, A. (personal observation); [5] Bravo 1953; [6] Mateo et al. 2001; [7] Hutterer 1985; [8] Hernandez-Divers et al. 2003; [9] Molina-Borja & Rodriguez-Dominguez 2004; [10] Mertens 1942; [11] Martín, A. (personal communication); [12] Martín 1985; [13] Mateo & López-Jurado 1992; [14] Michaux et al. 1991; [15] Lopez-Jurado & Mateo 1995; [16] Marquez et al. 1997

FIG. 2.S2. MAP SHOWING *N. pulverulenta* POPULATIONS SAMPLED IN THIS STUDY FROM GRAN CANARIA, TENERIFE, AND LA GOMERA.

Population codes are ordered from the easternmost to the westernmost island, and clockwise ordered within island. The codes correspond to those shown in the Table 2.S1. The potential geographic distribution of *N. pulverulenta* (dotted lines) was modified from Atlantis 3.1 (Gobierno de Canarias. Banco de Datos de Biodiversidad de Canarias; http//: www.biodiversidadcanarias.es/atlantis/).

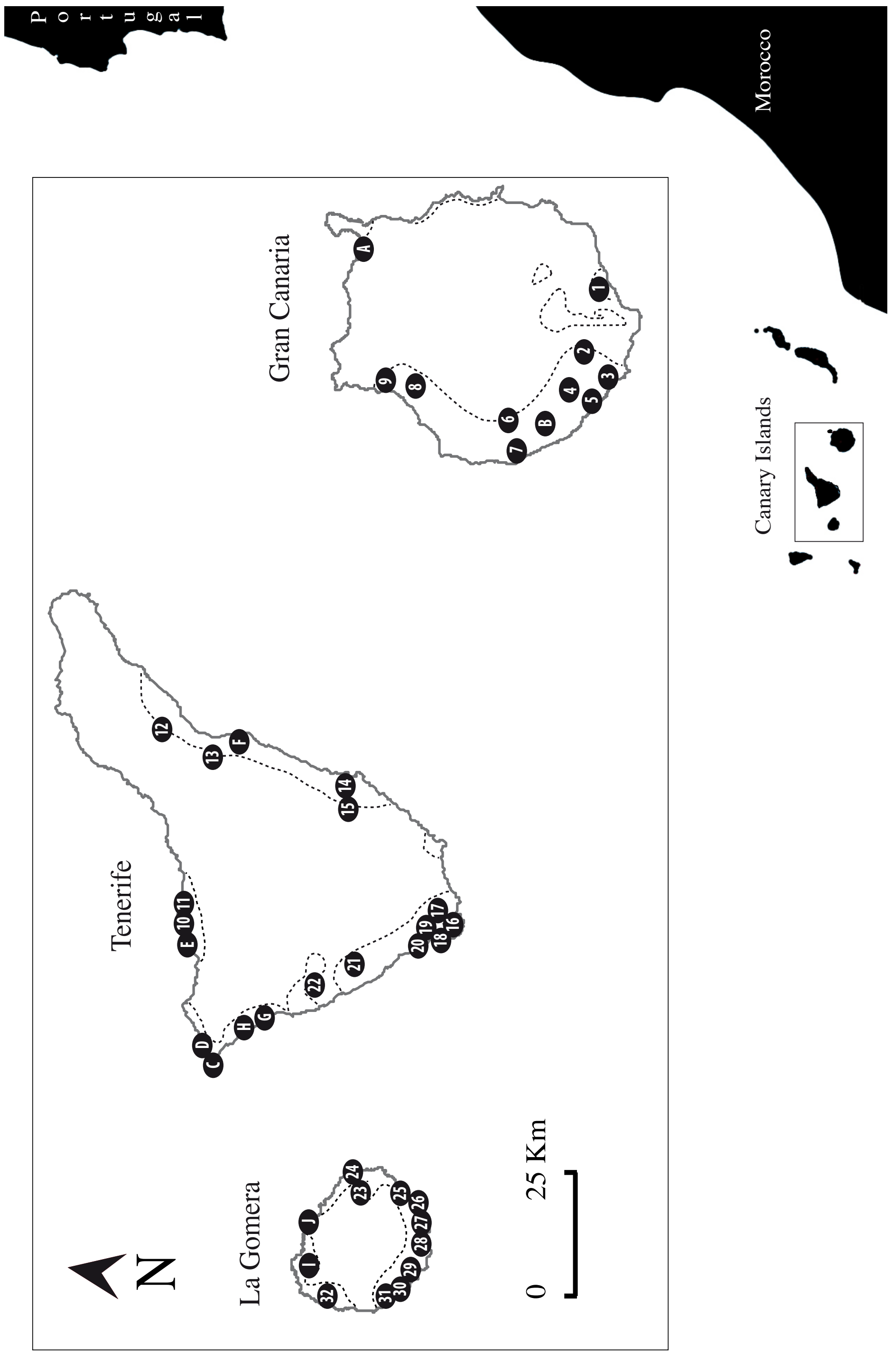


TABLE 2.S1. INFORMATION ABOUT THE 42 SAMPLED POPULATIONS OF *Neochamaelea pulverulenta* FROM THE CANARY ISLANDS.

The population codes match with those indicated in the Fig. 2.S2. Populations with letter codes were sampled only for age structure but not for the other demographic variables. Populations with asterisk (*) indicate the presence of G. galloti but also G. intermedia in the vicinity. The Emberger index is log-transformed (-*lnQ*). The number of individuals of each age class sampled in the populations, and the number of seedlings growing outside the canopy (within brackets) are shown. (d) indicates populations where at least 6 seedlings were measured (stem diameter).

Code	Population	Island	Lizard species	Latitude (N)	Longitude (W)	-*lnQ*	Adults	Juveniles	Saplings	Seedlings
1	Montaña de Tabaiba	Gran Canaria	*G. stehlini*	27°48'08"	15°31'04"	-5.9	88	10	2	1 (0)
2	Ayagaures	Gran Canaria	*G. stehlini*	27°48'36"	15°37'09"	-5.6	55	17	30	67 (4) d
3	Barranco de La Verga	Gran Canaria	*G. stehlini*	27°46'50"	15°41'42"	-5.9	61	11	35	6 (1) d
4	Barranco de Tauro	Gran Canaria	*G. stehlini*	27°49'07"	15°43'08"	-5.8	64	3	34	11 (0) d
5	Barranco de Tiritaña	Gran Canaria	*G. stehlini*	27°48'58"	15°44'36"	-5.7	79	5	16	33 (22) d
6	Barranco de Tasarte	Gran Canaria	*G. stehlini*	27°56'06"	15°45'12"	-6.1	47	8	54	121 (0)
7	Barranco de Güigüi	Gran Canaria	*G. stehlini*	27°56'01"	15°49'14"	-5.8	23	18	65	108 (0) d
8	Tirma	Gran Canaria	*G. stehlini*	28°02'05"	15°45'23"	-6.5	73	4	32	33 (5) d
9	La Laja del Risco	Gran Canaria	*G. stehlini*	28°03'33"	15°43'44"	-6.5	65	31	25	100 (10) d
A	Barranco de Tamaraceite	Gran Canaria	*G. stehlini*	28°07'31"	15°27'36"	-6.4	98	20	16	1
B	Barranco de Veneguera	Gran Canaria	*G. stehlini*	27°51'43"	15°46'21"	-5.7	516	96	175	NA
10	Punta de Juan Centella	Tenerife	*G. galloti*	28º23'32"	16º41'38"	-6.9	112	23	19	4 (0)
11	Charco de la Arena	Tenerife	*G. galloti*	28°23'58"	16°40'08"	-6.9	228	113	75	8 (2)
12	Tabaiba Alta	Tenerife	*G. galloti*	28°24'14"	16°20'21"	-6.9	56	11	41	76 (0) d
13	La Hidalga	Tenerife	*G. galloti*	28°19'53"	16°23'34"	-6.7	72	8	28	106 (0)
14	Abades	Tenerife	*G. galloti*	28°08'23"	16°27'24"	-6.4	73	12	10	47 (11) d
15	Arico	Tenerife	*G. galloti*	28°09'04"	16°28'17"	-6.4	35	5	42	131 (0) d
16	Malpaís Punta de Rasca	Tenerife	*G. galloti*	28º00'42"	16º41'54"	-6.0	54	2	3	272 (13) d
17	El Palm-Mar	Tenerife	*G. galloti*	28°01'23"	16°41'30"	-6.0	96	24	17	5 (1)
18	Montaña de Guaza I*	Tenerife	*G. galloti*	28°01'59"	16°42'26"	-5.9	31	5	24	511 (87) d
19	Montaña de Guaza II*	Tenerife	*G. galloti*	28°02'08"	16°42'29"	-5.9	29	13	13	45 (3)
20	Montaña de Guaza III*	Tenerife	*G. galloti*	28°02'54"	16°42'03"	-5.9	106	12	26	25 (11)
21	Los Menores	Tenerife	*G. galloti*	28°08'42"	16°45'26"	-6.5	23	14	109	247 (15) d
22	Barranco Guía de Isora	Tenerife	*G. galloti*	28°11'34"	16°46'22"	-6.5	67	15	44	90 (75) d
C	Teno Bajo I	Tenerife	*G. galloti*	28°21'08"	16°54'43"	-6.6	98	15	7	0
D	Teno Bajo II	Tenerife	*G. galloti*	28°21'13"	16°54'33"	-6.6	50	22	61	90
E	Cueva del Rey	Tenerife	*G. galloti*	28°22'59"	16°42'42"	-6.9	67	36	78	17
F	Malpaís de Güímar	Tenerife	*G. galloti*	28°18'37"	16°22'22"	-6.2	54	5	8	50
G	Barranco de Masca*	Tenerife	*G. galloti*	28°17'35"	16°50'56"	NA	83	37	33	28
H	Barranco Los Carrizales*	Tenerife	*G. galloti*	28°19'12"	16°52'17"	NA	97	17	12	24
23	Barranco de La Sabina	La Gomera	*G. caesaris*	28°07'54"	17°07'28"	-6.6	36	44	25	22 (1) d
24	Punta Llana	La Gomera	*G. caesaris*	28º07'41"	17º06'17"	-6.6	438	75	25	12 (0)
25	Roque de la Roja	La Gomera	*G. caesaris*	28°03'59"	17°09'11"	-6.4	20	10	72	125 (0) d
26	Barranco de Chinguarime	La Gomera	*G. caesaris*	28°02'31"	17°10'47"	-6.2	10	29	74	80 (0) d
27	Barranco Biguillo	La Gomera	*G. caesaris*	28°02'37"	17°11'03"	-6.2	82	21	7	30 (0)
28	Juan Barba	La Gomera	*G. caesaris*	28°02'11"	17°13'47"	-6.7	29	33	48	59 (1) d
29	La Caldera	La Gomera	*G. caesaris*	28°02'06"	17°16'00"	-6.6	26	11	64	88 (0) d
30	Barranco de la Negra	La Gomera	*G. caesaris*	28°03'04"	17°16'51"	-6.4	7	1	225	527 (0) d
31	Lomo Gerián	La Gomera	*G. caesaris*	28°04'28"	17°18'48"	-6.2	39	4	57	14 (3) d
32	Lomo del Balo	La Gomera	*G. caesaris*	28°09'21"	17°18'43"	-7.1	25	36	50	18 (0) d
I	Barranco de La Era Nueva	La Gomera	*G. caesaris*	28°11'01"	17°16'36"	-7.3	82	14	4	0
J	Cerro del Cepo	La Gomera	*G. caesaris*	28°11'51"	17°12'11"	-7.2	85	4	11	0

APPENDIX 2.S2. STATISTICAL ANALYSES

Several sampled populations of N. pulverulenta were geographically close, thus being subjected to similar biotic/abiotic conditions. This may result in similar values of the demographic variables, violating the required independence of data to properly apply generalized linear models. Thus, and as a complement of the performed analyses, we also tested for spatial autocorrelation among the residuals of the models used (the age structure patterns, the proportion of seedlings outside the canopy and the effective recruitment rate). Given that we did not record the geographic position of each sampled seedling (sample unit) we could not perform this analysis for the "seedling stem diameter" model (LMM). As an alternative, we pooled the individual data at the population level (population mean), performed a linear regression (LM) with "island" as a fixed factor and Emberger index as a covariate and we tested for spatial autocorrelation.

Spatial autocorrelation was assessed by computing multivariate Mantel correlograms following Matesanz *et al*. (2011). We tested at different distance classes including all pairs of populations located at a certain distance from each other (9 km among distance classes). This distance interval was selected in order to include a sufficient number of populations in the first distance class. We did not detect spatial autocorrelation for any of the fitted models (*p-values* > 0.05 after a progressive Bonferroni correction), thus geographic information of plant populations was not included as covariates. The results of these analyses are shown in Fig. 2.S3.

All analyses were carried out with R (R Development Core Team 2015). Specifically we used nlme (for GLM and LMM models; Pinheiro *et al*. 2014), multcomp (post-hoc Tukey tests; Hothorn *et al*. 2008) and mpmcorrelogram packages (Mantel correlograms; Matesanz *et al*. 2011).

FIG. 2.S3. RESULTS OF THE MULTIVARIATE MANTEL CORRELOGRAMS USING THE MANTEL STATISTIC (rM).

Empty squares indicate non-significant spatial autocorrelation at each distance class (9 km) for the dependent variables examined (see text for details): a) the proportion of seedlings relative to the other age classes, b) the proportion of pooled subadults (seedlings, saplings and juveniles) relative to the proportion of adults, c) the proportion of seedlings outside the canopy relative to the proportion of seedlings beneath the canopy, d) the effective recruitment rate of seedlings (i.e. the number of seedlings outside the canopy relative to the number of adults sampled), e) the averaged stem diameter at the population level.

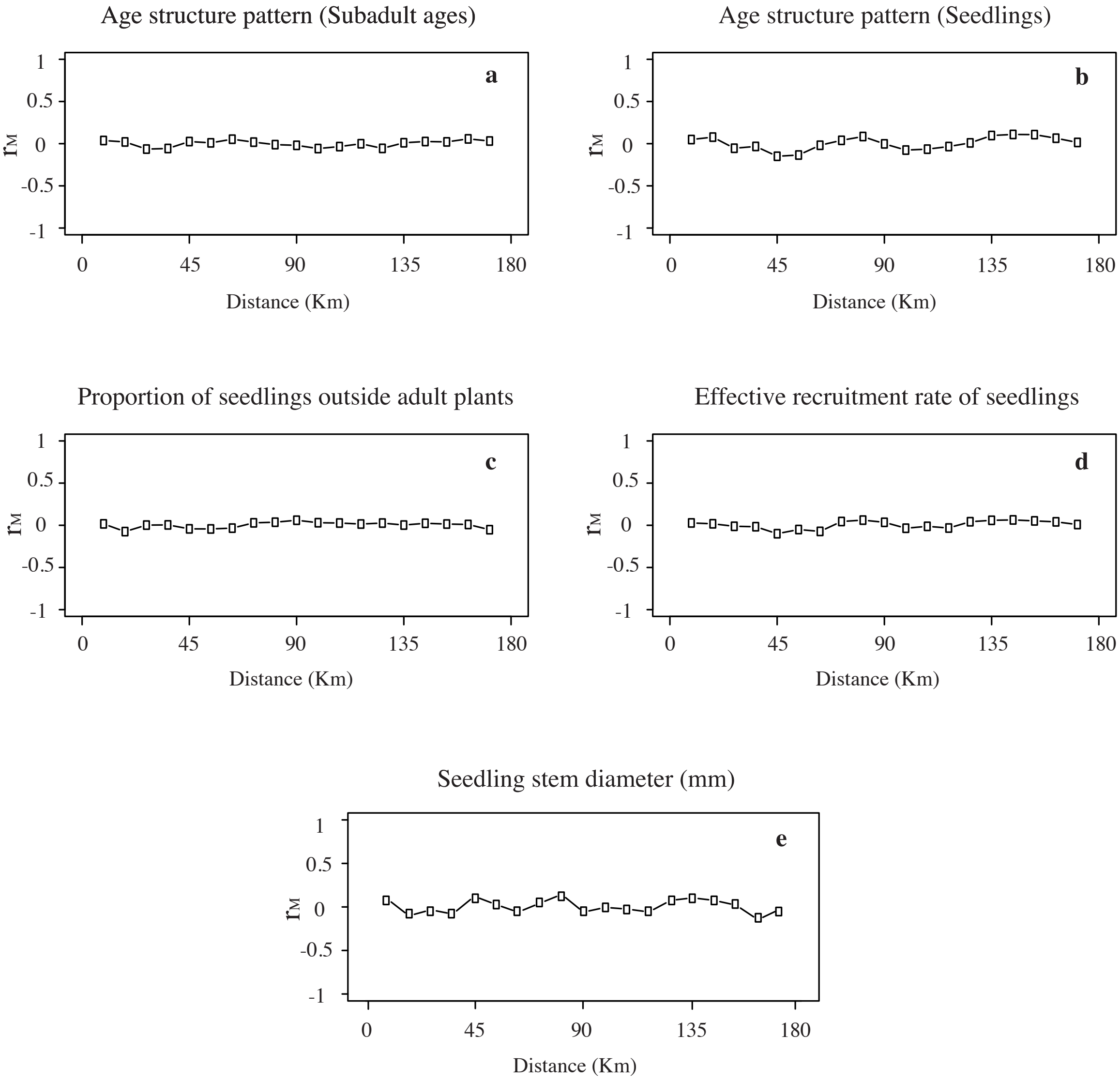


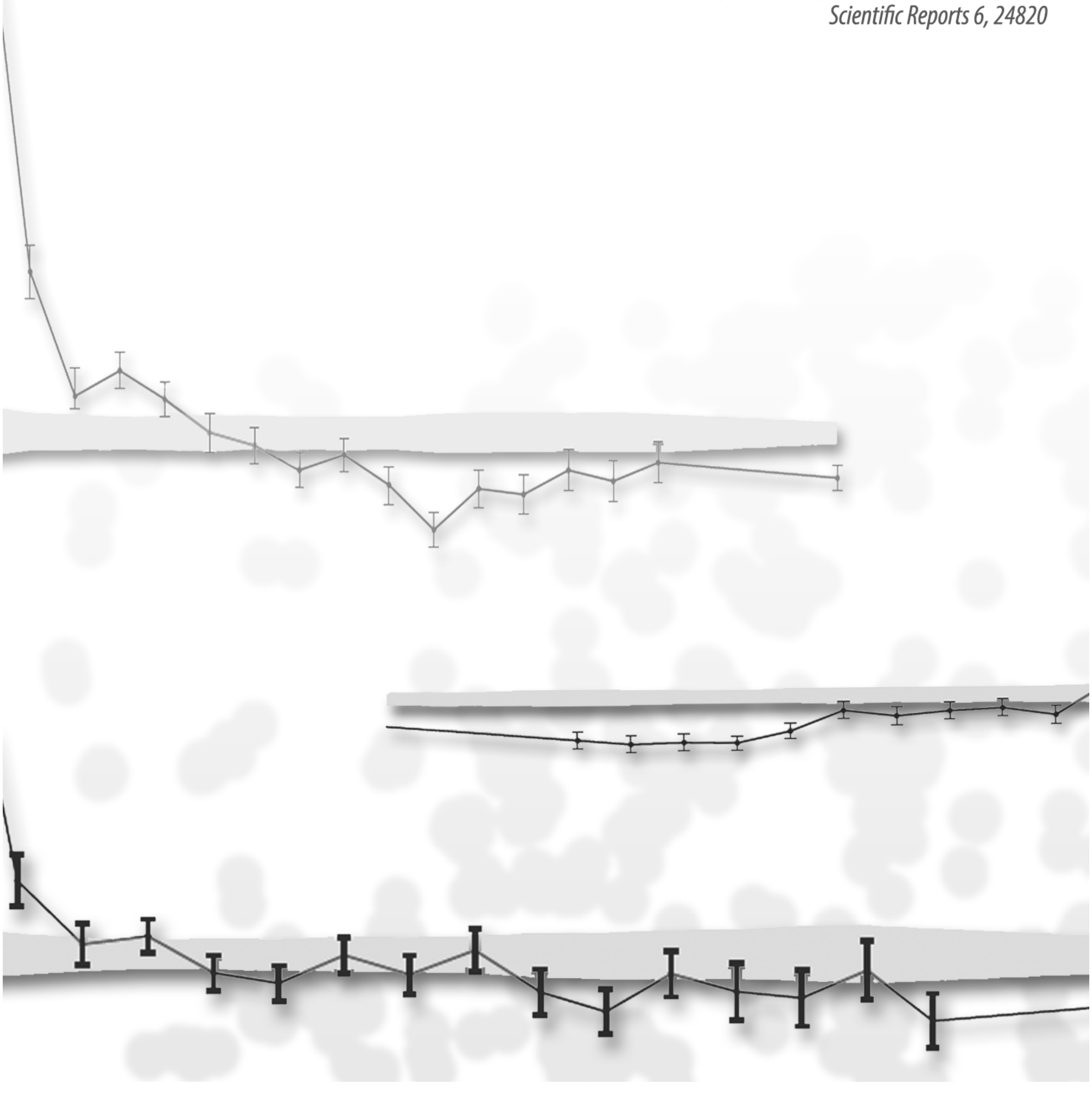
FIG. 2.S4. PHOTO-COLLAGE ILLUSTRATING THE STUDIED SYSTEM.

A) *Gallotia stehlini* (Ad male), the extant largest species of lizards from Gran Canaria (photo: P. Jordano). B) Medium-sized lizard *G. galloti* (Ad male) from Tenerife (photo: C. Camacho). C) Lowland xerophytic schrubland (Teno Bajo, Tenerife, photo: P. Jordano). D) *Neochamaelea pulverulenta* with ripe fleshy fruits (photo: A. Valido). E) Ripe fruits of *N. pulverulenta* (photo: A. Valido). F) Depulped seeds accumulating beneath the canopy of *N. pulverulenta* from La Gomera (photo: N. Pérez-Méndez). G) *G. galloti* droppings with dispersed seeds of *N. pulverulenta* from Tenerife (photo: N. Pérez-Méndez).

CAPÍTULO 3

The signatures of Anthropocene defaunation: cascading effects of the seed dispersal collapse

Pérez-Méndez N., Jordano P., García C., Valido A. (2016)
Scientific Reports 6, 24820



Abstract

Anthropogenic activity is driving population declines and extinctions of large-bodied fruit-eating animals worldwide. Loss of these frugivores is expected to trigger negative cascading effects on plant populations if remnant species fail to replace the seed dispersal services provided by the extinct frugivores. A collapse of seed dispersal may not only affect plant demography (i.e., lack of recruitment), but should also supress gene flow via seed dispersal. Yet little empirical data still exist demonstrating the genetic consequences of defaunation for animal-dispersed plant species. Here, we first document a significant reduction of seed dispersal distances along a gradient of human-driven defaunation, with increasing loss of large- and medium-bodied frugivores. We then show that local plant neighbourhoods have higher genetic similarity, and smaller effective population sizes when large seed dispersers become extinct (i.e., only small frugivores remain) or are even partially downgraded (i.e., medium-sized frugivores providing less efficient seed dispersal). Our results demonstrate that preservation of large frugivores is crucial to maintain functional seed dispersal services and their associated genetic imprints, a central conservation target. Early signals of reduced dispersal distances that accompany the Anthropogenic defaunation forecast multiple, cascading effects on plant populations.

Keywords.-Extinction; downsizing; lizard; long-distance dispersal; spatial genetic structure; genetic neighbourhood

INTRODUCTION

A myriad of vertebrate species have experienced population declines and eventual extinctions matching human expansion, i.e. the "Anthropocene defaunation" (Barnosky *et al.* 2011; Dirzo *et al.* 2014) Large-bodied species have been especially hardly hit, and as a result, many disturbed ecosystems currently host only small- to medium-bodied species (Peres & Dolman 2000; Hansen & Galetti 2009). If the remnant, extant species fail to provide pivotal services formerly assisted by vanishing large vertebrates, human-driven defaunation may trigger negative cascading effects on ecosystem dynamics (Duffy 2003; Estes *et al.* 2011; Brodie *et al.* 2014).

Large frugivores provide essential seed dispersal services to the plants they feed upon by the large proportions of seeds they disperse over long distances (Jordano *et al.* 2007; Bueno *et al.* 2013; Vidal *et al.* 2013). Thus, extinction of these larger species is expected to limit the extent of gene flow and, by doing so, to impact the spatial distribution of plant genetic variation (Hamrick *et al.* 1993; Sork & Smouse 2006). These effects are of paramount importance because the magnitude and distribution of the genetic variation within and among plant populations determine their evolutionary potential and the ability to cope with environmental disturbances (Hughes & Stachowicz 2004; Kremer *et al.* 2012).

Here we focus on a compelling case study where human-driven defaunation led to a significant downsizing of seed dispersers. Downsizing results from non-random extinction of large-bodied species or from the loss of large-bodied individuals within populations (Estes *et al.* 2011; Dirzo *et al.* 2014). We examine whether the downsizing of frugivorous lizards (*Gallotia*, Lacertidae) in the Canary Islands has progressively impaired their seed dispersal services. We further test if this process had a distinct signal on the fine-scale spatial genetic structure of *Neochamaelea pulverulenta* (Rutaceae), a plant species that relies exclusively on these lizards for dispersal (Valido & Nogales 1994). Large-bodied *Gallotia* lizards were abundant in the past but the arrival of first settlers (~ 2500 BP; see Material and Methods) unleashed a lizard defaunation process resulting in a marked, present-day gradient of seed disperser downsizing across islands (Barahona *et al.* 2000). Gran Canaria still preserves sizeable populations of a large-bodied species, *G. stehlini*, a setting close to the original situation; Tenerife hosts abundant populations of the medium-sized *G. galloti*; whereas La Gomera only has an abundant, small-sized lizard, *G. caesaris* (Fig. 3.1; see Material and Methods). Despite the high specificity of the mutualistic interaction we address in this study, we aim to document effects of frugivore downsizing which are generally relevant for more diversified assemblages characterized by limited functional redundancy among the mutualistic frugivores (McConkey & Brockelman 2011; Bueno *et al.* 2013)

We hypothesized that the reduction in frugivore body size would result in reduced dispersal distances. We further expected that these reductions would strengthen the fine-scale spatial genetic structure of the plants and, consequently, reduce effective population sizes mirroring the downsizing pattern. Our study sheds light on the (mis)functioning of increasingly defaunated ecosystems, ratcheting downwards in ecological and genetic diversity through the extinction of ecological interactions (Janzen 1974; Brodie *et al.* 2014; Valiente-Banuet *et al.* 2015).

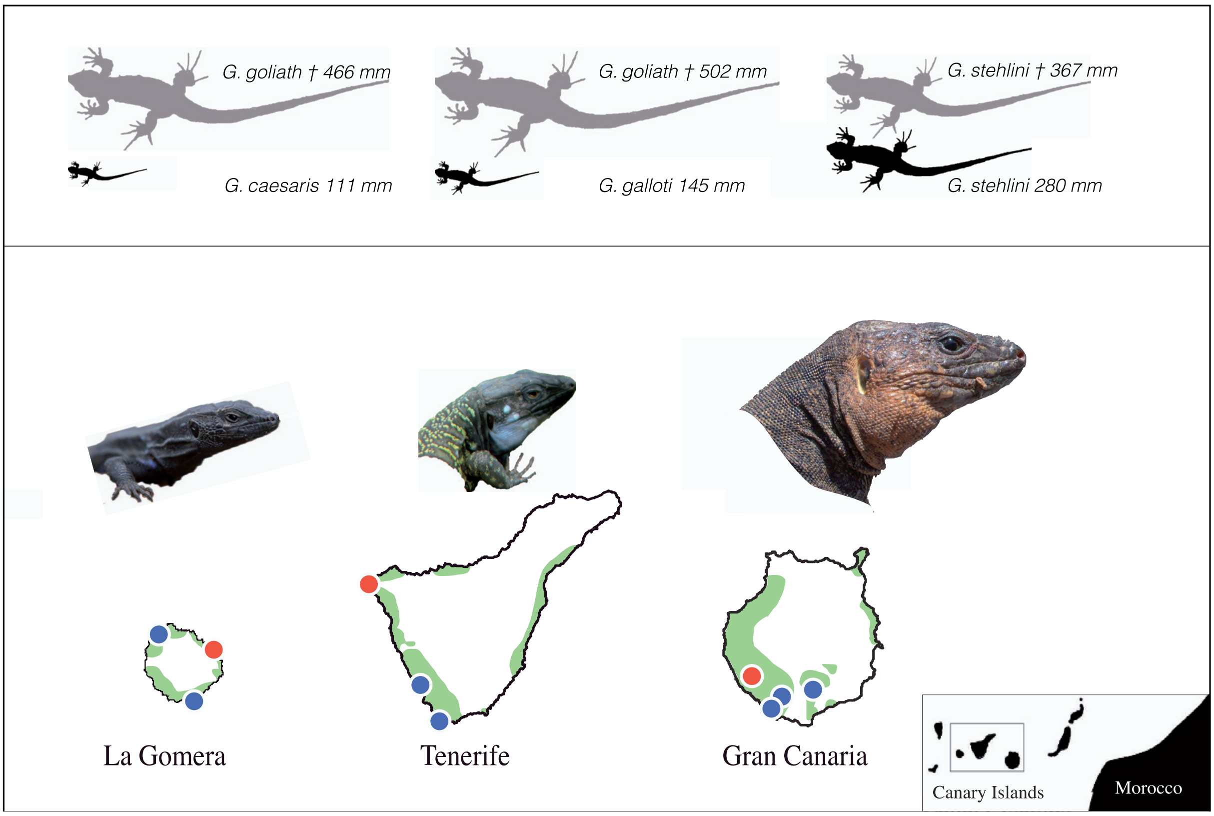


Fig. 3.1. The extinction-driven lizard downsizing gradient in the Canary Islands. Schematic representation of the human-driven lizard defaunation resulting in present- day variable frugivore body-sizes among islands. *Neochamaelea pulverulenta* (Rutaceae) relies exclusively on the lizards for seed dispersal and is only found in lowland areas of Gran Canaria, Tenerife, and La Gomera (geographic range shown in green) (Fig. 3.S4). Grey silhouettes illustrate extinct lizard taxa (†); black silhouettes represent the three extant, widely distributed species (photos). The maximum snout-to-vent length (SVL) is indicated. Red dots indicate locations of the main 1-ha study plots, whereas the blue ones indicate the replicated study populations (see Table 3.S1). The potential geographic distribution of *N. pulverulenta* (green) was redrawn from ATLANTIS 3.1 (available on line, Banco de Datos de Biodiversidad de Canarias; http://www.biodiversidadcanarias.es/atlantis/). For details about natural history of these lizard species see also Barahona *et al.* 2000 and Pérez-Méndez *et al.* 2015.

RESULTS AND DISCUSSION

To assess the consequences of lizard downsizing, we examined the extent of contemporary seed-dispersal distances (i.e., the linear distance between a dispersed seed and its assigned maternal plant) and effective-dispersal distances (i.e., distances between established juveniles and saplings, and their inferred maternal plants). Descriptors of seed dispersal distances (median, maximum) and frequency of long distance dispersal events (LDD hereafter; events > 30 m- 95th percentile) decreased significantly with the reduction of lizard body size ($P< 0.01$ for all comparisons among islands; Table 3.1). Similarly, the frequency of immigration events (i.e., seeds not from maternal plants within the plots) decreased in parallel to the reductions of lizard body size across islands (Table 3.1). In contrast, the frequency of short-distance dispersal (SDD hereafter; events < 5 m- 20th percentile- a distance threshold three times the canopy size of adult plants) significantly increased with lizard downsizing. These among islands differences are not attributable to differences in the spatial distribution of either individual plants or seed sampling points among plots (Fig. 3.S1). The longest dispersal distance events were detected in the population hosting the largest lizards (*G. stehlini*, Gran Canaria, Fig. 3.2; Table 3.1). In turn, the populations in Tenerife, and especially in La Gomera, showed an over-representation of SDD events assisted by the smaller-bodied *G. galloti* and *G. caesaris*, respectively, lacking LDD and immigration events in the latter (Fig. 3.2; Table 3.1). Furthermore, the effective dispersal distances also showed a similar trend to those of dispersed seeds (Fig. 3.S2). These effects were apparent even though by incorporating sub-adults we are including dispersal events from an unknown number of reproductive episodes. Thus, these effective dispersal patterns (Fig. 3.S2) are the result of a continuous history of seed dispersal and subsequent successful establishment in each population.

Population parameters	**Gran Canaria (Veneguera)**	**Tenerife (Teno)**	**La Gomera (Punta Llana)**
Median seed dispersal distance (m)	10.8 (10.7 ± 0.8) ^{a}	6.4 (6.2 ± 0.5) ^{b}	0.5 (0.5 ± 0.05) ^{c}
Maximum seed dispersal distance (m)	94.2 (84.3 ± 12.1) ^{a}	46.4 (37.1 ± 10.2) ^{b}	4.5 (3.6 ± 1.2) ^{c}
% dispersal events < 5 m (SDD)	17.2 (17.2 ± 2.5) ^{a}	41.6 (41.3 ± 2.8) ^{b}	100 (100 ± 0.0) ^{c}
% dispersal events > 30 m (LDD)	7.1 (7.4 ± 1.6) ^{a}	0.3 (0.4 ± 0.4) ^{b}	0 (0 ± 0.0) ^{c}
% immigration (plot)	19.1 (19.2± 2.3) ^{a}	11.4 (11.4 ± 1.7) ^{b}	0 (0 ± 0.0) ^{c}
% immigration (buffer)	14.7 (14.7± 2.1) ^{a}	4.6 (5.0 ± 1.2) ^{b}	0 (0 ± 0.0) ^{c}
Expected Heterozygosity (*uHe*)	0.64 ± 0.06	0.62 ± 0.06	0.64 ± 0.06
Observed Heterozygosity (*Ho*)	0.59 ± 0.06	0.60 ± 0.05	0.61 ± 0.06
Effective number of alleles (*Ae*)	3.5 ± 0.49	3.4 ± 0.54	4.0 ± 0.8
Allelic Richness (*AR*)	10.2 ± 1.6	10.2 ± 1.0	10.8 ± 1.4
Effective population size (*Ne/N*)	0.38 (0.35 - 0.42)	0.20 (0.18 - 0.22)	0.29 (0.27 - 0.32)

Fig. 3.2 Seed dispersal patterns of *Neochamaelea pulverulenta*. Frequency distributions of seed dispersal distances (2-m bins). Vertical marks along the distance axis represent unique documented dispersal events. We included a non-parametric smoothing spline fit (black line) to the empirical distance distribution together with bootstrapped estimates (grey lines) to allow comparisons across plots. Right inset bars indicate the percentage of seed immigration from plants growing outside the plots (white) and when plants growing in the plot buffer area were also considered (black) (see Material and Methods and Table 3.1 for sample sizes of dispersed seeds; maps shown in Figs. 3.S5 and 3.S6).

Differences among islands in the seed dispersal distances inferred from the 1-ha study plots are also generalizable up to the island scale, given the results from the additional replicated populations in each island (Table 3.S1). Frequencies of SDD events were consistently and significantly higher in populations hosting small- (La Gomera) to medium-sized (Tenerife) lizards when compared with populations hosting large-sized lizards (Gran Canaria) (Table 3.S1). Additionally, a previous demographic study encompassing 42 *N. pulverulenta* populations throughout the three islands (Pérez-Méndez *et al.* 2015) showed significantly reduced seedling recruitment >1 m away from the maternal canopy mirroring this lizard downsizing gradient.

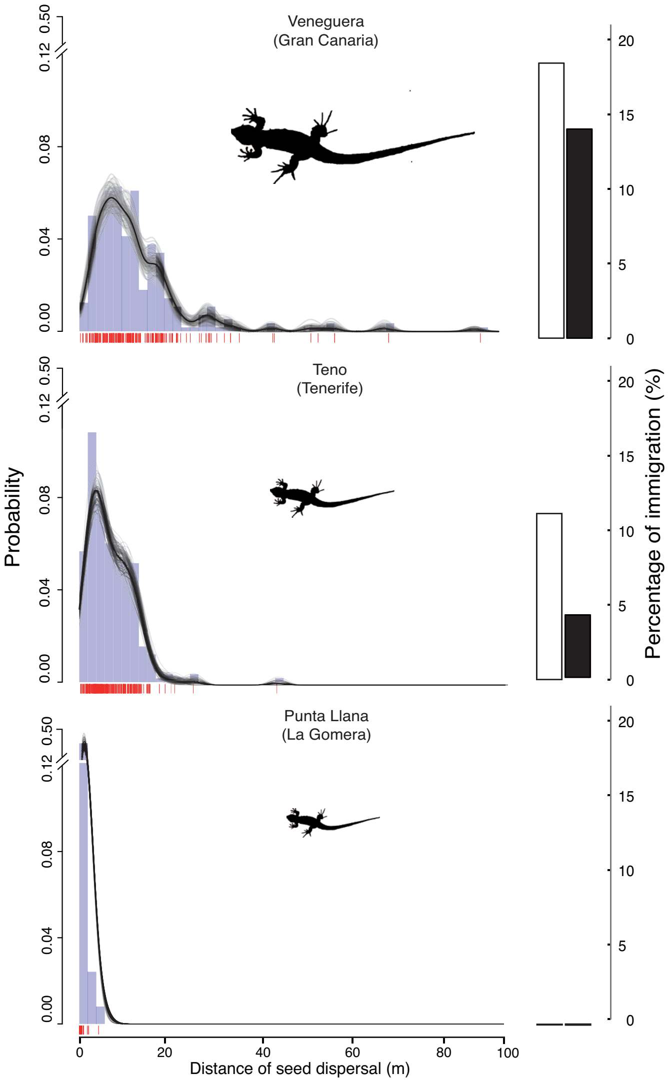


Fig. 3.2 Seed dispersal patterns of *Neochamaelea pulverulenta*. Frequency distributions of seed dispersal distances (2-m bins). Vertical marks along the distance axis represent unique documented dispersal events. We included a non-parametric smoothing spline fit (black line) to the empirical distance distribution together with bootstrapped estimates (grey lines) to allow comparisons across plots. Right inset bars indicate the percentage of seed immigration from plants growing outside the plots (white) and when plants growing in the plot buffer area were also considered (black) (see Material and Methods and Table 3.1 for sample sizes of dispersed seeds; maps shown in Figs. 3.S5 and 3.S6).

Variation in seed-dispersal and effective-dispersal distance distributions likely reflect contrasted movement patterns associated with the larger body size of lizards in Gran Canaria relative to the Tenerife and La Gomera species. Body size has been highlighted as an important trait positively co-varying with lizard home range area (Turner *et al.* 1969; Perry & Garland 2002). In addition, a number of studies have shown that these differences in lizard home range sizes translate into variation of seed dispersal distances

(Santamaría *et al.* 2007; Piazzon *et al.* 2012; Rodríguez-Pérez *et al.* 2012). The documented collapse of seed- and effective-dispersal distances in La Gomera relates not only to the reduced home range of *G. caesaris*, but also to the mismatch between the oversized fruits (see Material and Methods) and the lizards size (Fig. 3.1). Thus, only sporadic short-distance dispersal events still remain in this population, where the lizards just remove the fruit pulp and fail to ingest and disperse seeds.

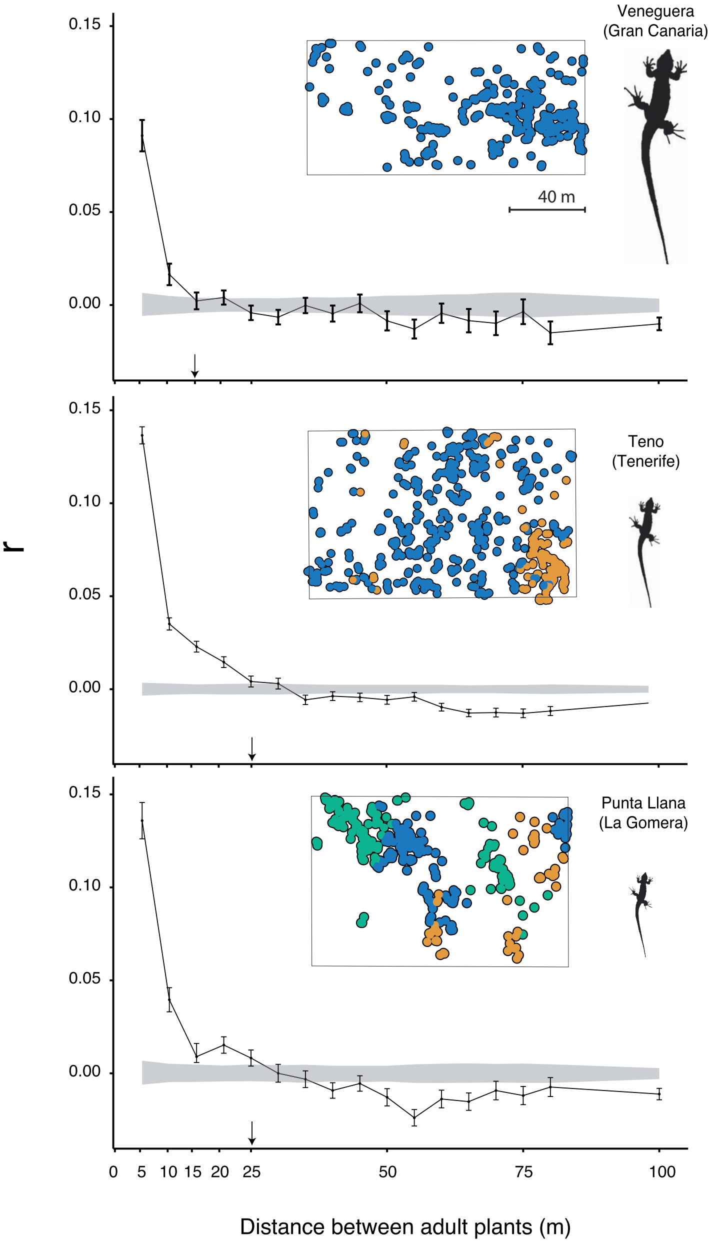


Fig. 3.3 Spatial patterns of fine scale genetic structure of *Neochamelea pulverulenta*. Autocorrelograms showing the variation of genetic similarity (rij) with geographic distance among pairs of adult plants (see Fig. 3.S3 for results with sub-adults). Grey areas represent the 95% confidence intervals for the null hypothesis calculated by permutations of the plant spatial coordinates. Arrows indicate the first distance class at which the spatial autocorrelation becomes non-significant. Insets show the 1-ha plots maps indicating the distribution of adult plants (dots). Colours indicate the assignment of each plant to genetic clusters inferred according to the posterior probability of cluster membership (see Material and Methods). All the study plots are represented at the same spatial scale.

Lost dispersal services in downsized populations may result in permanent shifts in dispersal patterns, with a legacy of persisting dispersal collapse. Previous studies (Voigt *et al.* 2009) have reported shorter distances of seedlings to maternal trees for two close related *Commiphora* species in Madagascar (where the species interact with small-sized frugivores) when compared to continental South Africa. In our study, the signal of seed dispersal collapse persisted to the established sub-adults, pointing to a long-lasting consequence of frugivore downsizing at the early demographic stages.

Estimates of plant genetic diversity in terms of expected and observed heterozygosity, effective number of alleles, and allelic richness were similarly high across studied plots regardless of the marked differences in the seed dispersal patterns (Table 3.1; see also Table 3.S2 for results with sub-adult plants). Yet extensive pollen-mediated gene flow might counterbalance the effect of the limited seed dispersal.

The absence of inter-island differences in the amount of within-plot plant genetic diversity contrasted with differences in its spatial distribution. When comparing the covariation of the genetic similarity (r_{ij}) with distance among pairs of adult plants, we detected significant values spanning longer distances in Tenerife and La Gomera (up to 25 m) than in Gran Canaria (up to 15 m) (Fig. 3.3). We obtained a similar pattern for the autocorrelograms derived from sub-adult individuals (saplings and juveniles) (Fig. 3.S3). These trends were also confirmed by using Bayesian clustering analysis, where clusters represent groups of individuals in Hardy-Weinberg equilibrium (Fig. 3.3). We found an increasing number of genetic clusters from Gran Canaria (K= 1) to Tenerife (K= 2), and La Gomera (K= 3) mirroring the lizard downsizing gradient (Fig. 3.3).

Our results suggest a functional link between defaunation-driven downsizing, reduced seed- and effective-dispersal distances, and increased intra-population spatial genetic structure (SGS). These effects showed up at the scale of the 1 ha plots where the potential confounding effects of other factors (e.g., differences in population fragmentation among islands) would have a marginal influence. The study plots were embedded within large preserved populations that far exceed the home range sizes of *Gallotia* lizard species (see Material and Methods). In addition, these plant populations present similar abiotic (temperature, rainfall regimes, soil type) and biotic characteristics (pollinator assemblages, vegetation composition, etc) (Pérez-Méndez *et al.* 2015). Thus, we conclude that enviromental effects do not account for the differences observed among islands, according to the spatial scale of the study. Note that *Gallotia* lizards are the only seed dispersers of *N. pulverulenta*; thus, the observed reduction of seed dispersal distances following the downsizing is expected to leave a footprint in the spatial distribution of the genetic variation in the long term (Nason *et al.* 1997). Estimates of effective population size (Ne/N) in the three situations confirmed this, with a significant reduction in the two islands with most downsized lizards (Tenerife and La Gomera; Table 3.1). The lowest value obtained in Tenerife is likely related to the fact that its demographic census (N) almost doubled the estimate for La Gomera, potentially influencing its smaller effective population size. Thus, defaunation may also impact the pollination stage by contributing to the emergence of local neighbourhoods with high genetic similarity. Mating events would preferentially occur among relatives within these small neighbourhoods (Neel *et al.* 2013).

Our results support predictions of increased genetic structure when seed-mediated gene flow is reduced (Hamrick *et al.* 1993; Pacheco & Simonetti 2000; Voigt *et al.* 2009; Calviño-Cancela *et al.* 2012). In addition, the results from Tenerife suggest that even moderate reductions in dispersal distances (i.e. medium sized lizards still providing sub-efficient seed dispersal) can impair the fine-scale genetic structure of *N. pulverulenta* populations. These findings warn of important alterations in the spatial distribution of plant genetic variation in systems apparently intact after frugivore defaunation, but with partially downgraded dispersal services.

In sum, the downgrading of seed dispersal services, following body-size reduction of mutualistic frugivores, cascades to the spatial genetic structure with potential negative consequences for plant populations in the long term. These effects include the collapse of the seed dispersal distances, which in turn enhanced fine-scale spatial genetic structure and reduced effective population sizes. We expect similar effects in more diversified frugivore assemblages where limited functional redundancy among species constrains possibilities for ecological replacement (McConkey & Brockelman 2011; Bueno *et al.* 2013). Preserving large frugivores is essential to maintain the functional seed dispersal services, their associated genetic imprints, and the potential for adaptation to environmental changes. Otherwise, the legacy effects of defaunation will eventually arise generations after the loss of important seed dispersers. Our study identified early signals of collapsed seed dispersal to forecast multiple, cascading effects on plant populations.

MATERIAL AND METHODS

Study Species

Neochamaelea pulverulenta (Rutaceae) (Vent) Erdtman is a fleshy-fruited shrub (up to 2.7 m height), endemic to the Canary Islands (Gran Canaria, Tenerife and La Gomera). It is found in lowland xerophytic areas (Fig. 3.S4), and is relatively abundant in some localities (Bramwell & Bramwell 2001). Ants and winged-insects (flies and bees) act as pollinators. Fleshy fruits include1-4 cocci, ripening from spring to early summer (Fig. 3.S4). Each coccus can be considered functionally a drupe (11.1 ± 1.6 mm in diameter), containing one large, hard-coated seed (8.6 ± 1.0 mm in diameter) (Valido 1999). Only medium- to large-bodied Canarian endemic lizards (*Gallotia* spp., Lacertidae) consume these fruits and act as legitimate seed dispersers (Valido & Nogales 1994; Valido 1999; Valido *et al.* 2003; Pérez-Méndez *et al.* 2015).

Giant *Gallotia* lizard species were relatively abundant in the Canary Islands before the arrival of first settlers (c. 2490 ± 60 BP according to C_{14} dating) (Onrubia-Pintado 1987). Nevertheless, the synergic combination of lizard hunting by humans and introduced mammals (e.g., feral cats), and a unique suite of life-history traits (e.g., low reproductive rate, absence of antipredatory strategies), most likely triggered the decline and/or extinction of these largest-sized species (Machado 1985; Barahona *et al.* 2000; Gonzalez *et al.* 2014). The magnitude of the extinction process and the resulting downsizing pattern notably varied among the three islands with *N. pulverulenta* populations (Barahona *et al.* 2000) (Fig. 3.1; see also Fig. 2.S1 in chapter 2). For example, lizard downsizing has been relatively subtle in Gran Canaria, from the large individuals of *G. stehlini* sub-fossils (maximum snout-to-vent length; *max* SVL= 367 mm) to the extant individuals (*max* SVL= 280 mm). Intermediate downsizing has occurred in Tenerife, from the extinct giant *G. goliath* species (*max* SVL= 502 mm) to the medium-sized *G. galloti* (*max* SVL=

144 mm). In turn, a substantial body size reduction has been recorded 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) (Fig. 3.1). Also, the critically endangered, *Gallotia intermedia* (*max* SVL= 174 mm) and *Gallotia bravoana* (*max* SVL= 212 mm) inhabit Tenerife and La Gomera, respectively, but they survive in extremely reduced, relict populations on very inaccessible cliffs (Hernández *et al.* 2000; Valido *et al.* 2000). This present-day biogeographic scenario allowed us to define a gradient of lizard downsizing, with three contrasted ecological settings illustrating the loss of large frugivores to a variable extent (Fig. 3.1). To assess the consequences of lizard downsizing we analysed the seed- and effective-dispersal distances in three representative 1-ha plots (one per island) illustrating the lizard body size gradient. We also studied the current fine-scale spatial pattern of plant genetic variation within those plots. To further test for differences among islands we also sampled two or three additional *N. pulverulenta* populations in each island (see below).

Study sites and sampling design.

We chose 10 plant populations encompassing the full geographic range of the plant to represent typical, well-preserved xerophytic scrublands comparable in biotic and abiotic conditions (Pérez-Méndez *et al.* 2015) (Fig. 3.1, Table 3.S1). In three of these populations we set a representative 1-ha plot for exhaustive sampling and intensive study located at: Gran Canaria (65 x 154 m; Barranco de Veneguera), Tenerife (80 x 125 m; Teno Bajo) and La Gomera (80 x 125 m; Punta Llana). The goal was to obtain genotypic data for all plants and extensive seed sampling to assess dispersal distances. The remaining populations received more restricted sampling to obtain replicated seed dispersal data (see below). *N. pulverulenta* plants and *Gallotia* lizards are relatively abundant in all the study sites. In order to increase the likelihood to infer the mother plant of dispersed seeds, the main plot size (1 ha) was established according to previous information on lizard movement patterns (Molina-Borja 1985). We georreferenced (Leica GPS 1200+; ± 1 cm accuracy), tagged, measured (stem basal diameter, height, horizontal canopy projection area) and collected fresh leaves for all *N. pulverulenta* individual plants within the study plots. Individuals plants were classified as adult (both reproductive and non-reproductive adults), and sub-adult individuals (juveniles and saplings) according to previous information (Pérez-Méndez *et al.* 2015). In addition, in order to increase the likelihood of determining the maternal source of dispersed seeds and determine to what extent the immigration events were mediated by long dispersal distances, all adult individuals located within a 12-m buffer area around the perimeter of the studied plots were also tagged, geo-referenced and sampled for leaves. Given previous evidences that legitimate dispersal does not occur in La Gomera (Valido 1999; Pérez-Méndez *et al.* 2015), these buffer areas were only sampled in Gran Canaria and Tenerife plots, where seeds are effectively dispersed by lizards (see below). All collected leaves were silica-dried and stored until lab processing. We sampled leaves from 675 individuals in Gran Canaria (adults= 409; juveniles= 102; saplings= 164), 987 in Tenerife (adults= 778; juveniles= 148; saplings= 61) and 509 in La Gomera plots (adults= 397; juveniles= 86; saplings= 26). Also, 229 and 525 adult plants were sampled from the buffer areas in the Gran Canaria and Tenerife plots, respectively (Fig. 3.S5 for spatial distribution of plants).

The characterization of seed dispersal patterns of *N. pulverulenta* was performed by a regular sampling of lizard-mediated dispersed along 10-m wide transects on Gran Canaria (13 60-m transects), Tenerife (9 125-m transects), and La Gomera (10 80-m transects). Transects spanning the whole of the studied area. Along these transects we regularly established and geo-referenced sampling points every 5 m along the progression line. Around these points we established a circular sampling area of 1 m^2 where we collected and silica-dried stored all fresh lizard droppings containing seeds of *N. pulverulenta*. In addition, these 10 m wide transects were carefully scanned by 2-3 people to increase the number of sampled seeds (Fig. 3.S6 for spatial distribution of dispersed seeds and adult plants). Numbers of seed sampling points were *n*= 408, 612, and 414 for Gran Canaria, Tenerife and La Gomera plots, respectively. We found no evidence of legitimate, lizard-dispersed seeds (i.e., defecated seeds within lizard droppings) in La Gomera plot. The small-sized lizards in this population (*G. caesaris*) can not swallow *N. pulverulenta* fruits, but may sporadically disperse some seeds when they handle fruits while biting off the fleshy pulp (Valido 1999; Pérez-Méndez *et al.* 2015); therefore, we collected and geo-referenced all seeds manipulated by lizards found along linear transects within this plot. Overall, for the main 1-ha plots we obtained the genotypes for 326 endocarps in Gran Canaria, 305 in Tenerife, and 62 in La Gomera.

The exhaustive sampling required for the 1-ha plot maternity assignments of dispersed seeds limited logistically our ability to achieve a fully replicated sampling design within islands. We designed a complementary sampling protocol to allow comparisons of seed dispersal patterns among islands with adequate replication. We selected seven additional *N. pulverulenta* populations, 3 in Gran Canaria (2 in Barranco de Arguineguín, 1 in Barranco de Fataga), 2 in Tenerife (Malpaís de Punta de Rasca, La Caleta de Adeje), and 2 in La Gomera (Barranco del Medio, Taguluche) (see Fig 3.1 for locations). In each population we set up 1-3 linear transects to scan for fresh lizard droppings containing *N. pulverulenta* seeds. For each replicated sampling areas of 1 m^2 we collected all dispersed seeds and sampled leaves from all the adult plants present within a 5-m radius plot. We sampled 5-20 plots and 15-35 seeds per population (see Table 3.S1 for details). We obtained the frequency of SDD events for each population by averaging the percentage of seeds whose assigned mother plant was found within the 5-m radius.

Microsatellite genotyping

DNA was isolated from dried leaves of plants and from endocarps of dispersed seeds (the woody part of the seed, which is maternally originated tissue, surrounding the embryo) (Godoy & Jordano 2001). DNA extraction of leaves and endocarps was performed following protocols for this species (Rigueiro *et al.* 2009). Briefly, DNA extraction of leaves was performed with a modified CTAB extraction method. In the case of DNA extraction from endocarps, we carefully separated them from the embryos, before breaking it into small fragments with a pair of pliers. This woody tissue was immersed in liquid nitrogen, grinded in a ball-mill (Mixer Mill MM301, Retsch, Germany), and a Qiagen DNeasy Plant Mini Kit was used for DNA extraction. 5 μL of DNA extraction at different concentrations for leaves and endocarps (DNA: $H_2 0$; 2:13 and 3:2 respectively) were used for the polymerase chain reaction (PCR). Plants and endocarps were genotyped for 12 and 10 specific microsatellite markers, respectively, specially designed for parentage assignment of dispersed seeds, and analysis of spatial genetic structure (Rigueiro *et al.* 2009). Two markers were excluded for endocarps due to a high rate of amplification errors. 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. The scoring was performed independently by two people and crosschecked. The identity probability (*i.e.* the average probability that two unrelated individuals share the same multilocus genotype) based on the set of polymorphic microsatellite markers ranged between 6×10^{-10} in Punta Llana and 6×10^{-8} in Teno.

Maternity and parentage analyses, seed- and effective-dispersal distances, LDD, and immigration rate

As endocarps are tissues of maternal origin, the source plant for all dispersed seeds was assigned by a full matching of the multilocus genotypes of endocarps and any of the candidate mother plants. Because obtaining high-quality DNA from thick-coated endocarps recovered from faeces after some period in the ground, we needed a criterion allowing the inference of a matched genotype given a maximum number of mismatches among paired leaf-endocarp. To do that, we used the algorithm implemented in the *ALLELEMATCH* package (Galpern *et al.* 2012) in R 3.1.1 (R Development Core Team 2015), which groups samples (plants and endocarps) sharing an unique genotype profile. This algorithm finds similarities between samples accounting for missing or mismatch data. Then it uses this information to apply a hierarchical clustering method to identify groups of similar genotypes (unique genotype profiles) at an optimal threshold of missing/mismatches (Fig. 3.S7). Thus, seeds were assigned to a maternal shrub when both adult plants and seeds shared the same genotype profile. All analysed endocarps were assigned to a single adult individual, as multiple matches were not detected. Seeds with genotype profiles not matching any candidate mother plant were considered as immigrant seeds, i.e., dispersed from non-sampled plants growing in the surroundings of the study plots, as these are embedded in extensive continuous populations. Immigration rate is expected to be high in a plot surrounded by dense plant populations given that many more potential reproductive plants remain non-sampled. The buffer area of Teno exhibited a higher plant density than the Veneguera one, however immigration rate was significantly lower. This suggests that our assessments of the effects of lizard downsizing on immigrant events are conservative.

Based on the spatial coordinates of both dispersed seeds and their assigned maternal plant we firstly measured the linear seed dispersal distances (± 1 cm resolution).

Secondly, we estimated the frequency distribution of dispersal distance events and the frequency of immigrant events. We tested for differences in the population-level, aggregated, frequency distributions of seed dispersal distances and immigration events resulting from multiple inferences of dispersal events sampled from dispersed seeds. To do that we resampled 999 times the empirical seed dispersal vectors (including immigrant seeds) to obtain bootstrapped estimates of dispersal distances and their associated SD. Then we applied linear models with island as fixed factor and a post-hoc Tukey test to evaluate among-island dispersal distance differences in terms of *i*) median, *ii*) maximum, *iii*), but also *iv*) frequency of short distance dispersal (SDD; events < 5 m), *v*) frequency of long distance dispersal (LDD; events > 30m), *vi*) frequency of immigrant events, and *vii*) frequency of immigrant events when accounting for plants growing in the12 m buffer. Finally, we adjusted a non-parametric function (smooth spline curve) and its bootstrap-estimated CI interval to the empirical frequency distribution of seed dispersal distances for each study plot to graphically represent the probability distribution of dispersal events (*n*= 99 resamplings; Fig. 3.2). Moreover in order to control for differences among plots in the aggregation and distribution of both reproductive plants and the sampling areas to collect dispersed seeds, we also calculated an expected distribution of seed dispersal distances. We estimated all the possible pairwise distances between reproductive plants and seed sampling points (Fig. 3.S1). These expected distributions reflect the variable correspondence between the distribution of the plants and the sampling points (Robledo-Arnuncio & García 2007) due to simple positional effects. Among island differences in expected dispersal distributions (Fig. 3.S1) are much subtler than differences between the expected and empirical seed dispersal distance distributions within islands. This suggests that differences among islands in the plants-dispersal sampling points arrangements (i.e., purely geometric effects) were not influential on the observed patterns.

The frequency distribution of effective dispersal distances were also estimated by applying parentage analyses to sub-adults individuals (saplings and juveniles separately) in 1-ha study plots. We used a maximum-likelihood framework as implemented in CERVUS 3.0 software (Kalinowski *et al.* 2007) to estimate both paternal and maternal parent for saplings and juveniles. Only reproductive individuals were considered as candidate parents. We used a relaxed 80% confidence interval for the parentage assignment and run 10,000 simulations with the following parameters: minimum number of matching loci= seven loci; error rate= 0.01; proportion of candidate parents sampled= 0.90; proportion of loci typed= 0.99. In addition, sample size (number of reproductive adults) and allele frequency were accommodated in the simulations for each study plot. As CERVUS does not differentiate between maternal and paternal parents, we assumed that the nearest reproductive individual was the maternal plant, whereas the most distant parent was the paternal plant. Our decision was based on information obtained in the Tenerife 1-ha plot (Teno) from a random subset of 40 dispersed seeds for which embryo genotypes were also obtained. Realized pollen dispersal distances, estimated from the embryo genotypes, were longer (median= 40.2 m) than their corresponding seed dispersal distances (3.2 m). Based on the spatial coordinates of sub-adult individuals and their inferred maternal plants, we also obtained the frequency distribution and the smoothing spline curve of effective dispersal distances of juvenile and sapling individuals in each studied plot (Fig. 3.S2). The confidence intervals for the empirical seed dispersal distance distribution curves by bootstrapping were also estimated, as previously described.

Genetic diversity, fine-scale spatial genetic structure and genetic clustering

We determined among-plot differences on the amount of plant genetic diversity in terms of expected heterozygosity (uH_e), observed heterozygosity (H_o), effective number of alleles (A_e) and average allelic richness (AR) for each age class (i.e., adult, juvenile and sapling individuals) by applying GeneAlex 6.5 (Peakall & Smouse 2012). In addition, we computed autocorrelograms for each plot and age class to compare the magnitude and spatial scale of the spatial genetic structure (SGS)(Smouse & Peakall 1999; Peakall & Smouse 2012). This approach uses multilocus genotype information to calculate pairwise genetic autocorrelation coefficient (r_{ij}) among pairs of individuals for a set of specified distance classes. Associated standard errors of r_{ij} were estimated using bootstrapping procedures. The selection of distance classes was calibrated to result in similar sample sizes. We used the same distance classes (5 m) in the three locations to allow comparisons among them. To test departures from the null-hypothesis (*i.e.,* for each distance class, two randomly chosen individuals are not more genetically similar than two individuals randomly chosen outside that given distance interval, $r_{ij} = 0$) we permuted the spatial position of individuals (*n* permutations= 999) and then calculated the mean of the 999 permuted values and generated a 95 % confidence envelope around that null-hypothesis mean. Plant density is expected to enhance the spatial genetic structure (SGS) of plant populations, however our results showed that the studied plot in Tenerife (with medium-size lizards) showing the highest plant density exhibited lower SGS than La Gomera plot (small-sized lizards). Thus, variation in plant density seems not to obscure the signal imprinted by body-size reduction of lizards and the associated downgrading of dispersal functions. Finally, the effective population size (*Ne/N*) was calculated from genotype information of reproductive individuals by using the NeEstimator software (Do *et al.* 2014). We applied the linkage disequilibrium method excluding rare alleles occurring at lower frequencies than 0.01 (critical value).

We also explored fine-scale genetic structure within plots by searching for genetic clusters of adult plant. We first applied a Bayesian-clustering algorithm, as implemented in *GENELAND* R package (Guillot *et al.* 2005) that splits each plot into *K* clusters in Hardy-Weinberg-Linkage-Equilibrium (HWLE). We applied a two-step procedure (Gilbert *et al.* 2012). Firstly, we performed 25 independent runs and checked for convergence issues after setting the following parameters: no admixture model; null allele model= TRUE; spatial model= TRUE; number max of nuclei= 778; uncertainty coordinates= 0; K_{min}= 1; K_{max}= 50 for 100000 sweeps; thinning= 100; burning= 100. Spatially-explicit models take into account the spatial location of the individuals to improve the inference power of the substructure when differentiation occurs by limited gene flow driven by the presence of physical barriers. The number of genetic clusters (*K*) was inferred as the modal number of genetic groups of the best run (based on posterior density values). Secondly, we fixed the number of genetic clusters inferred in the previous step for each study plot and performed 25 independent runs to assign each individual to a cluster. The assignment of each individual to a cluster was set according to the posterior probability of the cluster membership of the best run (threshold= 0.8). Then, we mapped the each individual sorted by clusters by using R 3.1.1 (R Development Core Team 2015). When we inferred the genetic clustering for sub-adult individuals (sapling, juveniles), models did not converge properly, possibly because of low samples sizes. Thus, because the number of estimated clusters for each run varied considerably, we did not include the results for these age classes.

ACKNOWLEDGEMENTS

We are in debt with G. Pérez and Y. Arjona for their assistance during the fieldwork, and J.M. Arroyo, C. Rigueiro, M. Carrión and R. Gutiérrez for laboratory assistance. The insular cabildos from Gran Canaria, Tenerife, and La Gomera for granting the sampling permits. The owners of Agrícola Luz Teno (Tenerife) and Cañada Honda (Gran Canaria) permitted the study on their properties. The manuscript benefitted from constructive comments by M. Galetti, Gregory S. Gilbert, J.J. Robledo-Arnuncio, and J.N. Thompson. This study was supported by Ministerio de Economía y Competitividad (CGL2009-09715 and CGL2013-47429-P) and Junta de Andalucía (RNM-5731). N.P.-M. was supported by an FPI-fellowship (BES-2010-041463), C.G. was supported by Fundação para a Ciência e a Tecnologia the FI Investigador Programme (FCT- Research grant (FCT-ANR/BIA-BIC/0010/2013). A.V. is supported by the R&C programme (RYC-2007-00620), as well as a Severo Ochoa Excellence Award (SEV-2012-0262).

Apéndices

CAPÍTULO 3: The signatures of Anthropocene defaunation: cascading effects of the seed dispersal collapse

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FIG. 3.S1. FREQUENCY DISTRIBUTIONS OF EXPECTED AND EMPIRICAL, LIZARD-MEDIATED, SEED DISPERSAL DISTANCES

Expected dispersal distances are distances among all pairwise reproductive *Neochamaelea pulverulenta* adult plants within plots to all the seed sampling points (grey bars, 5 m binning) in the three 1-ha study plots. Empirical seed dispersal distances (black bars) are distances among all dispersed seeds within plots to their assigned reproducive plants. Note the similarity of expected distances distributions among plots. Lizard silhouettes are scaled to the maximum SVL.

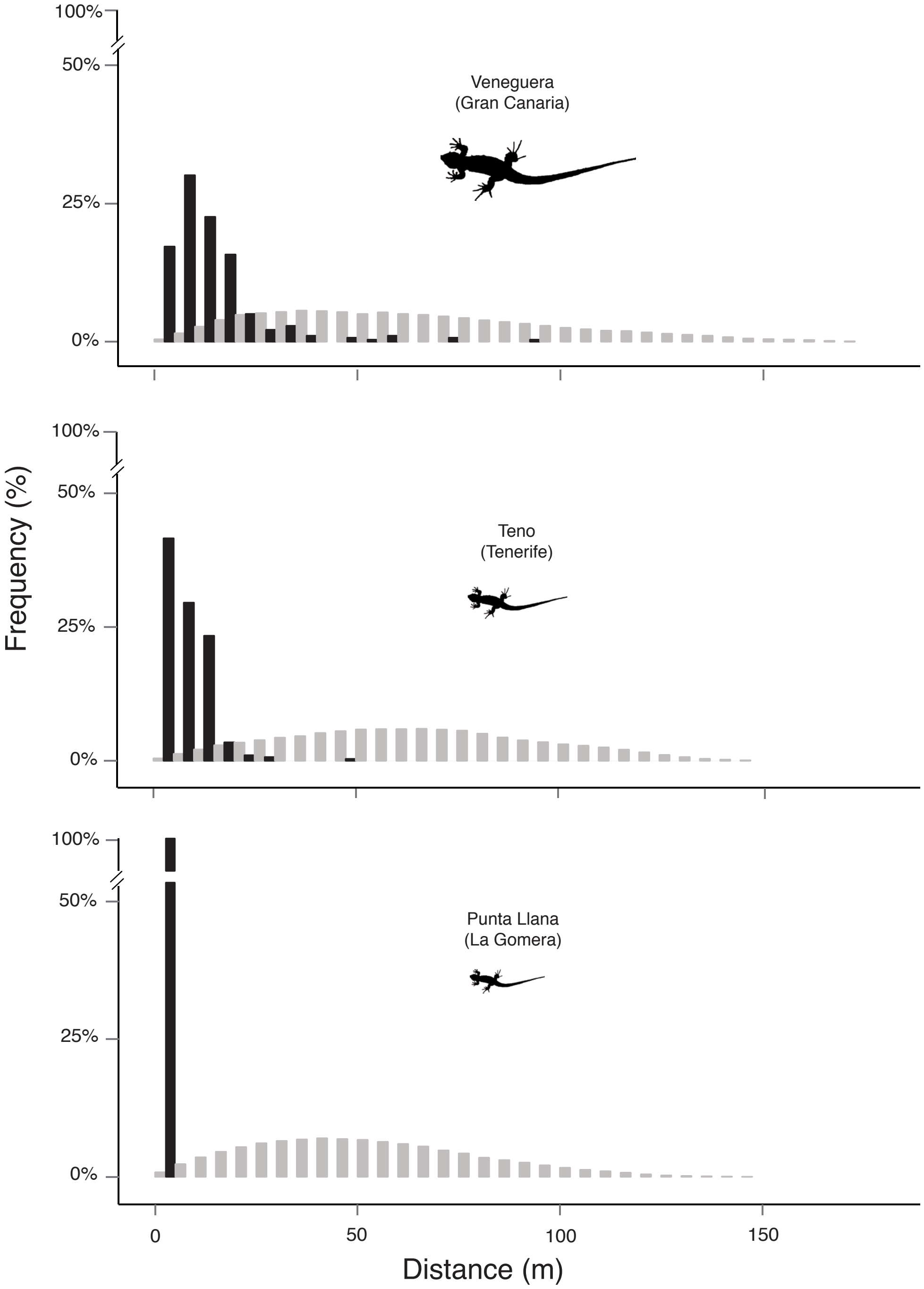


FIG. 3.S2. FREQUENCY DISTRIBUTIONS AND SMOOTHING CURVES OF EFFECTIVE DISPERSAL

Effective dispersal distances are distances between established saplings and juveniles and their assigned most likely maternal plant based on parentage analyses in the three 1-ha study plots. Sample sizes (number of assigned recruits) are indicated in each panel. Maximum dispersal distances are: Veneguera (juveniles= 94.8 m; saplings= 81.9 m), Teno (juveniles= 55.4 m; saplings= 55.4 m) and Punta Llana (juveniles= 64.4 m; saplings= 38.7 m). We assumed that the maternal plant was the nearest candidate parent based on data about pollen and seed dispersal distances obtained from Teno (Tenerife) (see Material and Methods). Lizard silhouettes are scaled to the maximum SVL.

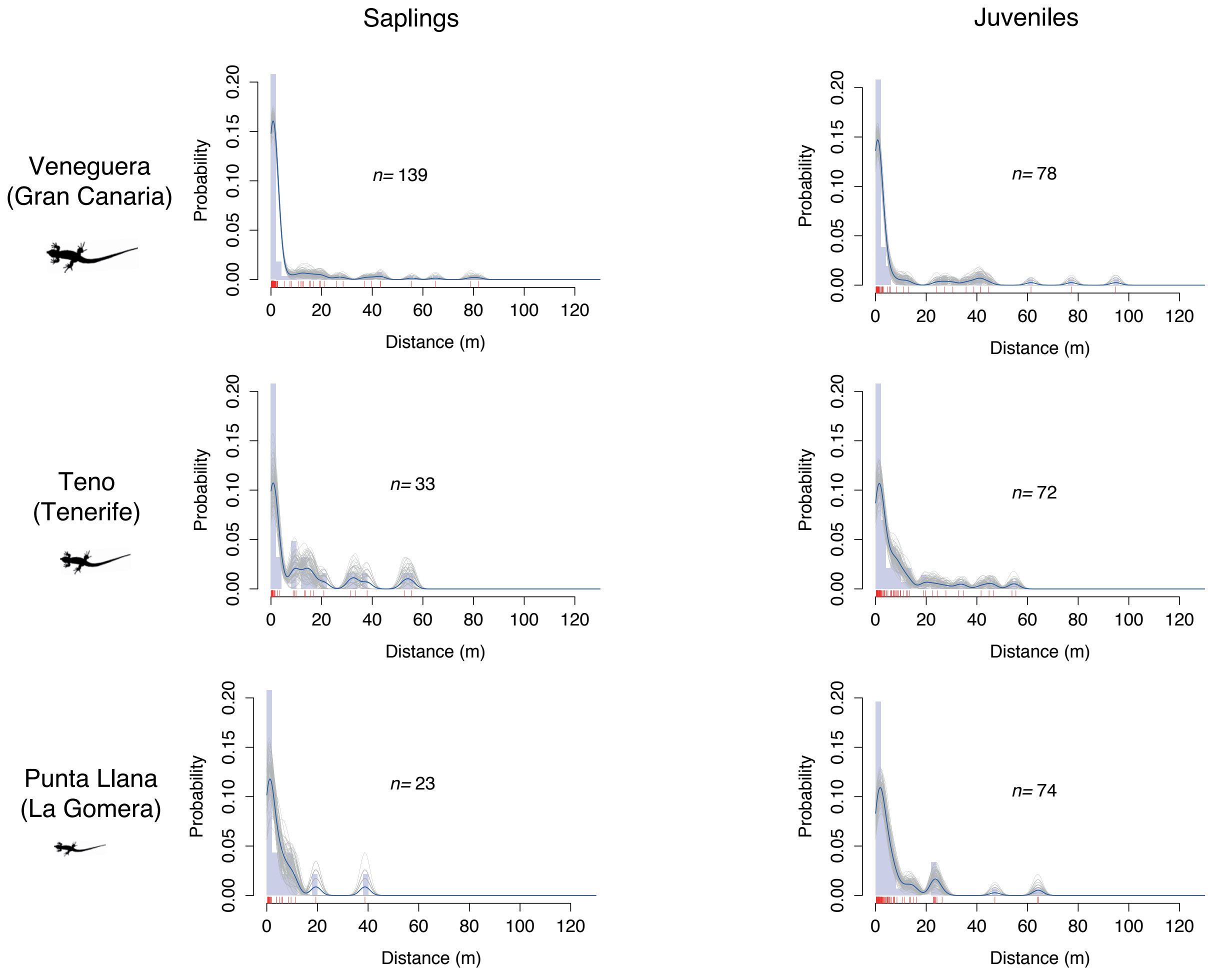


FIG. 3.S3. AUTOCORRELOGRAM OF GENETIC SIMILARITY

Autocorrelograms of genetic similarity among pairs of *Neochamaelea pulverulenta* individual plants. Genetic similarity (rij) and error bars were determined by bootstrap resampling (n= 999) Grey areas represent the 95% confidence intervals for the null hypothesis calculated by permutations of the plant spatial coordinates. Distance classes are 1 m, 2 m, and successive 5 m intervals up to 80 m, and 100 m. Only plants growing within the study 1-ha plots were considered in this analysis. Lizard silhouettes are scaled to the maximum SVL.

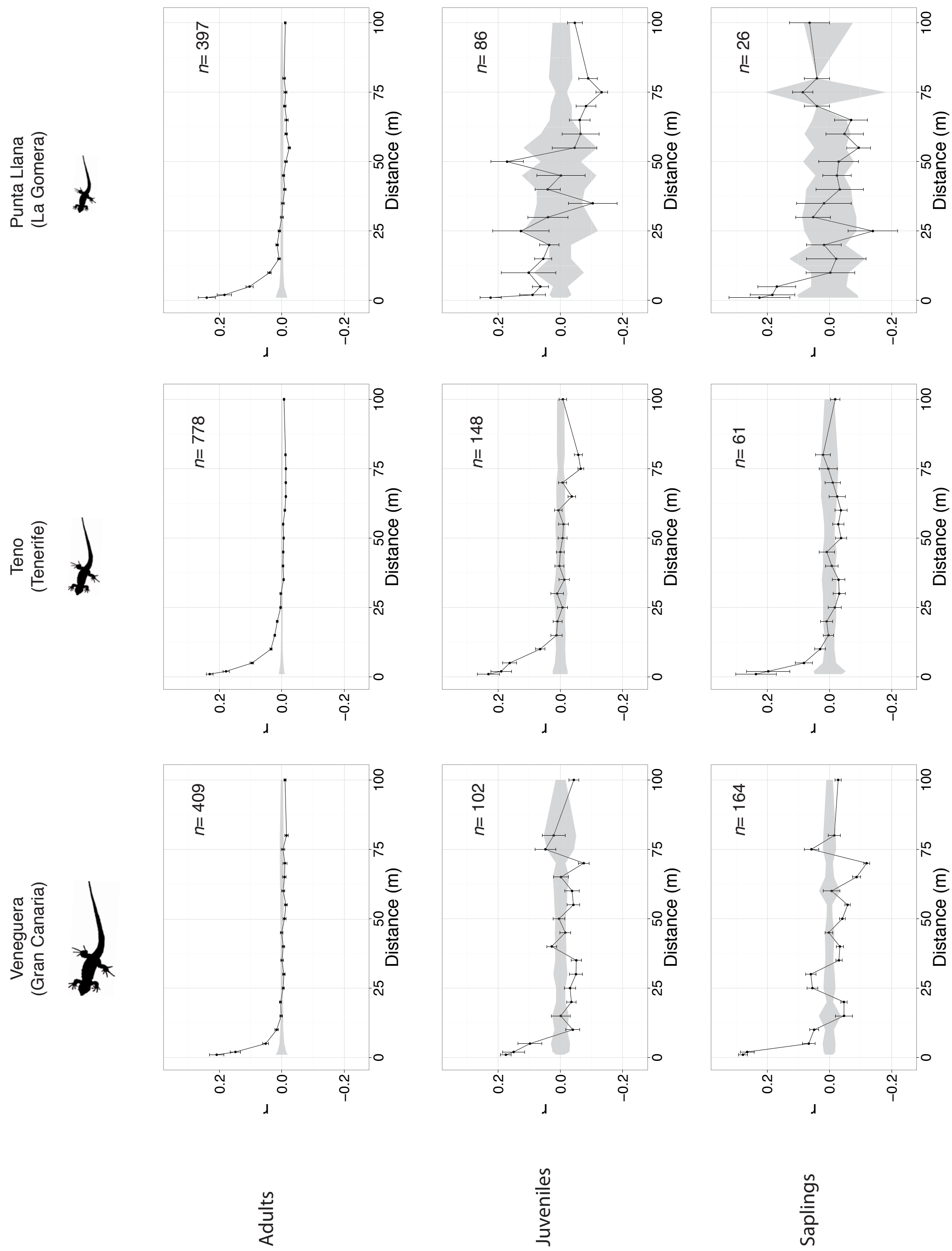
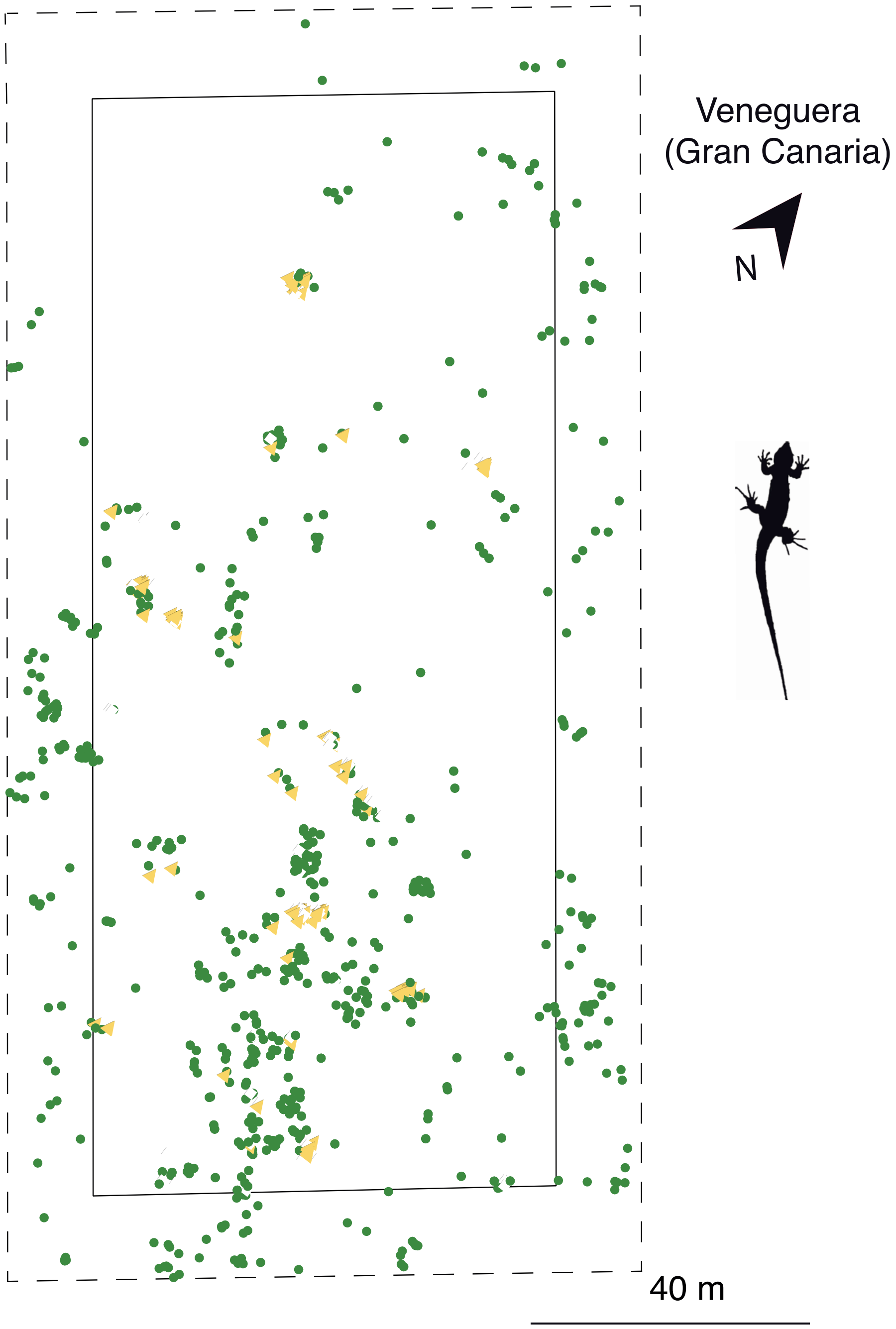


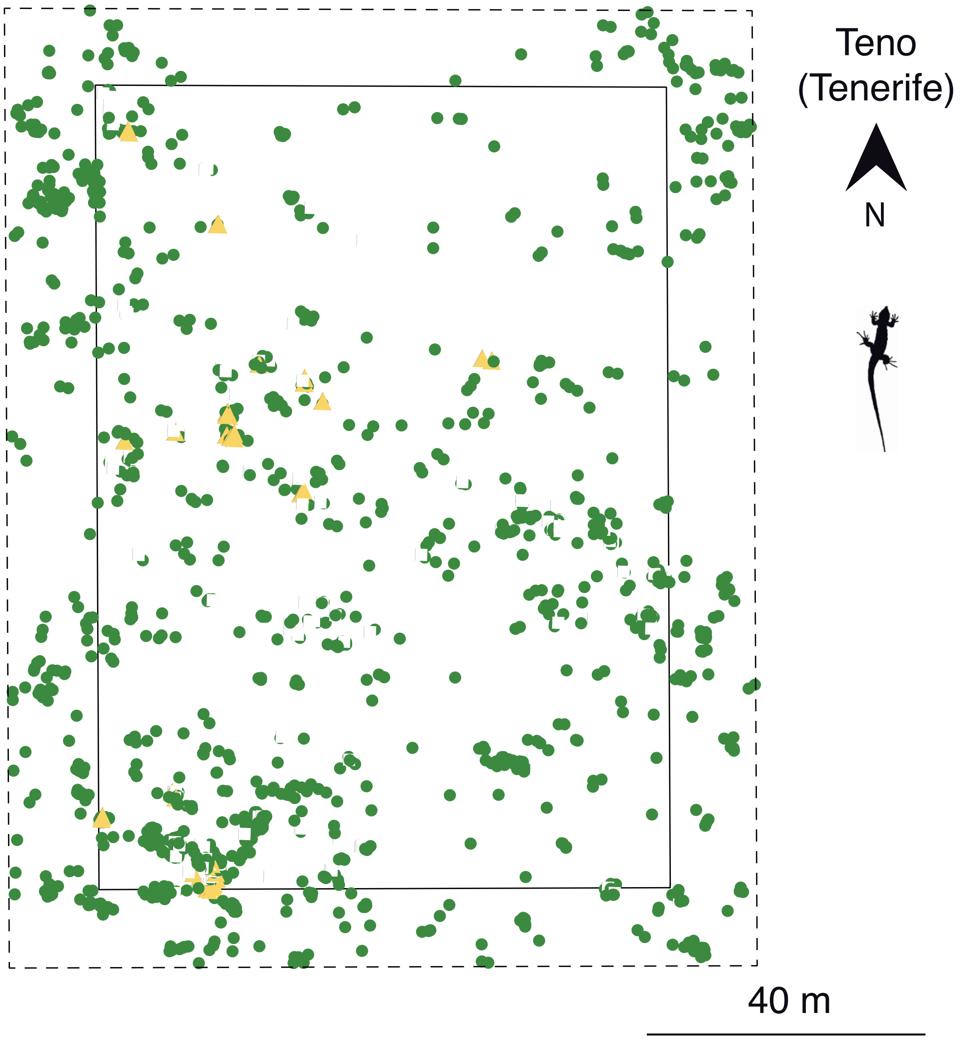
FIG. 3.S4. PHOTO-COLLAGE OF THE STUDY SYSTEM

Upper-left, *N. pulverulenta* individual growing close to an *Euphorbia balsamifera* and *E. canariensis* (Euphorbiaceae); upper-center, *N. pulverulenta* with ripe fruits; upper-right, details of a fruit formed by three cocci (functionally drupes); lower-left, study plots in Punta Llana (La Gomera); lower-center, Teno (Tenerife); lower-right, Veneguera (Gran Canaria).

FIG. 3.S5. SPATIAL DISTRIBUTION OF *Neochamaelea pulverulenta* INDIVIDUAL PLANTS

Adult individuals are shown as green circles, juveniles as white squares and sapling as yellow triangles in the three 1-ha study plots. The adult individuals sampled in the buffer area were also included as potential maternal plants for dispersed seeds sampled within the plot (n= 229 and 525 adults in Veneguera and Teno, respectively). The 1-ha plots are represented with continuous lines and the buffer limits with dashed lines. Veneguera (409; 102; 164, respectively), Teno (778; 148; 61, respectively), and Punta Llana (397; 86; 26, respectively).Lizard silhouettes are scaled to the maximum SVL.




Teno
(Tenerife)
N
40 m

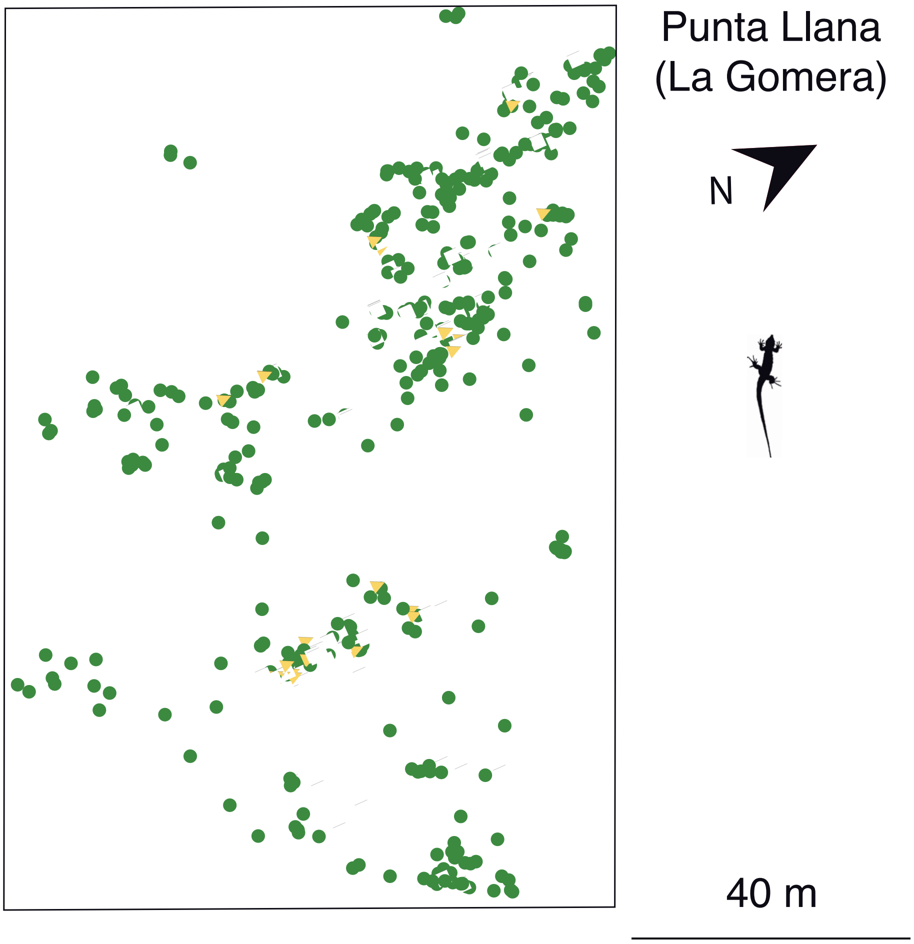
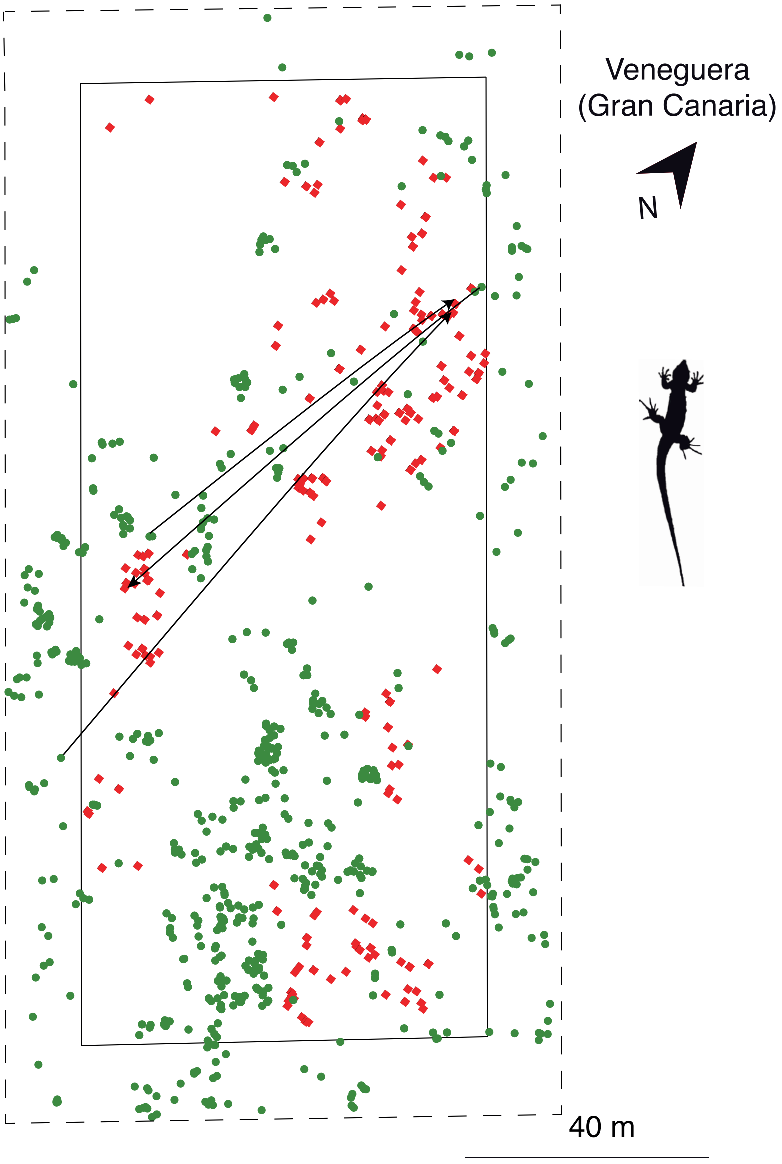
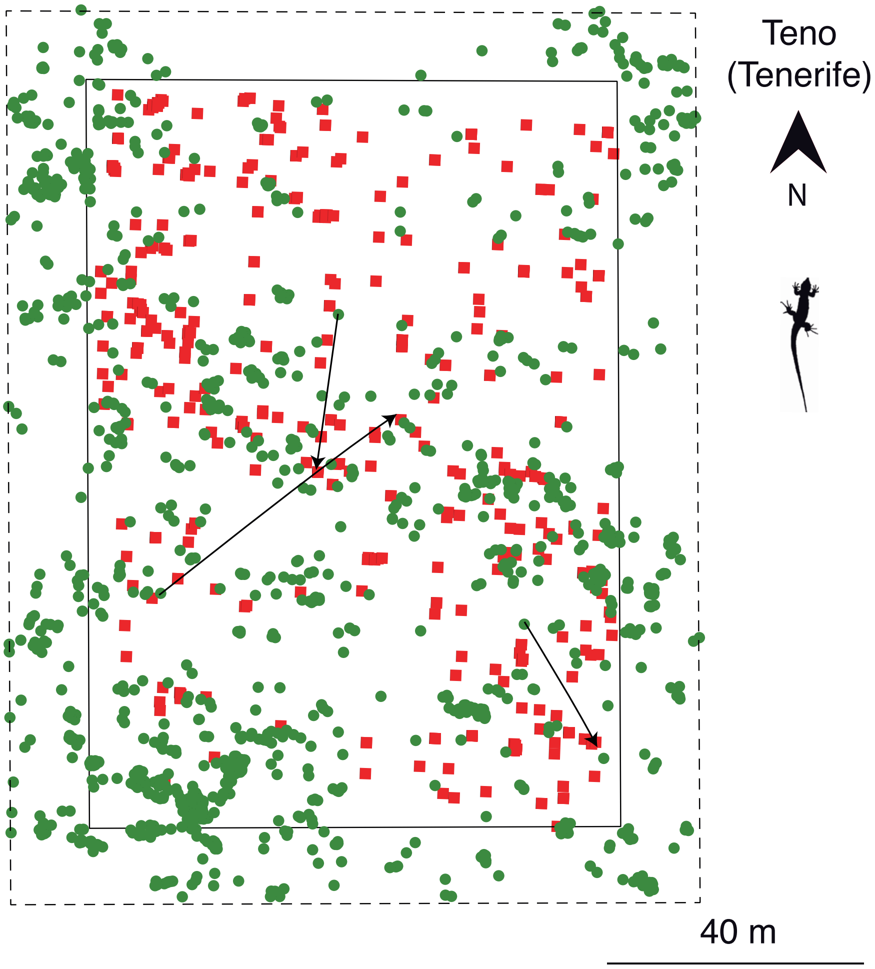

Punta Llana
(La Gomera)
N
40 m

FIG. 3.S6. SPATIAL DISTRIBUTION OF BOTH ADULT INDIVIDUAL PLANTS OF *Neochamaelea pulverulenta* AND SEEDS DISPERSED BY LIZARDS

Individual plants are shown as green circles and dispersed seeds as red squares at each 1-ha study plot. Veneguera (Gran Canaria; n= 409 adults, 326 seeds), Teno (Tenerife; n= 778 adults, 305 seeds), and Punta Llana (La Gomera; n= 397 adults, 62 seeds). The adult plants sampled in the buffer area were also included as potential maternal plants for dispersed seeds sampled within the plot (n= 229 and 525 adults in Veneguera and Teno, respectively). The 1-ha plots are represented with continuous lines and the buffer limits with dashed lines. The three longest seed dispersal events in each plot are also indicated with lines (94.2 m, 72.7 m, 59.9 m, Veneguera; 46.4 m, 26.7 m, 22.4 m, Teno; 4.5 m, 2.2 m, 1.9 m, Punta Llana), arrows show the direction of the dispersal event. Lizard silhouettes are scaled to the maximum SVL.




Teno
(Tenerife)
N
40 m

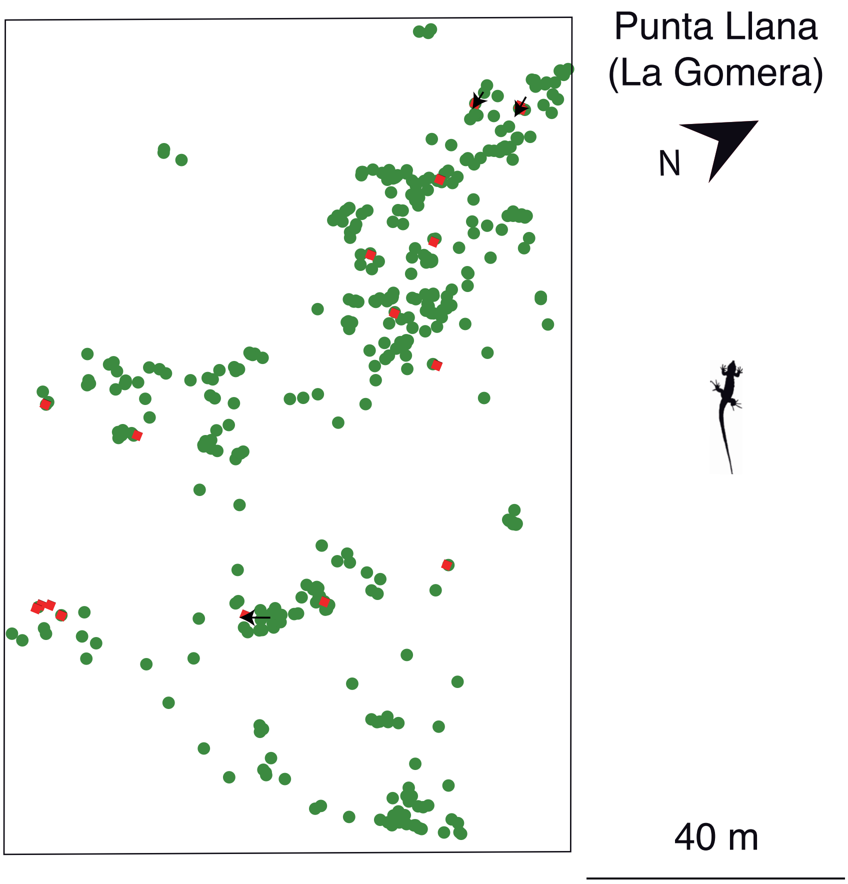

Punta Llana
(La Gomera)
N
40 m

FIG. 3.S7 OUTPUT FROM ALLELEMATCH

The optimal number of mismatches (black arrow) among multiple genotypes for robustly inferring unique individuals at each 1-ha study plot. Sampled genotypes matching below this threshold values were considered as unique genotypes. The curves represent the decay in the frequency of unique mismatches (dots) and the increase in the frequency of multiple matches (squares) between target seed endocarp and adult plant genotypes when increasing the number of mismatches.

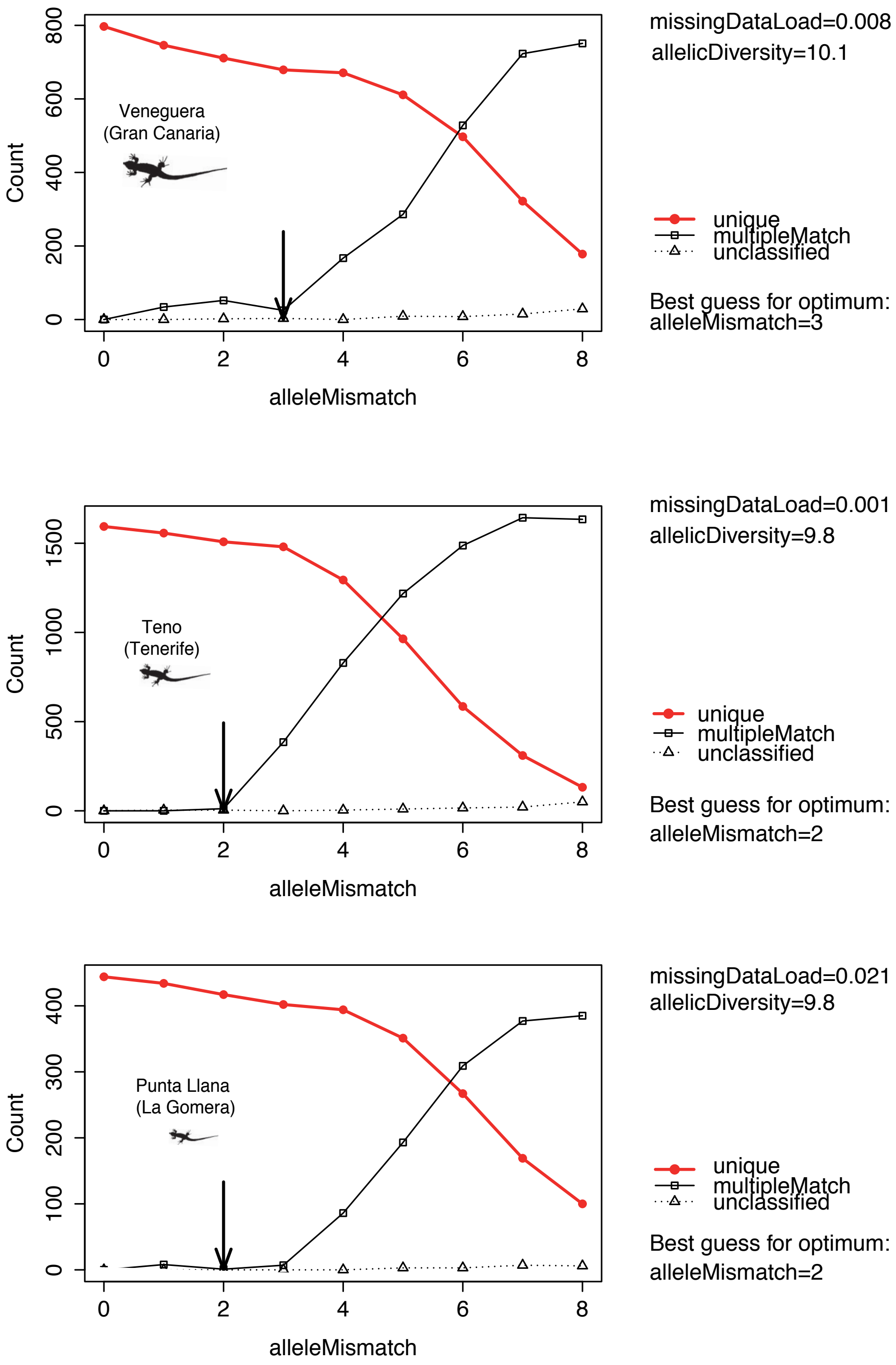


TABLE 3.S1. SHORT-DISTANCE DISPERSAL EVENTS IN ALL STUDIED POPULATIONS

Percentage of short-distance dispersal events (% SDD; percentage of seeds whose assigned mother plant was found within a 5-m radius) obtained in all studied populations. Percentages of SDD in the main study 1-ha plots (*) were determined by resampling (n= 999) a similar number of 5-m plots and seeds (n= 40) to those sampled in the replicated populations. Average values (± 1 SE) for each island are also indicated (see Fig. 3.1 for locations). Differences among islands in averaged % SDD are significantly different (Chi-sq= 8.02, p< 0.05; K-Wallis test).

Island	Locality	Coordinates	N plots	N seeds	% SDD
Gran Canaria			84	123	10.2 ± 5.9
	Barranco de Veneguera*	27°51'43" N 15°46'21" W	40	40	18.6 ± 5.7
	Barranco de Arguineguín 1	27º49º45” N 15º40’46” W	16	33	9.9
	Barranco de Arguineguín 2	27º48’13” N 15º40’23” W	8	15	6.7
	Barranco de Fataga	27º50’20” N 15º34’10” W	20	35	5.7
Tenerife			66	97	43.0 ± 6.5
	Teno Bajo*	28°21'13" N 16°54'33" W	40	40	41.9 ± 7.2
	Malpaís Punta de Rasca	28º00’42” N 16º42’15” W	16	35	37.1
	Caleta de Adeje	28º06’33” N 16º44’26” W	10	22	50.0
La Gomera			52	102	92.5 ± 6.5
	Punta Llana*	28º07'41" N 17º06'17" W	40	40	100 ± 0.0
	Taguluche	28º09’51” N 17º19’66” W	5	28	89.2
	Barranco del Medio	28º02’02” N 17º11’64”W	7	34	88.2

TABLE 3.S2. SUMMARY OF GENETIC DIVERSITY PARAMETERS

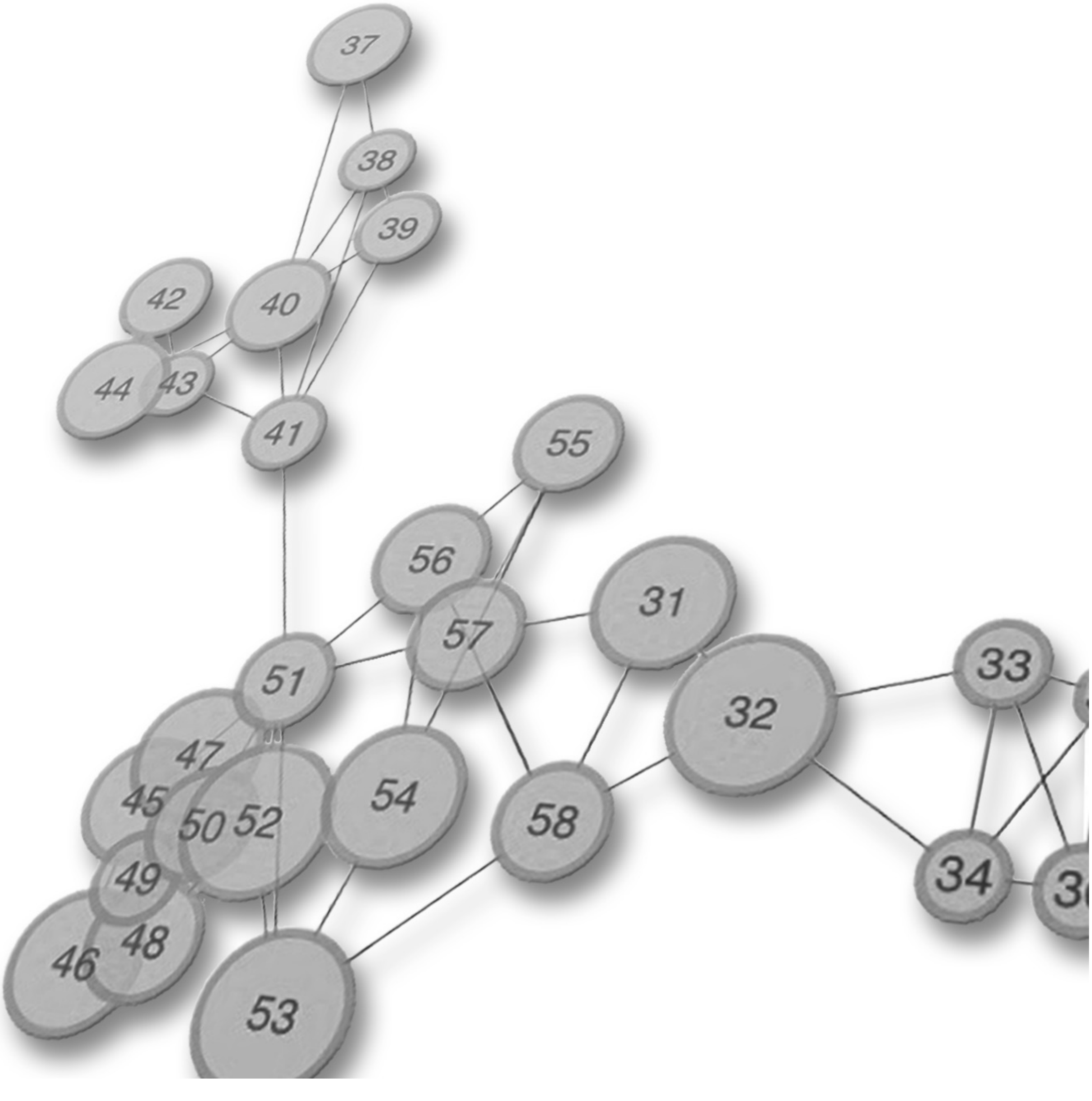
Genetic diversity parameters were obtained for different age classes of *Neochamaelea pulverulenta* across the 1-ha plots: Veneguera (Gran Canaria), Teno (Tenerife), and Punta Llana (La Gomera). *n* (adults; juveniles; saplings); Veneguera (409; 102; 164), Teno (778; 148; 61), and Punta Llana (397; 86; 26). Data are means ± 1 SE.

Population parameters		Veneguera (Gran Canaria)	Teno (Tenerife)	Punta Llana (La Gomera)
Expected Heterozygosity *(uHe)*	Adults	0.64 ± 0.06	0.62 ± 0.06	0.64 ± 0.06
	Juveniles	0.64 ± 0.05	0.62 ± 0.06	0.63 ± 0.07
	Saplings	0.61 ± 0.06	0.60 ± 0.06	0.62 ± 0.07
Observed Heterozigosity *(Ho)*	Adults	0.59 ± 0.06	0.60 ± 0.06	0.61 ± 0.06
	Juveniles	0.62 ± 0.06	0.57 ± 0.05	0.62 ± 0.07
	Saplings	0.58 ± 0.06	0.57 ± 0.06	0.61 ± 0.07
Effective number of alleles *(Ae)*	Adults	3.47 ± 0.49	3.42± 0.54	4.03 ± 0.79
	Juveniles	3.45 ± 0.45	3.38 ± 0.53	4.10 ± 0.77
	Saplings	3.17 ± 0.43	3.21 ± 0.44	3.48 ± 0.53
Allelic Richness *(AR)*	Adults	10.2 ± 1.60	10.2 ± 1.00	10.8 ± 1.40
	Juveniles	8.17 ± 1.19	7.75 ± 1.00	8.58 ± 1.23
	Saplings	8.67 ± 1.26	6.42 ± 0.64	6.83 ± 0.92

CAPÍTULO 4

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

Pérez-Méndez N., Jordano P., Valido A. (2016)
Under review



Abstract

Defaunation of large-bodied frugivorous vertebrates is causing severe losses of crucial ecosystem functions such as dispersal of seeds. An immediate consequence might be an alteration or a severe collapse of gene-flow among fleshy-fruited plant populations, with marked effects on the spatial distribution of genetic variation at regional scales. Yet these consequences of the defaunation phenomenon remain understudied. Here we tested whether human-induced downsizing of frugivorous lizards (*Gallotia*, Lacertidae) from the Canary Islands progressively impaired plant genetic diversity, within-island population connectivity, and patterns of genetic isolation by distance of *Neochamaelea pulverulenta* (Rutaceae), which relies exclusively on these lizards for seed dispersal. Our study entails an extensive genetic sampling of plant populations along a gradient of defaunation with an increasing loss of large- and medium-sized frugivorous lizards and spanning the whole geographic range of the plant. We found no differences in genetic diversity patterns among islands, but a marked reduction of overall plant population connectivity within islands, and a significant increase of isolation by distance following the extirpation of large- and medium-sized lizards. Our results indicate that conservation of large-bodied frugivores is essential because their irreplaceable mutualistic dispersal services maintain an extensive movement of seeds across the landscape, crucial for the maintenance of genetic cohesiveness of metapopulations and the adaptive potential of plant species across their entire geographic range.

Keywords.- Extinction, landscape genetics, lizards, Canary Islands, isolation by distance, gene flow

INTRODUCTION

Defaunation, the sustained loss of distinct groups of animals (e.g., top predators, megafauna) in contemporary landscapes (Dirzo & Miranda 1991), is causing added losses of crucial ecosystem functions (Dirzo *et al.* 2014; McCauley *et al.* 2015; Malhi *et al.* 2016), such as dispersal of seeds for animal-dependent plants (Markl *et al.* 2012; Fontúrbel *et al.* 2015). Central consequences of these cascading effects includes changes in the vegetation structure (Johnson 2009; Harrison *et al.* 2013; Bakker *et al.* 2016), reductions of plant regeneration (Chapman & Chapman 1995; Cordeiro & Howe 2003; Terborgh *et al.* 2008), and carbon storage potential (Bello *et al.* 2015; Peres *et al.* 2016; Doughty *et al.* 2016), and altered evolutionary regimes (Galetti *et al.* 2013). Additional outcomes may arise following defaunation, such as the alteration or collapse of gene flow, 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 their consequences, ultimately leading to significant reductions of population-scale genetic diversity (i.e., the amount and distribution of genetic variation across populations) (Pacheco & Simonetti 2000; Pérez-Méndez *et al.* 2016). Yet the consequences at larger, regional scales remain understudied (Voigt *et al.* 2009; Calviño-Cancela *et al.* 2012).

Long-distance seed dispersal (LDD) assisted by vertebrate animals (Nathan 2008), is a crucial ecological process for maintaining the cohesiveness of the genetic makeup of fleshy-fruited plant species 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), as anthropogenic defaunation selectively removes large-bodied vertebrates (Koch & Barnosky 2006; Wright *et al.* 2007; Barnosky *et al.* 2011; Estes *et al.* 2011; Dirzo *et al.* 2014), which in turn, are primarily responsible for long distance seed dispersal across landscapes (Jordano *et al.* 2007). The deterioration of the dispersal process (i.e. the reduction of seed dispersal distances) following defaunation-driven downsizing of frugivore assemblages, is then expected to impair and eventually collapse genetic connectivity of plant populations, with pervasive effects on the regional distribution of genetic variation. Specifically, we should expect a loss of genetic diversity and increased isolation by distance across the landscape that mirrors the progressive extirpation of large frugivores. These genetic effects of human-induced disturbances remain understudied despite their potential for triggering losses of ecological functionality (Janzen 1974; Western 2001; Valiente-Banuet *et al.* 2015).

Here we use a comparative approach to address these questions within a biogeographic experiment occurring on the Canary Islands. We take advantage of a gradient of human-driven frugivore downsizing across Gran Canaria, Tenerife and La Gomera islands. We focus on the specific mutualistic interaction between *Neochamaelea pulverulenta* (Rutaceae), an endemic, fleshy-fruited shrub species which is only present in these islands, and their unique seed dispersers, the endemic frugivorous lizards of the genus *Gallotia* (Lacertidae). Large-bodied lizard species were abundant in these islands until the first arrival of Aborigines (dated ~2500 years BP) triggered a defaunation process that impacted the insular lizard fauna (Onrubia-Pintado 1987; Barahona *et al.* 2000; Gonzalez *et al.* 2014). Yet the magnitude of the impact was markedly different in each island, defining a downsizing gradient with three contrasted ecological situations (Pérez-Méndez *et al.* 2015, 2016): *i*) Gran Canaria, an island close to the original situation; i.e., preserving large-sized lizards (*G. stehlini*) that facilitate long-distance dispersal (up to 98 m), *ii*) Tenerife, hosting sub-efficient seed dispersers; i.e. medium-sized lizards (*G. galloti*) dispersing seeds over shorter distances (up to 48 m) and *iii*) 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 5 m (see Material and Methods, Fig. 4.1 , and Pérez-Méndez *et al.* 2015, 2016 for details).

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 genetic structuring (Pacheco & Simonetti 2000; Pérez-Méndez *et al.* 2016). Here we examine how the loss of mutualistic species and interactions resulting from defaunation may 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 both at population and landscape scales in islands where large-sized lizards have been extirpated. In addition, we expect a reduction of within-island population connectivity and increased genetic isolation by distance among populations mirroring the reduction of lizard-mediated dispersal distances.

To test our hypothesis we firstly compare the amounts of genetic diversity in the three scenarios, with an extensive sampling of plant individual genotypes within populations distributed across the three islands. Then, we apply population graph theory (Dyer & Nason 2004; Dyer *et al.* 2010; Dyer 2015) to look for differences in the topology of genetic connectivity networks and identify signals of collapsed connectivity at the whole scale of the species distribution. The large-scale effects of connectivity loss due to collapse of seed-mediated gene flow may not be evident by using traditional pairwise contrasts among populations. Rather, with a population graph approach we focus on changes at the whole scale of insular meta-populations. Non-defaunated landscapes will preserve population connectivity and population pairs exchanging migrants will exhibit significant conditional covariance, being linked in the network by edges with length inversely proportional to this genetic covariance (Dyer et al. 2010). Conversely, populations in more defaunated landscapes will show reduced exchange of migrants, and thus conditional independence, not being connected to each other in the network given the set of intervening populations through which they exchange migrants. Finally, we determine and compare the patterns of genetic isolation by distance (IBD; Wright 1943) across the three scenarios of defaunation. 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 (height= 1.1 ± 0.5 m; mean ± SD, *n*= 2139 adult plants) from the central islands of the Canarian archipelago (Gran Canaria, Tenerife and La Gomera). It is distributed along a coastal belt of xerophytic lowlands (< 400 m a.s.l.; Fig. 4.S1), where mean temperature is 21 ºC and annual precipitation < 200 mm (AEMET-IP 2012). This vegetation is also dominated by *Euphorbia* spp. (Euphorbiaceae), *Lycium intricatum* (Solanaceae), *Periploca laevigata* (Asclepiadaceae), *Plocama pendula*, *Rubia fruticosa* (Rubiaceae), and *Schyzogine sericea* (Asteraceae), among others. Both ants and winged-insects (ca. 12-14 species) are their main pollinators. From spring to early summer it bears fleshy fruits, which are composed of 1-4 cocci (functionally drupes). Each drupaceous coccus contains one large, hard-coated seed (8-10 mm in diameter) (Valido 1999).

The only legitimate seed dispersers of *N. pulverulenta* are medium- to large-bodied endemic lizards (g. *Gallotia*, Lacertidae) (Valido & Nogales 1994). There is a gradient of progressive reduction of both body-size of lizards and seed dispersal distances across the three islands where *N. pulverulenta* is distributed (Barahona *et al.* 2000; Pérez-Méndez *et al.* 2015, 2016). This is the result of a historical defaunation 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 the islands (Barahona *et al.* 2000; see also Fig.1 in Pérez-Méndez *et al.* 2015). In Gran Canaria lizard downsizing 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 m (Pérez-Méndez *et al.* 2016). 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 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*), yet they are critically endangered and only present with extremely reduced population sizes in relictual sites on very inaccessible cliffs (Hernández *et al.* 2000; Valido *et al.* 2000). Although only frugivorous lizards legitimately disperse seeds of *N. pulverulenta*, common kestrels (*Falco tinnunculus*, Falconidae), which prey on *Gallotia* lizards, have been also cited as potential secondary seed dispersers when discarding lizard prey remains containing seeds (Padilla *et al.* 2012). Thus, they may eventually mediate some long distance dispersal events of *N. pulverulenta* seeds.

Sampling design and microsatellite genotyping

We sampled leaves of adult individual plants from 30, 28 and 22 *N. pulverulenta* populations in Gran Canaria, Tenerife, and La Gomera, respectively (Table 4.S1), spanning the whole distribution range of the species (see potential distribution and sampled populations in Fig. 4. S1). Each population was separated by at least 0.75 km from any other population (within-island range: 0.75 – 57 km). Within each population we followed 3-4 linear transects covering an area of ~1 ha. Along these transects we sampled fresh leaves of approximately 30 adult plants per population (range: 12-33 individuals/population; Table 4.S1) totalling 2358 individuals. Individual plants were sampled at least 5 m distant from any other. Plant leaves were labelled and stored silica-dried until lab processing.

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; Pérez-Méndez *et al.* 2016). 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 for each population to assess the accumulation of sampled alleles with increasing sampling effort. We did that to ensure that our sampling effort was appropriate to characterize intra-population genetic diversity in all sampled populations (Fig. 4.S2). The amount of within population genetic diversity was estimated as the expected heterozygosity (*He*), observed heterozygosity (*Ho*), and average allelic richness (*AR*) for each population (mean ± SD) by using HIERFSTAT package (Goudet 2005) for R (R Development Core Team 2015). To test for differences in genetic diversity parameters among pairs of 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; Excoffier *et al.* 1992) with three hierarchical levels (among islands, among populations within islands, and among individuals within populations). The analysis was conducted with the R package *ade4* (Dray & Dufour 2007).

Landscape genetic connectivity

To understand how defaunation of large-bodied lizard species affects the movement of genes across the landscape we used the population genetic information (multilocus genotypes) to create a network of genetic connections (Dyer 2007) among populations for each island. 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 (edges) denoted the presence of significant genetic covariance among nodes (populations) after accounting for overall genetic covariation of the network. 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 (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 (for a detailed account of the Population Graph method see Dyer & Nason 2004; Dyer 2007). We used the popgraph package (Dyer 2014), which is implemented in R, to build the networks. We compared the resulting networks for each island using several parameters as proxies of their genetic population connectivity. *1*) *Degree*: the number of edges a population has to other populations; thus we estimated the average degree across populations on each island. A high number of edges for a given node indicates a high population connectivity, with a higher average degree across nodes suggesting a higher overall connectivity. *2*) *Edge length*: a proxy of conditional genetic dependence among pairs of populations; the shorter is the link between two populations the smaller is their genetic differentiation (conditional genetic distance, cGD) after controlling for the differences with the rest of populations (higher connectivity); we estimated the average edge length across populations on each island. *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 *igraph* package (Csárdi & Nepusz 2006) for R to calculate these parameters. 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 (Fig. 4.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.

Finally, we assessed how genetic connectivity among populations co-varies with the geographic distances in each of the three scenarios. We firstly generated an adjacency matrix *R*, where its elements $r_{ij}= 1$ if the population v_i is connected with the population v_j in the empirical networks and $r_{ij}= 0$ otherwise. Additionally we calculated a geographic distance matrix *D*, where d_{ij} is the euclidean geographic distance between populations v_i and v_j. We then categorized the distances of matrix *D* in a set of distance classes with values of 4, 6, 8, 10, 15, 20, 25, 35 and 65 km. Finally, we calculated the proportion of actual connections among populations ($r_{ij} = 1$) for each distance class. To estimate the departure of these proportions from a null expectancy, we permuted 1000 times the empirical adjacency matrix *R* and estimated the 95% confidence interval for each distance class. If the pattern of connectivity is driven by purely distance effects, we should obtain a marked departure from the random expectation and higher than expected connectivity values for specific distance intervals.

Genetic structure

A Bayesian clustering method implemented in the STRUCTURE software v.2.3 (Pritchard *et al.* 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 (30 in Gran Canaria; 28 in Tenerife; 22 in La Gomera). The optimal number of K was estimated following the method of Evanno (Evanno *et al.* 2005), implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012). We ran this model 5 independent times, and we averaged multiple runs 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) (Sokal & Sneath 1963) and displayed it as a phylogram (Fig. 4.S4). We used the *poppr* package (Kamvar *et al.* 2015) in R to perform this analysis.

	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 ± 0.27 ^{a}	4.4 ± 0.28 ^{a}	4.3 ± 0.17 ^{a}
Edge length	6.7 ± 0.34 ^{a}	7.2 ± 0.30 ^{a}	8.5 ± 0.25 ^{b}
Closeness	0.053 ± 0.002 ^{a}	0.039 ± 0.002 ^{b}	0.048 ± 0.001 ^{a}
C) IBD parameters			
Intercept	0.266 (0.256-0.276) ^{a}	0.249 (0.223-0.275) ^{a}	0.300 (0.282-0.319) ^{b}
Slope	0.0096 (0.0092-0.0104) ^{a}	0.0101 (0.0093-0.0109) ^{a}	0.0167 (0.0154-0.0181) ^{b}

Table 4.1. Summary of main genetic diversity (*He*: expected heterozygosity; *Ho*: observed heterozygosity; *AR*: average allelic richness), network (*degree, edge length, closeness*), and IBD (*intercept* and *slope* of the regression between geographic distance and between-population genetic distance) parameters of *Neochamaelea pulverulenta* (Rutaceae) populations across the three islands. For the genetic diversity parameters, data are mean ± 1 SD. For the network parameters data are mean ± 1 SE. For the IBD parameters the 95 % confidence intervals are indicated within brackets. Different letters in superscripts indicate significant differences among islands. The numbers of sampled populations (and individual plants) are 30 (883) in Gran Canaria, 28 (823) in Tenerife, and 22 (652) in La Gomera. SeeTable 4.S1 and Fig. 4.S1 for population details. Genetic diversity parameters for each population are also shown in Table 4.S1.

Isolation by distance (IBD)

Reduction of genetic connectivity among plant populations is expected to increase the effects of genetic isolation. We tested for evidence and variation in the strength of genetic isolation by distance (IBD hereafter) in the three islands. IBD was tested using a Mantel test on Euclidean genetic distances against Euclidean geographic distances between all pairs of populations. We used the isolation by distance web service (IBDWS) version 3.23 (Jensen *et al.* 2005). The significance of Mantel's Z test statistics and the estimation of the Mantel *r* coefficient were based on 10,000 permutations. We additionally tested whether the patterns depart from the expectations of a Wright null model (regression slope; ß ~ 0) or of a stepping-stone model of IBD (ß ~ 1). Under a Wright (Wright 1943) N-island model, the expectation of the regression coefficient is ß= 0.0 as all populations are equally likely to exchange genes. However, if populations are exchanging genes only

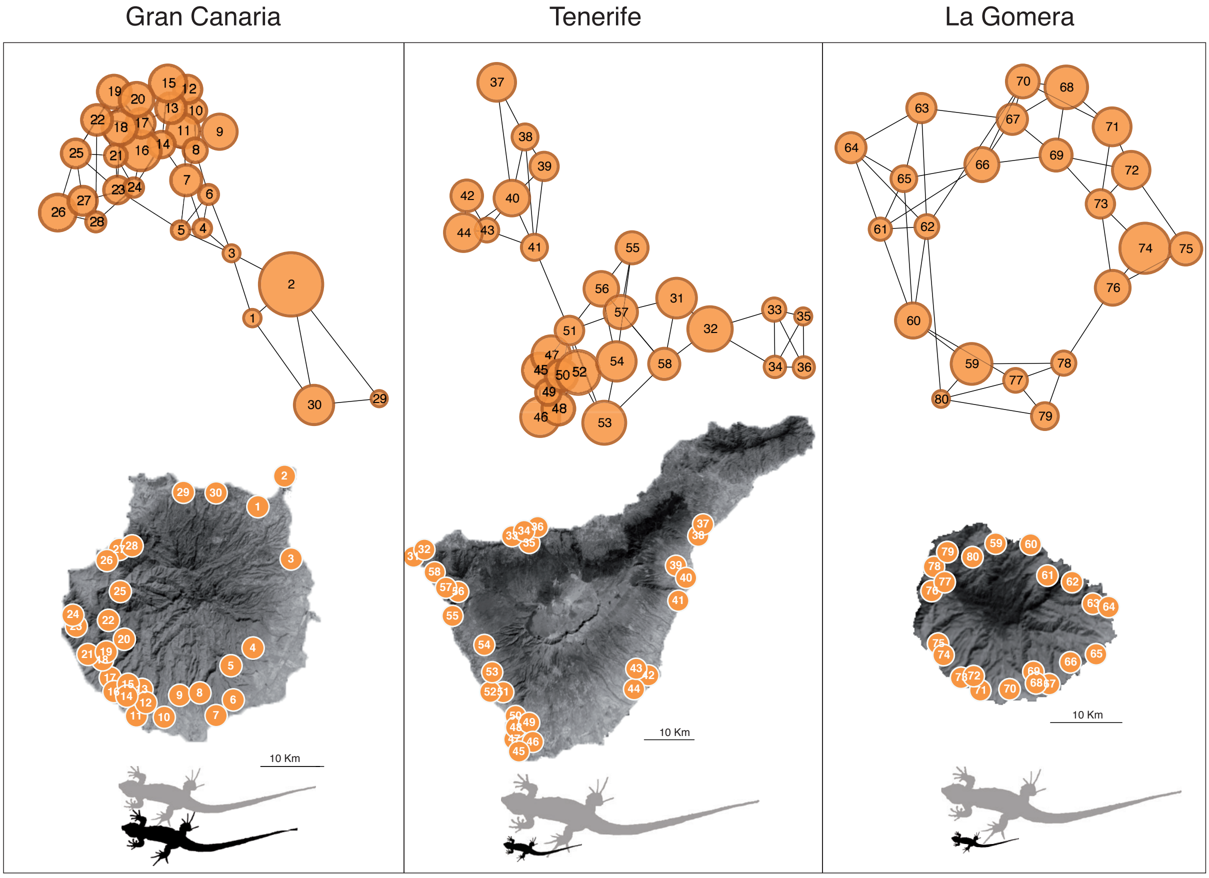


Fig. 4.1. Population graphs showing genetic connectivity patterns for *Neochamaelea pulverulenta* (Rutaceae) in Gran Canaria, Tenerife and La Gomera (Canary Islands). Nodes represent sampled populations with node sizes representing relative differences in within population genetic variation. Edge lengths show the among population genetic distances. See Table 4.S1 and Fig. 4.S1 for a detailed account of sampled populations. Grey silhouettes illustrate extinct lizard taxa in each island; black silhouettes represent the three extant, widely distributed species (photos in Fig.4.S1). Lizard silhouettes are scaled to indicate relative body sizes (largest silhouette= 502 mm SVL, Gallotia goliath from Tenerife; see Material and Methods for lizard sizes). The spatial extent of this map encompasses the entire species geographic distribution of *N. pulverulenta* in the Canary Islands (see Fig. 4.S1).

with their most immediate neighbours, as in a one-dimensional stepping stone model (Kimura & Weiss 1964), the expectation for the regression coefficient is ß= 1.0. For this we applied a reduced major axis regression. We calculated the 95% confidence intervals for both the intercepts and slopes of the regression 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, we estimated the percentage of pairs of populations showing smaller genetic distances than expected by the IBD patterns in each island; i.e. those pairs of populations located beneath the 95% confidence interval of the linear regression. We applied a chi-square to test whether these percentages were higher than expected (2.5 % of the total number of pairs of populations) and different among islands.

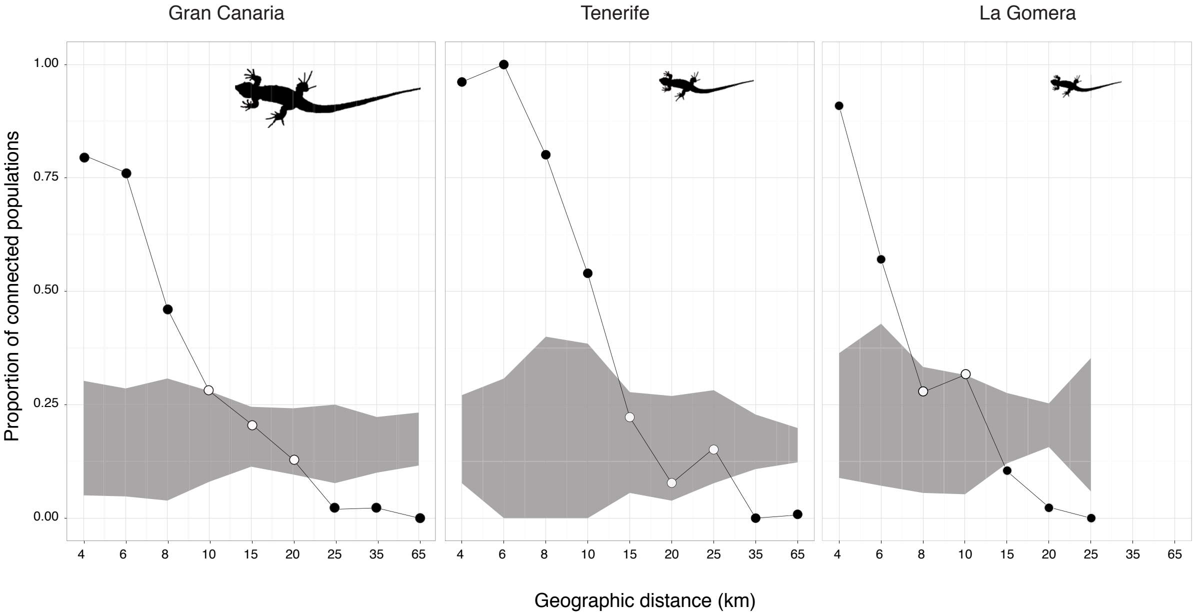


Fig. 4.2. Population genetic connectivity trends in relation to geographic distance. Points show the proportion of connected populations in the empirical networks for a set of distance classes. Black dots indicate departure from of the null hypothesis (grey areas), which is derived from randomly connected networks (1000 permutations of the empirical networks; see material and methods for a detailed account). White dots indicate no departure from the null hypothesis. Lizard silhouettes are scaled to relative body sizes (largest silhouette = 280 mm SVL, *G. stehlini* from Gran Canaria; see Material and Methods for lizard sizes)

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, 34, 35, 36) to others with much higher values (e.g. populations 16, 67, 68) (Table 4.S1). Unexpectedly, the most defaunated La Gomera scenario showed the highest genetic diversity at the island level (i.e. average genetic diversity of populations within islands), with all parameters (except *He*) being significantly higher than Gran Canaria and Tenerife ($p< 0.05$ for all pairwise contrasts; Table 4.1). In turn, *He* was similar between La Gomera and Gran Canaria, and both islands showed higher values than Tenerife (Table 4.1). The AMOVA indicates that among the spatial hierarchical levels examined, most variation is explained by among-island differences (17.6%), with lower values 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, within-individual variation (65.3%) (Table 4.S2).

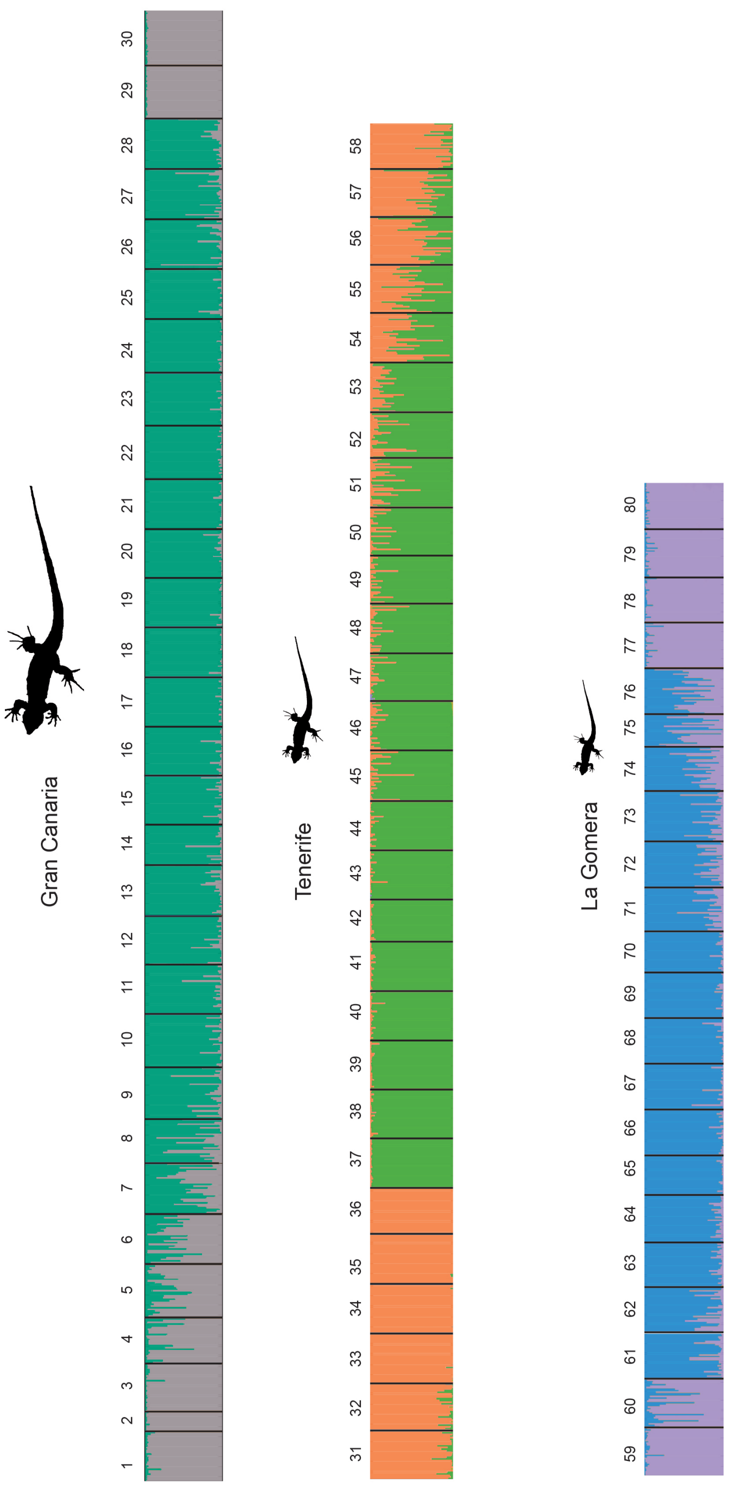


Fig. 4.3. Estimated insular population structure for *Neochamaelea pulverulenta* (Rutaceae) according to Bayesian clustering analysis performed in the STRUCTURE package (Pritchard *et al.* 2000). Bars of the histograms represent individuals of *N. pulverulenta* within populations (numbers match with population codes of Table 4.S1 and Fig. 4.S1). Colours indicate the membership probability of individuals to each cluster ($K= 2$ in each island; see Fig. 4.S5 for details). Lizard silhouettes are scaled to relative body sizes (largest silhouette = 280 mm SVL, *Gallotia stehlini* from Gran Canaria; see Material and Methods for lizard sizes).

Landscape genetic connectivity

The network topology parameters differ markedly among islands (Table 4.1; Fig. 4.1). First, *degree* decreases from Gran Canaria to Tenerife and to La Gomera, although no significant statistical differences were found ($p > 0.05$). Secondly, the average *edge length* markedly increases from Gran Canaria to Tenerife, and to La Gomera, although significant statistical differences were found only 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 only in Tenerife ($p < 0.01$). Overall, all the parameters tested suggest the highest connectivity in Gran Canaria. Moreover, the reduction of genetic connectivity in relation to the geographic distance between plant populations was less pronounced in both Gran Canaria and Tenerife than in La Gomera, where it drops sharply beyond 4 km (Fig. 4.2).

Isolation by distance (IBD)

The STRUCTURE analysis following the Evanno method identified two distinct genetic clusters ($K = 2$) in each island (Figs. 4.4 and 4.S5). The assignment probability of individuals in each detected cluster showed a gradual decrease in a clockwise direction suggesting a pattern of progressive genetic differentiation among populations (Fig. 4.4). This pattern was consistent across the three islands. In turn, the UPGMA cluster analysis also

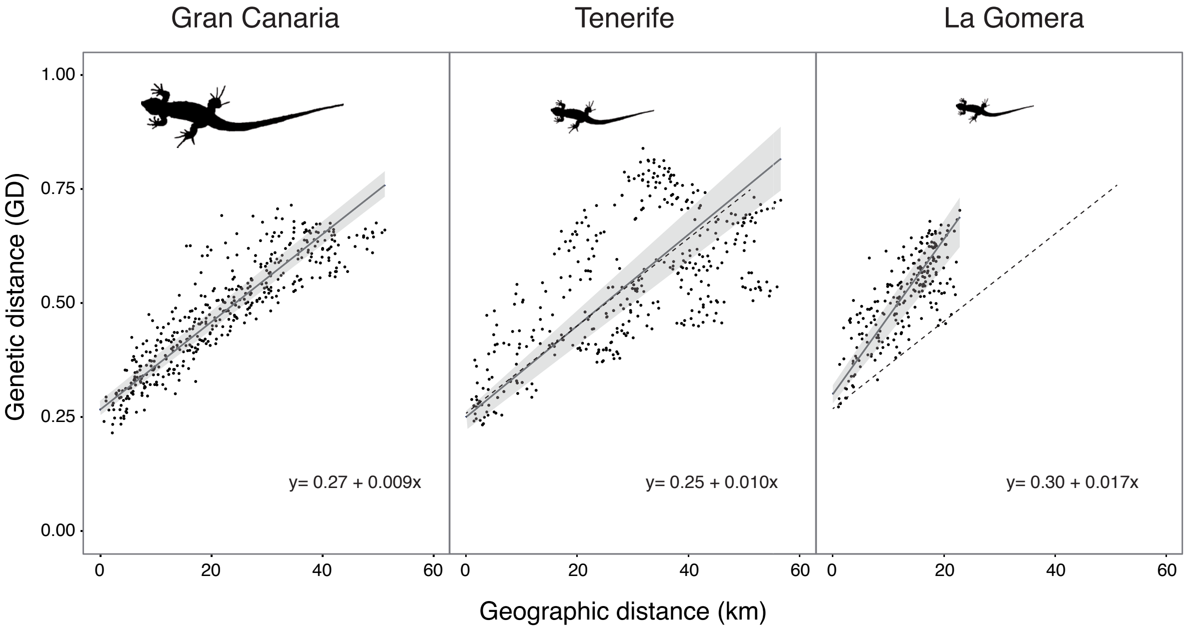


Fig. 4.4. Isolation by distance (IBD) patterns for *Neochamaelea pulverulenta* (Rutaceae) populations in the three islands. Representation of the Euclidean genetic distance and the Euclidean geographic distance (km). The reduced major axis regression line (solid line) and the bootstrapped (1000 resamplings) 95% confidence interval (grey area) are shown. The regression line of Gran Canaria is projected in panels of Tenerife and La Gomera to allow among island comparisons (dashed lines). The regression equations are also shown within panels ($p < 0.01$). Lizard silhouettes are scaled to indicate relative body sizes (largest silhouette = 280 mm SVL; *Gallotia stehlini* from Gran Canaria; see Material and Methods for lizard sizes).

grouped populations according to their relative geographic position (Fig. 4.S4).

A model of IBD fits the patterns of genetic differentiation found in the three islands very well and supports the results of the analysis of genetic population connectivity. Both the Mantel tests ($p< 0.01$ for the three islands) and the reduced major axis regressions (Gran Canaria, $r^2= 0.81$, $p< 0.01$; Tenerife, $r^2= 0.41$, $p< 0.01$; La Gomera, $r^2= 0.61$, $p< 0.01$) indicate more marked IBD pattern in La Gomera when compared to Gran Canaria and Tenerife. The slightly worse fit in Tenerife (41 %) mostly results from the deviation of a small set of isolated populations from the North of the island (Population 33-36; see Fig. 4.S1). When we excluded these populations from analyses, the amount of explained variation increased to 67% (Fig. 4.S6 for IBD pattern). The magnitude (i.e. the slope) of IBD was significantly higher in La Gomera than in Gran Canaria and Tenerife, as shown by the largest slope and intercept of the regression (t-test; $p< 0.01$) (Fig. 4.3). This suggests that La Gomera situation was closer to a stepping-stone model (ß~1) whereas Gran Canaria and Tenerife were closer to a Wright null model (ß~0). Finally, the percentages of population pairs with lower genetic distances (suggesting greater connectivity) than expected by the IBD patterns (i.e., number of population pairs with significant outlier values below the IBD slope, Fig. 4.3, relative to all possible pairs) were higher in Gran Canaria (34 %) and Tenerife (35%) than in La Gomera (19%) (chi-square; $p< 0.05$; Fig. 4.3). The observed pattern of IBD across the three islands supports the large scale patterns of genetic structure revealed by STRUCTURE and UPGMA analyses.

DISCUSSION

Defaunation-mediated downsizing of frugivorous vertebrates may trigger cascading effects with a myriad of consequences for plant demography (Janzen 1986; Cordeiro & Howe 2003; Traveset & Riera 2005; Wotton & Kelly 2011; Traveset *et al.* 2012; Costa-Pereira & Galetti 2015) and plant genetics (Pacheco & Simonetti 2000; Voigt *et al.* 2009; Calviño-Cancela *et al.* 2012; Pérez-Méndez *et al.* 2016). Here we demonstrate that when mutualistic interactions are functionally disrupted (La Gomera) pervasive genetic impacts may spread well beyond the local population scale. Although genetic diversity appear to be not affected by reduced seed dispersal, the contrasting topology of the population genetic networks revealed a significant reduction of genetic connectivity among plant populations within the more defaunated islands, especially in La Gomera, where dispersal has collapsed. Accordingly, we also found the strongest pattern of isolation by distance in this island when compared with islands preserving the functionality of lizard-mediated seed dispersal (Gran Canaria and Tenerife). Our previous study (Pérez-Méndez *et al.* 2016) showed a progressive truncation of seed dispersal distances of *N. pulverulenta* within local populations, mirroring the lizard downsizing with marked implications for the fine-scale spatial genetic structure. Here we show that these effects extend beyond local population limits to have consequences for the spatial distribution of genetic diversity at much broader regional 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 (White *et al.* 2002; England *et al.* 2003). The reduction, an eventual collapse, of seed-mediated gene flow in populations hosting small- to medium-sized lizard species may result in an increase of genetic isolation and genetic drift, with added reductions of genetic variation across *N. pulverulenta* populations. Instead, genetic 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 indicates 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 centre of high genetic diversity and source of propagules for the other islands, as reported for several Canarian plant species (García-Verdugo *et al.* 2010; Salvo *et al.* 2010). 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, pollinator guilds in xerophytic areas are quite similar among these islands (Trøjelsgaard *et al.* 2015), thus we have no evidences supporting this. Finally, differences may arise as the result of a higher human pressure and habitat fragmentation (Fig. 4.S1) in the most populated Gran Canaria (population density = 546 inhabitants/km^2) and Tenerife (population density = 442 inhab./km^2) islands relative to the less populated La Gomera island (population density = 60 inhab./km^2) (Instituto Canario de Estadística, http://www.gobiernodecanarias.org/istac) . Whatever the explanation, the high levels of genetic diversity found in the island with the most downsized disperser suggest a delayed effect of defaunation relative to other anthropogenic pressures such as fragmentation, which is usually accompanied by a rapid depauperation of genetic pools (Young *et al.* 1996).

Landscape genetic connectivity

The differences in topology of the population graphs corresponding to the different island scenarios suggest that, despite no differences in overall genetic diversity, marked variation exists in the spatial structure of the three genetic pools. This variation was reflected in changes among islands in overall connectivity and relative isolation among populations.

Our results derived from population graph analyses suggest that gene flow of *N. pulverulenta* was mostly restricted among populations separated by no more than 10 km (Fig. 4.2), a very short distance when compared with other animal-dispersed plant species (Dyer & Nason 2004; Sork *et al.* 2010; Herrera-Arroyo *et al.* 2013; Dyer 2015). For example, the population graph reported by Sork *et al.* (2010) for *Quercus lobata* in California shows several edges connecting populations geographically separated by much further distances (> 400 km). Thus, low genetic connectivity detected in the three study islands indicates overall highly restricted gene flow among *N. pulverulenta* populations. This is most likely the result of the specific interaction with their unique primary seed dispersers (Valido 1999; Pérez-Méndez *et al.* 2015) and a small set of pollinator species, as well as the potential barriers for gene flow in the insular environments (with deep valleys and sharp ridges characteristic of these volcanic islands). Fruit-eating lizards (*Gallotia* spp.) have very limited home ranges (Molina-Borja 1985) with reported seed dispersal distances below 100 m (Pérez-Méndez *et al.* 2016). Likewise, the species-poor assemblage of pollinators is composed by several species of ants, small bees, and flies, which seem to exhibit restricted foraging patterns according with pollination distances (median: ~ 40 m) estimated in a previous study by using parentage analysis of embryos in Tenerife (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 dispersers remains unstudied.

Despite this general trend, topologies of the genetic networks also indicate that genetic connectivity markedly varied according with the defaunation status of the islands. As we hypothesized the meta-population of Gran Canaria, which still preserves large-size lizards, showed the highest connectivity; i.e. the largest number of connections among populations, the shortest links, the largest closeness and a slight decrease of connectivity with distance. In contrast, the same parameters indicate signs of reduced connectivity in Tenerife and suggest more marked isolation and severe loss of connectivity in the defaunated La Gomera. These results are conservative as La Gomera 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 smaller in La Gomera (13 km ± 6 km; mean ± 1 SD) than in Gran Canaria (22 ± 12 km) and Tenerife (29 ± 15 km). As previously discussed (Pérez-Méndez *et al.* 2015, 2016), these differences in connectivity could not be interpreted in terms of variation in abiotic conditions among islands, given the similar climate (AEMET-IP 2012), and soil properties of these lowland arid scrublands inhabited by *N. pulverulenta* (Fernández Caldas *et al.* 1987). In addition, habitat fragmentation might have been more severe in the most developed Gran Canaria and Tenerife islands according with the current distribution of *N. pulverulenta* (Fig. 4.S1). Thus, our results are very conservative supporting the conclusion of a severe impact of defaunation in La Gomera. Given the evidences of seed dispersal collapse in this island, the most parsimonious explanation for the observed patterns of reduced population connectivity and large IBD is the collapse of dispersal driven by defaunation process. 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 in this island, one wonders how *N. pulverulenta* preserves residual connectivity among their populations. First, despite pollen movement not being very extensive, several species such as honey bees or native bees may eventually move over large distances (Greenleaf *et al.* 2007), providing opportunities for gene exchange among relatively distant populations. 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).

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. We would expect similar genetic outcomes in other insular ecosystems of low functionally redundancy, where key large frugivores have become extinct (Meehan *et al.* 2002; McConkey & Drake 2006, 2015; Wotton & Kelly 2011). Even more diversified ecosystems with low functional redundancy among frugivore species are prone to experience similar impacts (Janzen 1974; McConkey & Brockelman 2011; Bueno *et al.* 2013). For example, Mueller *et al.* 2014, by combining gut retention times and movement data, demonstrate that the largest frugivorous birds (hornbills) disproportionally contribute to connectivity of fragmented landscapes in a subtropical forest of South Africa. Functional extinction of these species is expected to collapse seed dispersal beyond local patches, triggering a parallel reduction of overall landscape genetic connectivity.

Isolation by distance (IBD)

Genetic isolation by distance (IBD) (Wright 1943) is a common pattern among plant species (Sexton *et al.* 2014), which theoretically arises as the outcome of a limited exchange of genes among nearby populations, independently of their environmental differences (Bradburd *et al.* 2013; Sexton *et al.* 2014; Wang & Bradburd 2014). Given the short dispersal distances previously reported for our study system (Pérez-Méndez *et al.* 2016), we can predict a marked pattern of IBD in the three islands (Hamrick *et al.* 1993). Accordingly, geographical distance explained a large proportion of the spatial distribution genetic variation of *N. pulverulenta*, especially in Gran Canaria and La Gomera (r^2= 81% and 61%, respectively). Overall this suggests a marked IBD pattern in the three island scenarios, yet with interesting differences among them.

The magnitude of IBD (i.e., the slope of the regression) was much larger in the most defaunated island, lacking the efficient, large dispersers (La Gomera) than in the islands hosting medium- and large-sized lizards (Tenerife and Gran Canaria, respectively). This result is not surprising, when considering the documented absence of long distance dispersal events (Pérez-Méndez *et al.* 2016). However, contrary to our predictions, the slopes of the IBD regression, together with the percentage of pairs of populations showing smaller genetic distances than expected from purely geographic distances, were similar in Gran Canaria and Tenerife. As discussed before pollen movement may be more extensive in Tenerife than Gran Canaria, compensating the loss of largest lizards, however similar pollinator assemblage are present in both islands (Trøjelsgaard *et al.* 2015). Even though primary seed dispersal distances being almost half in Tenerife than in Gran Canaria (Pérez-Méndez *et al.* 2016), secondary dispersal by predatory birds (Padilla *et al.* 2012) may counterbalance this reduction by promoting gene flow among nearby populations. However, if this is true, Gran Canaria and Tenerife should also exhibit similar patterns of genetic connectivity, which is not the case. Therefore, limitation of seed dispersal driven by lizard defaunation appears to have affected the severely defaunated La Gomera island, with effects also evident in the lower connectivity (especially the lowest closeness) among Tenerife populations compared to the non-defaunated Gran Canaria populations.

The few previous studies assessing the impact of defaunation on the patterns of genetic differentiation at the landscape scale (Voigt *et al.* 2009; Calviño-Cancela *et al.* 2012) have not shown results consistent with those reported here. Calviño-Cancela *et al* (2012) found that extensive pollen-mediated gene flow counterbalanced the extinction of the only seed dispersers (frugivorous lizards) of the shrub *Daphne rodriguezii* (Thymelaeaceae) preventing high genetic differentiation among populations. In turn, Voigt *et al* (2009) detected less genetic differentiation among the *Commiphora guillauminii* (Burseraceae) tree populations from Madagascar (lacking large-bodied frugivores) than in *Commiphora harveyi* populations from South Africa (preserving large species). They attribute this unexpected result to differences in the historical distribution of the two species. However, given that they compare two distinct species, this pattern may arise as the outcome of different phylogeographic histories. Thus, more studies are needed to clarify the potential impact of frugivore downsizing (and reduced gene-flow) on the distribution of genetic variation across landscapes. As far as we know, this is the first study that, by including three scenarios with a gradient of progressive downsizing, demonstrates a pervasive impact of defaunation on large-scale genetic structuring of a plant species.

Our findings suggest that downsizing of mutualistic vertebrate frugivores, that results from the selective defaunation of large-bodied species, hinders seed-mediated gene flow among plant populations. The deterioration of this process 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. These effects will be especially important for long-lived, large-seeded woody plant species of insular systems. The synergistic combination of the reduced functional redundancy (Woodward *et al.* 2005; González-Castro *et al.* 2015) and the dramatic, size-specific anthropogenic defaunation associated to these environments (Hansen & Galetti 2009) make them especially vulnerable to the extinction of crucial mutualistic interactions.

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Apéndices

CAPÍTULO 4: Persisting in defaunated landscapes: reduced plant population connectivity after seed dispersal collapse

TABLE 4.S1. INFORMATION OF SAMPLED POPULATIONS

Location, number of plants sampled, and genetic diversity indices of all sampled populations (n= 80) of *Neochamaelea pulverulenta* (Rutaceae) across the three Canarian islands (Gran Canaria, Tenerife, La Gomera). Geographical coordinates are expressed in decimal. Data of genetic diversity indices are mean ± SD of expected heterozygosity (He), observed heterozygosity (Ho), and allelic richness (AR). See Fig. 4.S1 for locations on the map.

Population	**Code**	**Island**	**Latitude**	**Longitude**	**Sample size**	**He**	**Ho**	**AR**
Los Giles	1	Gran Canaria	28.125347	-15.460028	30	0.49 ± 0.29	0.48 ± 0.3	3.71 ± 1.76
La Isleta	2	Gran Canaria	28.175283	-15.421117	12	0.47 ± 0.27	0.37 ± 0.24	2.88 ± 1.34
Jinámar	3	Gran Canaria	28.03735	-15.408592	29	0.48 ± 0.23	0.48 ± 0.24	3.34 ± 1.39
Barranco de Tirajana	4	Gran Canaria	27.888915	-15.480562	28	0.57 ± 0.19	0.56 ± 0.21	3.85 ± 1.26
Los Gallegos	5	Gran Canaria	27.859346	-15.522248	32	0.58 ± 0.19	0.57 ± 0.19	4.08 ± 1.26
Montaña de Tabaiba	6	Gran Canaria	27.8023	-15.517867	30	0.61 ± 0.14	0.54 ± 0.13	4.42 ± 1.25
Barranco de las Burras	7	Gran Canaria	27.776124	-15.550269	30	0.66 ± 0.16	0.65 ± 0.14	4.87 ± 1.71
Montañeta Redonda	8	Gran Canaria	27.814059	-15.580894	27	0.64 ± 0.18	0.62 ± 0.19	4.82 ± 1.7
Barranco de Ayagaures	9	Gran Canaria	27.810056	-15.61905	31	0.69 ± 0.13	0.64 ± 0.16	5.27 ± 2.04
Cuartería de los Indígenas	10	Gran Canaria	27.77325	-15.648168	32	0.65 ± 0.2	0.66 ± 0.23	4.95 ± 2
Barranco de la Verga I	11	Gran Canaria	27.780511	-15.695091	29	0.68 ± 0.19	0.64 ± 0.2	5.33 ± 2.12
Barranco de la Verga II	12	Gran Canaria	27.797568	-15.682778	30	0.67 ± 0.16	0.62 ± 0.19	5.3 ± 2.38
Barranco de La Perra	13	Gran Canaria	27.814776	-15.695991	30	0.68 ± 0.19	0.65 ± 0.2	5.41 ± 2.07
Barranco de Tauro I	14	Gran Canaria	27.818639	-15.718887	24	0.66 ± 0.17	0.61 ± 0.15	5.22 ± 2.42
Barranco de Tauro II	15	Gran Canaria	27.828422	-15.716317	30	0.68 ± 0.16	0.62 ± 0.14	5.44 ± 2.49
Barranco de Tiritaña	16	Gran Canaria	27.816082	-15.74321	29	0.67 ± 0.17	0.65 ± 0.14	5.76 ± 2.57
Barranco de Mogán I	17	Gran Canaria	27.839225	-15.750136	30	0.65 ± 0.18	0.57 ± 0.17	5.29 ± 2.25
Barranco de Veneguera I	18	Gran Canaria	27.869183	-15.764745	30	0.67 ± 0.19	0.63 ± 0.2	5.49 ± 2.04
Barranco de Veneguera II	19	Gran Canaria	27.882428	-15.757628	29	0.64 ± 0.2	0.59 ± 0.21	5.44 ± 2.25
Barranco de Veneguera III	20	Gran Canaria	27.90361	-15.723602	30	0.67 ± 0.18	0.64 ± 0.19	5.36 ± 2.16
Barranco de Tasarte I	21	Gran Canaria	27.878133	-15.792349	30	0.63 ± 0.19	0.61 ± 0.2	4.86 ± 2.29
Barranco de Tasarte II	22	Gran Canaria	27.935047	-15.753334	32	0.63 ± 0.18	0.62 ± 0.23	4.75 ± 1.48
Barranco de Tasartico	23	Gran Canaria	27.92515	-15.814221	32	0.64 ± 0.22	0.61 ± 0.21	5.64 ± 2.21
Barranco de Güi-Güi	24	Gran Canaria	27.933504	-15.820674	32	0.62 ± 0.24	0.62 ± 0.24	4.83 ± 2.29
Barranco de la Aldea	25	Gran Canaria	27.983044	-15.731114	30	0.6 ± 0.15	0.58 ± 0.13	4.11 ± 1.39
Tirma	26	Gran Canaria	28.034736	-15.756321	30	0.62 ± 0.24	0.54 ± 0.24	4.82 ± 2.27
El Risco	27	Gran Canaria	28.053333	-15.732778	30	0.55 ± 0.23	0.49 ± 0.25	4.16 ± 1.99
Guayedra	28	Gran Canaria	28.059221	-15.72901	30	0.55 ± 0.21	0.51 ± 0.21	4.05 ± 1.93
El Salado	29	Gran Canaria	28.145995	-15.603889	32	0.43 ± 0.29	0.40 ± 0.26	2.7 ± 1.22
Lomo de La Guancha	30	Gran Canaria	28.141794	-15.554902	33	0.46 ± 0.17	0.47 ± 0.16	3.1 ± 0.89
Punta de Teno Bajo I	31	Tenerife	28.347297	-16.913317	30	0.61 ± 0.19	0.53 ± 0.18	4.74 ± 1.76
Punta de Teno Bajo II	32	Tenerife	28.359722	-16.900833	29	0.69 ± 0.13	0.67 ± 0.14	5.15 ± 1.57
Cueva del Rey	33	Tenerife	28.383056	-16.711667	30	0.37 ± 0.28	0.38 ± 0.26	2.73 ± 1.38
Punta de Juan Centella	34	Tenerife	28.392222	-16.693889	30	0.4 ± 0.2	0.39 ± 0.22	2.69 ± 1.25
Barranco de las Ánimas	35	Tenerife	28.390033	-16.683754	30	0.36 ± 0.24	0.29 ± 0.21	2.28 ± 0.89
Punta Charco del Viento	36	Tenerife	28.399167	-16.673333	28	0.3 ± 0.25	0.25 ± 0.21	2.12 ± 0.91
Tabaiba Alta	37	Tenerife	28.403889	-16.339167	30	0.59 ± 0.12	0.52 ± 0.13	3.67 ± 1.29
Barranco Hondo	38	Tenerife	28.389167	-16.348611	29	0.51 ± 0.18	0.42 ± 0.17	3.63 ± 1.22
La Hidalga	39	Tenerife	28.331491	-16.392895	29	0.55 ± 0.23	0.45 ± 0.23	4.11 ± 1.22
Malpaís de Güímar	40	Tenerife	28.310278	-16.372778	30	0.64 ± 0.16	0.61 ± 0.17	4.74 ± 1.6
Punta Prieta	41	Tenerife	28.270331	-16.388206	30	0.56 ± 0.22	0.52 ± 0.23	4.42 ± 2.08

Abades	42	Tenerife	28.139758	-16.456732	26	0.59 ± 0.16	0.59 ± 0.15	4.44 ± 1.81
Villa de Arico	43	Tenerife	28.151277	-16.471664	30	0.57 ± 0.16	0.56 ± 0.19	4.35 ± 1.52
Barranco de Tajao	44	Tenerife	28.115475	-16.476023	30	0.6 ± 0.16	0.55 ± 0.17	4.65 ± 1.55
Malpaís Punta de Rasca	45	Tenerife	28.011664	-16.698341	30	0.64 ± 0.13	0.57 ± 0.16	4.84 ± 1.6
El Palm-Mar	46	Tenerife	28.02317	-16.691881	30	0.64 ± 0.14	0.59 ± 0.16	4.94 ± 1.7
Montaña de Guaza I	47	Tenerife	28.033075	-16.707498	29	0.65 ± 0.14	0.59 ± 0.16	5.2 ± 1.5
Montaña de Guaza II	48	Tenerife	28.048539	-16.700953	30	0.65 ± 0.11	0.62 ± 0.15	4.94 ± 1.68
Montaña de Guaza III	49	Tenerife	28.056667	-16.69	29	0.61 ± 0.15	0.58 ± 0.19	4.75 ± 1.5
Arona	50	Tenerife	28.068611	-16.710833	29	0.65 ± 0.14	0.62 ± 0.14	4.93 ± 2.04
Caleta de Adeje I	51	Tenerife	28.110278	-16.744444	30	0.66 ± 0.13	0.65 ± 0.15	4.85 ± 1.58
Caleta de Adeje II	52	Tenerife	28.110833	-16.761111	28	0.68 ± 0.16	0.65 ± 0.15	5.33 ± 1.73
Los Menores	53	Tenerife	28.145222	-16.757279	30	0.63 ± 0.21	0.64 ± 0.26	4.77 ± 1.9
Barranco de Guía	54	Tenerife	28.192996	-16.77286	30	0.71 ± 0.14	0.74 ± 0.17	5.23 ± 1.82
Los Gigantes	55	Tenerife	28.243778	-16.835992	29	0.63 ± 0.18	0.59 ± 0.24	4.52 ± 1.67
Barranco del Natero	56	Tenerife	28.288611	-16.833746	30	0.62 ± 0.19	0.53 ± 0.18	4.68 ± 2.13
Barranco de Masca	57	Tenerife	28.293082	-16.848972	30	0.53 ± 0.21	0.52 ± 0.2	4.25 ± 1.49
Barranco de Los Carrizales	58	Tenerife	28.320202	-16.871472	28	0.51 ± 0.26	0.48 ± 0.25	3.97 ± 1.55
Lomo de La Sepultura	59	La Gomera	28.198683	-17.2474	32	0.65 ± 0.17	0.62 ± 0.18	4.77 ± 1.94
Cerro del Cepo	60	La Gomera	28.19775	-17.203267	32	0.62 ± 0.17	0.58 ± 0.18	4.82 ± 1.82
El Palmar	61	La Gomera	28.163056	-17.181944	30	0.6 ± 0.24	0.6 ± 0.26	4.85 ± 1.99
Barranco de Juel	62	La Gomera	28.155833	-17.150833	30	0.6 ± 0.25	0.63 ± 0.29	4.72 ± 2.05
Aluse	63	La Gomera	28.131716	-17.124567	29	0.64 ± 0.16	0.66 ± 0.17	5.47 ± 2.23
Punta Llana	64	La Gomera	28.128056	-17.104722	31	0.65 ± 0.18	0.66 ± 0.21	5.72 ± 2.6
Punta de Juan Daza	65	La Gomera	28.075333	-17.12045	26	0.58 ± 0.24	0.6 ± 0.26	4.68 ± 2.1
Roque de la Roja	66	La Gomera	28.066399	-17.152998	30	0.69 ± 0.15	0.69 ± 0.17	5.88 ± 1.9
Barranco de Chinguarime	67	La Gomera	28.042102	-17.179768	30	0.68 ± 0.18	0.64 ± 0.2	6.5 ± 2.49
Barranco de Tapahuga I	68	La Gomera	28.046389	-17.190556	30	0.66 ± 0.23	0.65 ± 0.25	6.12 ± 2.62
Barranco de Tapahuga II	69	La Gomera	28.055833	-17.199722	30	0.68 ± 0.18	0.64 ± 0.22	5.59 ± 2.04
Antoncojo	70	La Gomera	28.036309	-17.229809	26	0.67 ± 0.18	0.64 ± 0.23	5.62 ± 2.12
Barranco de Quise	71	La Gomera	28.035083	-17.266825	29	0.7 ± 0.12	0.7 ± 0.15	5.83 ± 2.13
Las Negras	72	La Gomera	28.05105	-17.280967	30	0.69 ± 0.13	0.66 ± 0.19	5.45 ± 2.11
Barranco de Arguayoda	73	La Gomera	28.049144	-17.291173	33	0.68 ± 0.1	0.63 ± 0.15	4.99 ± 1.94
Lomo Gerián	74	La Gomera	28.074495	-17.313254	29	0.72 ± 0.11	0.73 ± 0.13	5.41 ± 2.34
Argaga	75	La Gomera	28.083808	-17.320161	21	0.64 ± 0.13	0.67 ± 0.13	4.6 ± 1.8
Riscos de Heredia	76	La Gomera	28.145833	-17.328333	30	0.68 ± 0.12	0.66 ± 0.13	5.44 ± 1.96
Taguluche	77	La Gomera	28.15575	-17.311867	30	0.58 ± 0.22	0.6 ± 0.24	4.7 ± 2.48
Barranco de Los Monos	78	La Gomera	28.172783	-17.325317	30	0.6 ± 0.21	0.6 ± 0.21	5.02 ± 2.34
Tazo	79	La Gomera	28.189233	-17.308483	32	0.61 ± 0.18	0.61 ± 0.21	5.21 ± 2.65
La Era Nueva	80	La Gomera	28.183617	-17.276733	31	0.55 ± 0.2	0.54 ± 0.2	3.7 ± 1.71

TABLE 4.S2. ANALYSIS OF MOLECULAR VARIANCE (AMOVA)

Results of the analysis of molecular variance (AMOVA) for *Neochamaelea pulverulenta* (Rutaceae) performed with 12 specific microsatellite markers for the whole data set (2358 individual plants from 80 populations in three islands) and considering three hierarchical spatial scales. Degrees of freedom, sum of squares, mean of squares, and percentage of explained variance are referred to as *df, SS, MS*, and *% Var,* respectively. * $p < 0.05$.

Source of variation	df	SS	MS	% Var
Among islands	2	2293	1497	17.6
Among populations: within islands	77	3636	47	13.9*
Among individuals: within populations	2278	8652	4	3.2
Error	2358	8158	3	65.3

FIG. 4.S1. POTENTIAL DISTRIBUTION OF *Neochamaelea pulverulenta* (RUTACEAE)

The potential geographic distribution (blue areas) of *Neochamaelea pulverulenta* (Rutaceae) in the Canary Islands (modified from ATLANTIS 3.1; http//: www.biodiversidadcanarias.es/atlantis/). Orange dots indicate sampled populations (*n*= 80; numerical codes match those of Table 4.S1). *N. pulverulenta* relies exclusively on lizards for seed dispersal and is only found in lowland areas (< 400m a.s.l.) of Gran Canaria (*Gallotia stehlini*), Tenerife (*G. galloti*), and La Gomera (*G. caesaris*). Black silhouettes (indicating relative sizes) represent the three extant, widely distributed, lizard species (photos) on each island (largest silhouette = 280 mm SVL for *G. stehlini* in Gran Canaria). For details about natural history and the extinction patterns of lizard species in the Canary Islands see also Barahona *et al.* 2000; Pérez-Méndez *et al.* 2015, 2016. The spatial extent of this map encompasses the entire species distribution of *N. pulverulenta.*

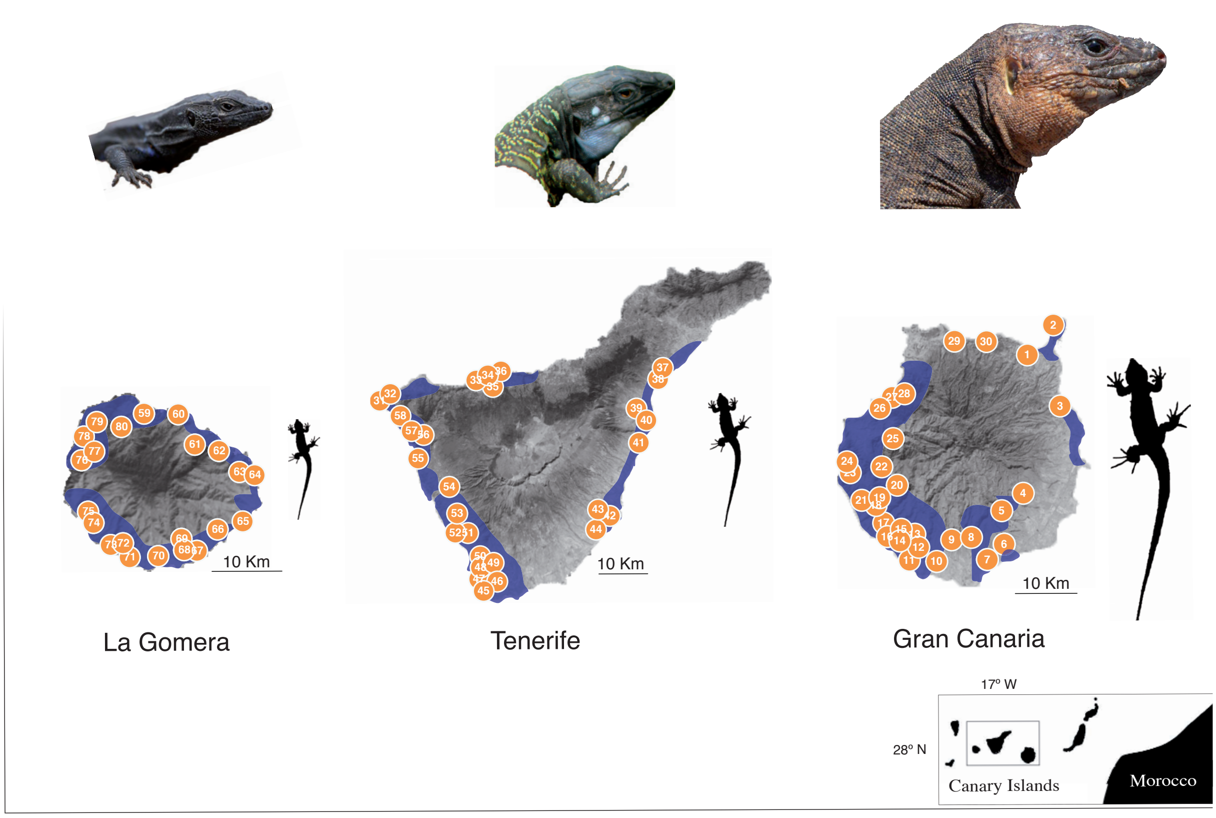
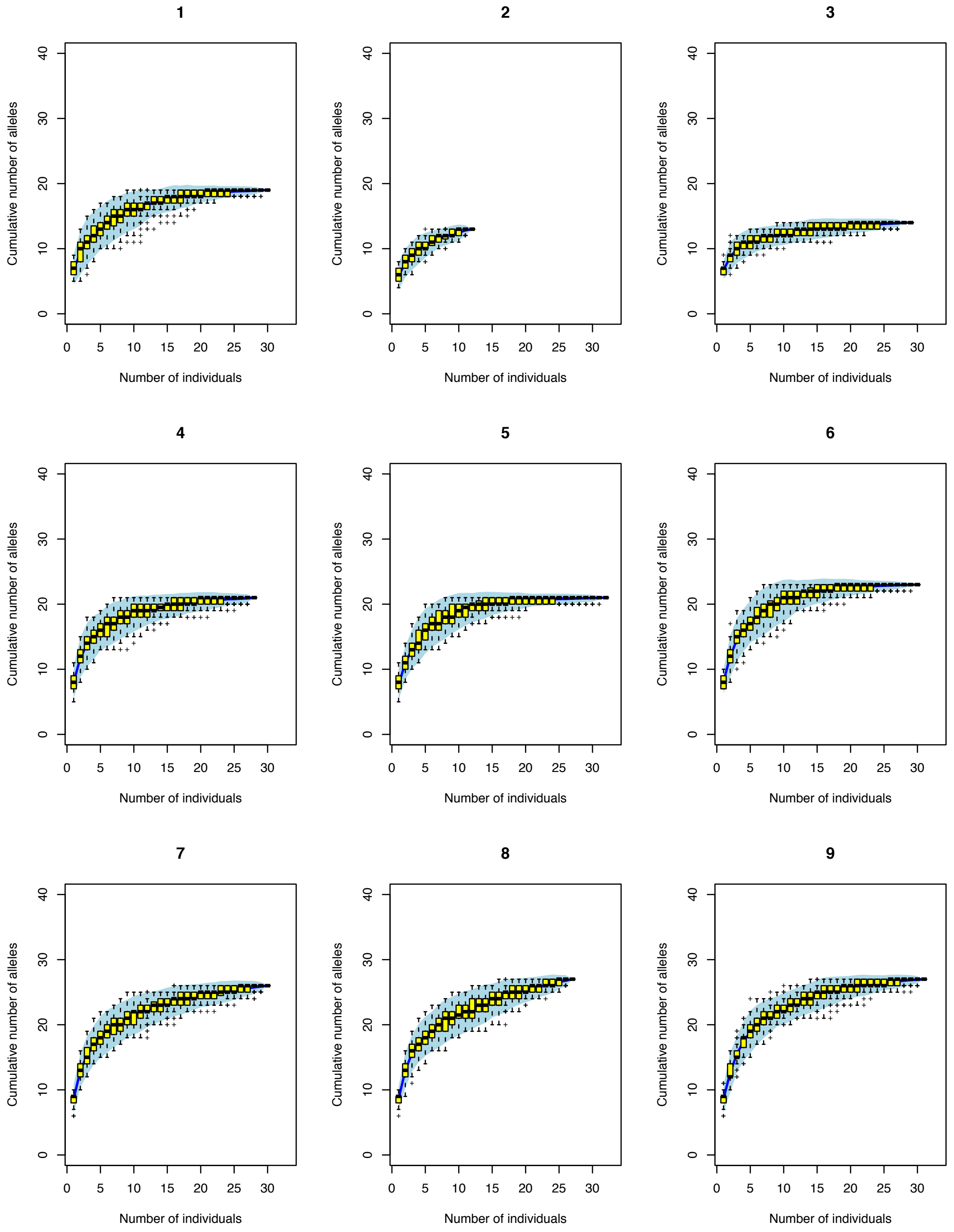


FIG. 4.S2. SAMPLE-BASED ACCUMULATION CURVES OF ALLELIC DIVERSITY

Sample-based accumulation curves of allelic diversity in relation to the number of individual plants genotyped for each studied population of *Neochamaelea pulverulenta* (Rutaceae). Curves indicate the accumulation of distinct alleles with increasing sampling effort. Population codes are indicated above each panel (see also Fig. 4S1 and Table 4.S1 for geographic locations of each population). Population codes 1-30 correspond to Gran Canaria; 31-58 to Tenerife; and 59-80 to La Gomera. In light blue, 95% confidence interval; box plots in yellow indicate median, 25-75 percentiles and outlier values (+).



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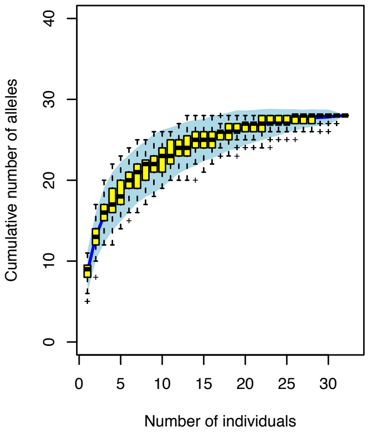

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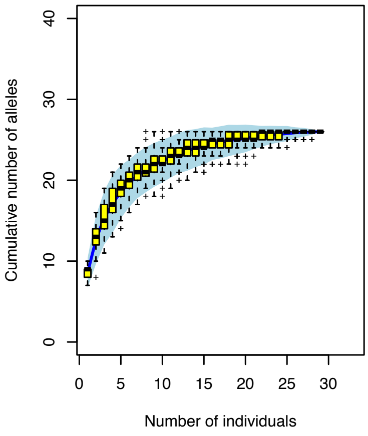

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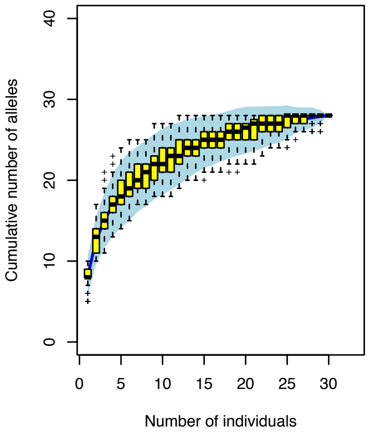

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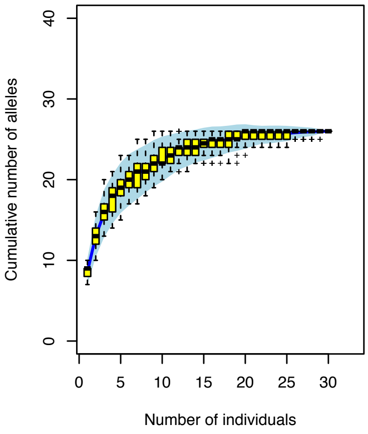

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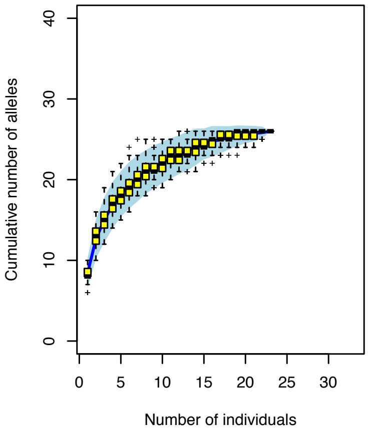

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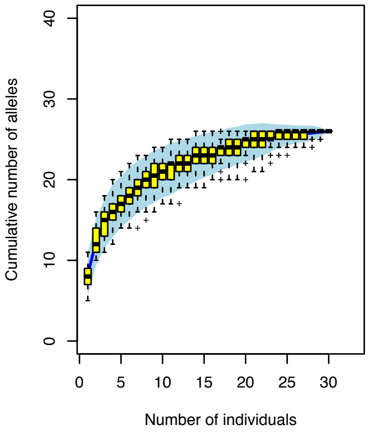

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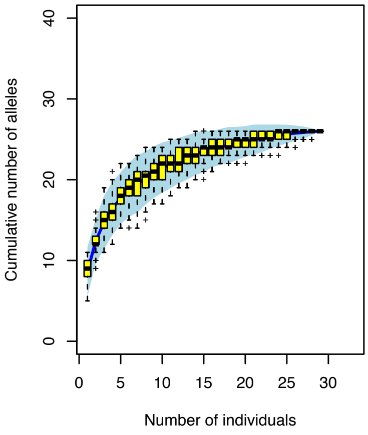

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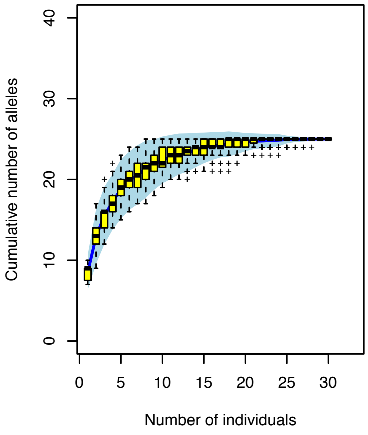

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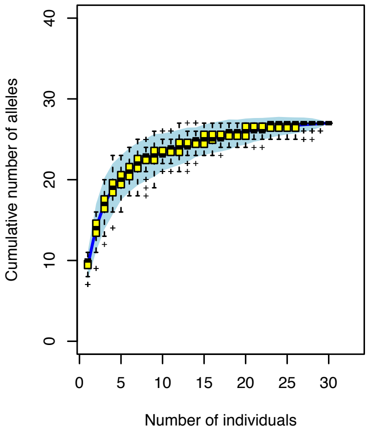

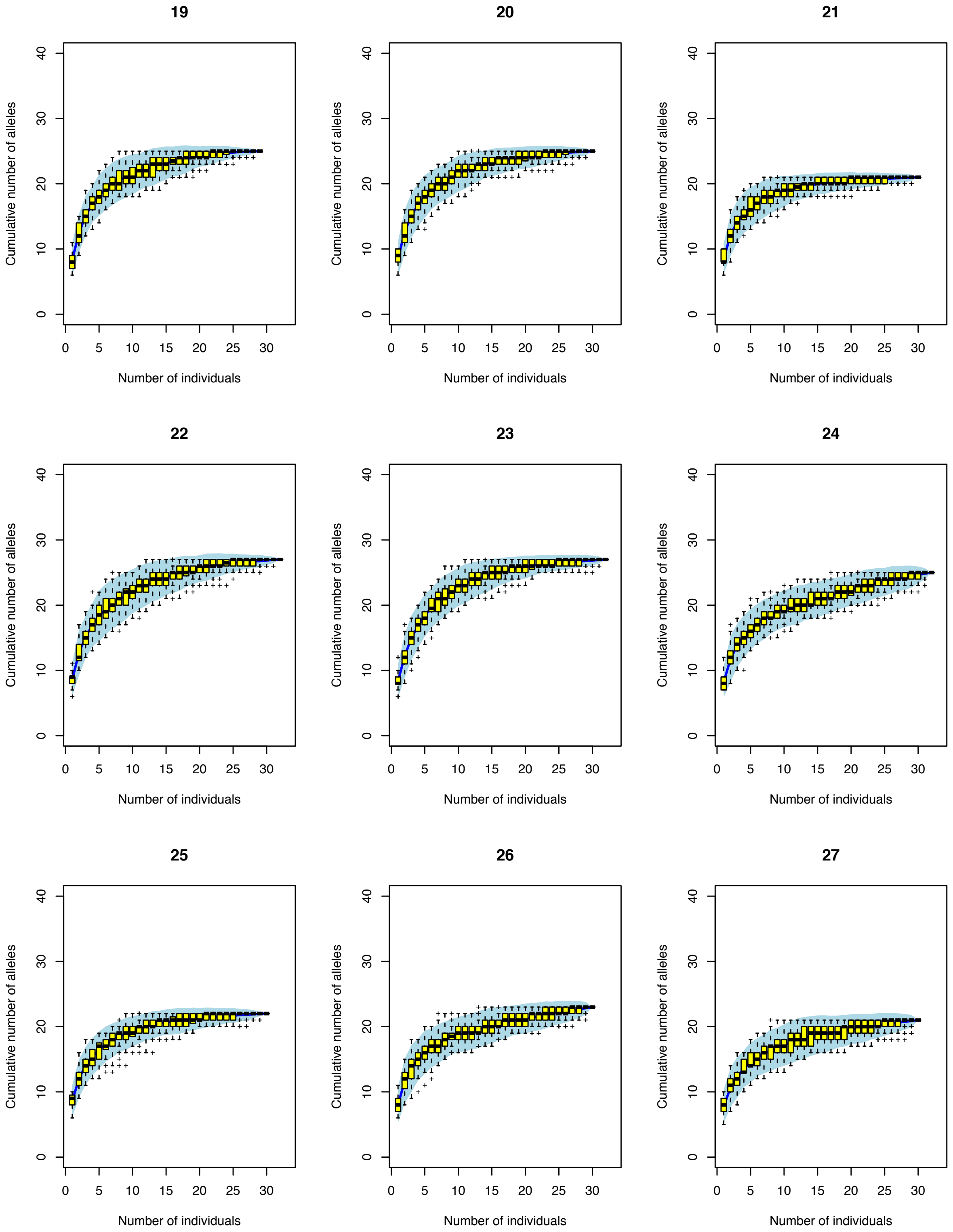
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Number of individuals
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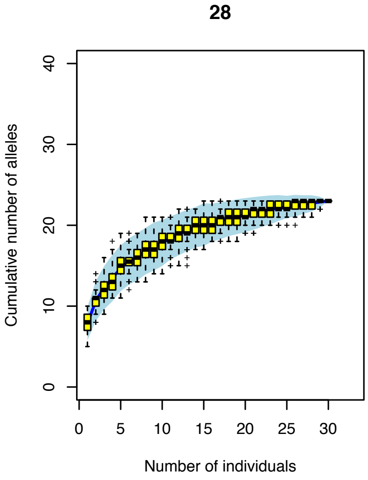
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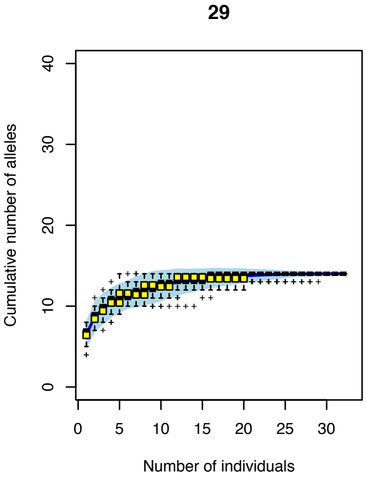
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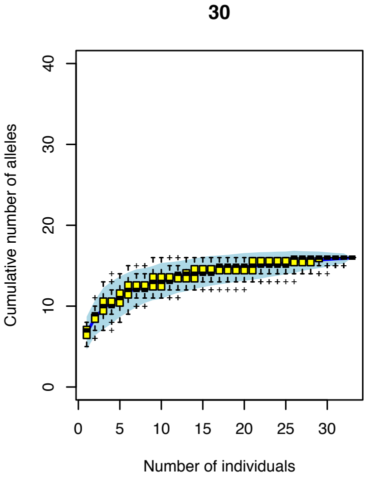
Number of individuals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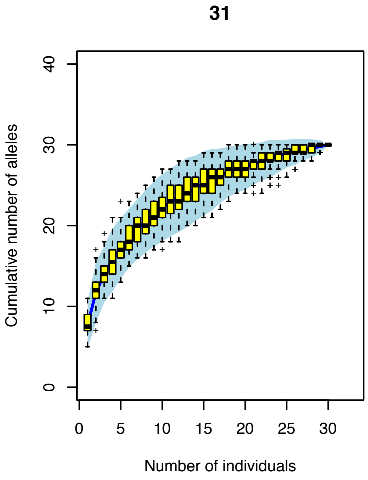
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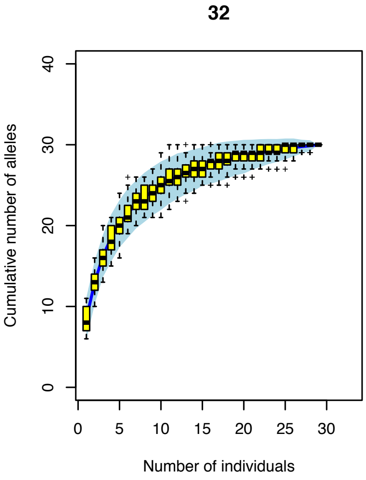
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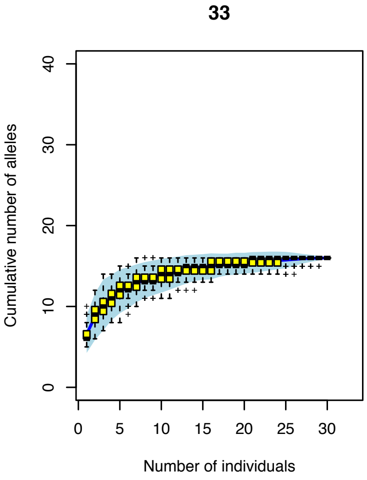
Number of individuals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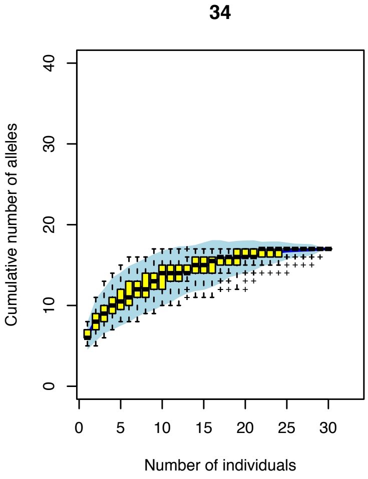
Number of individuals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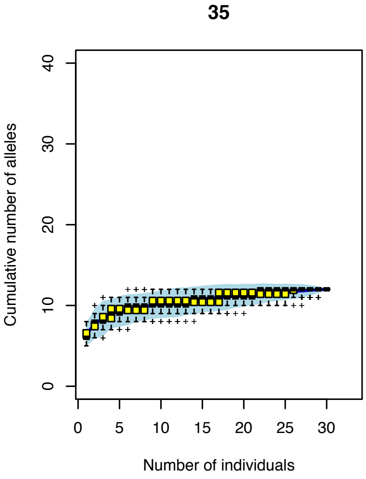
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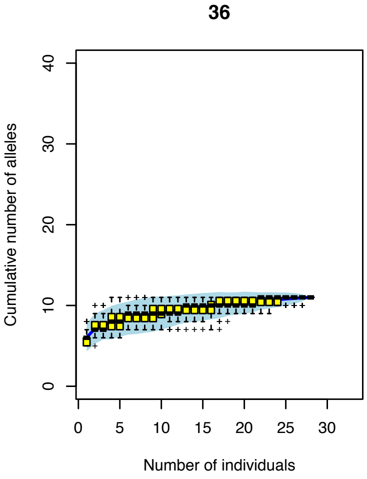
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Number of individuals

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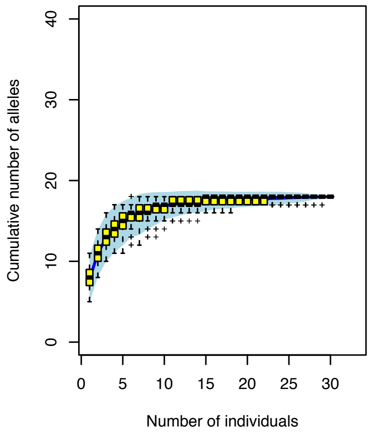

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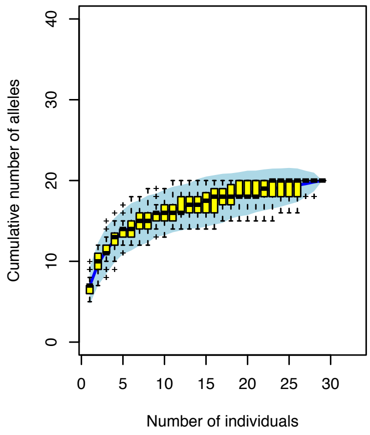

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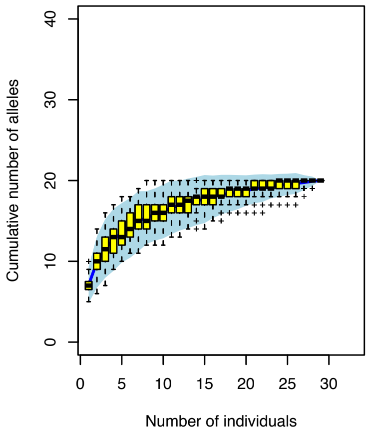

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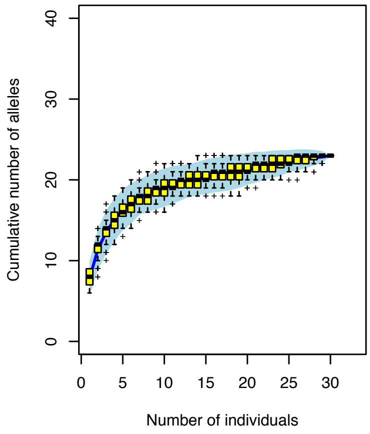

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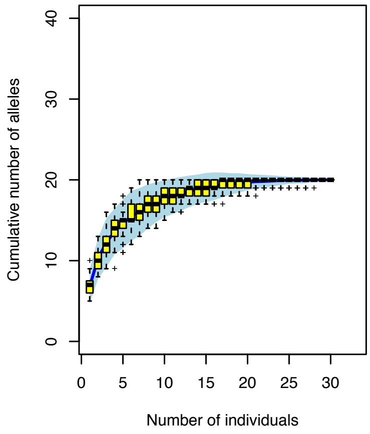

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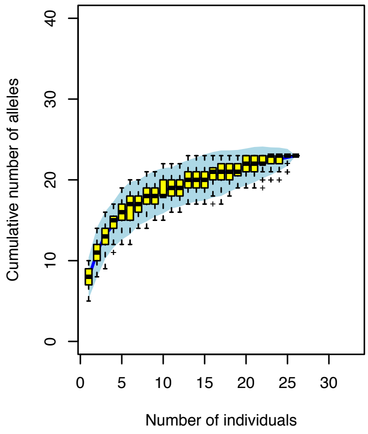

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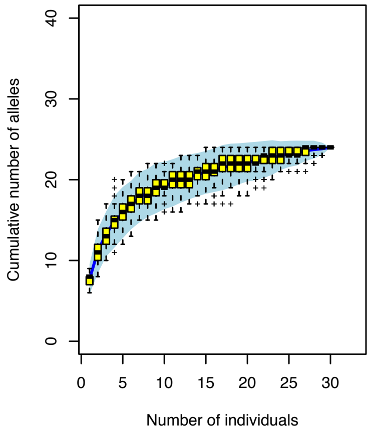

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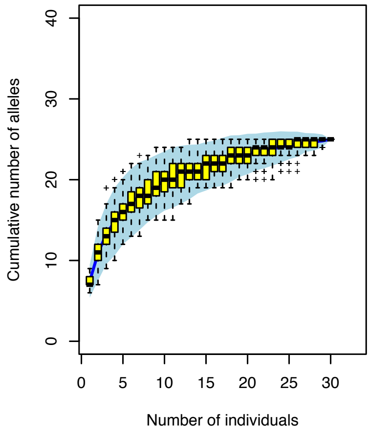

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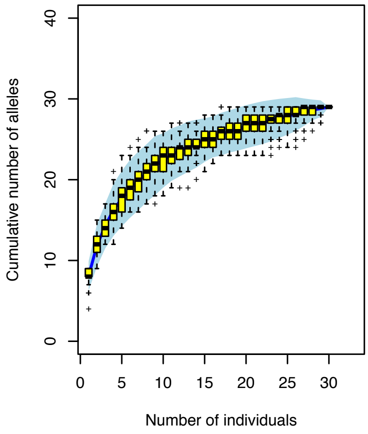

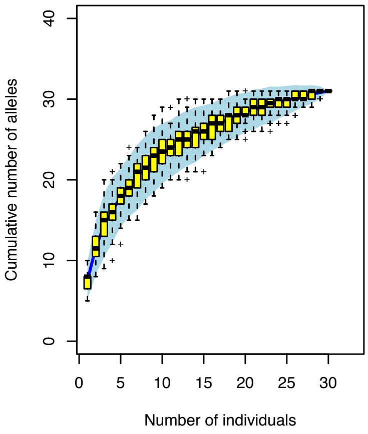
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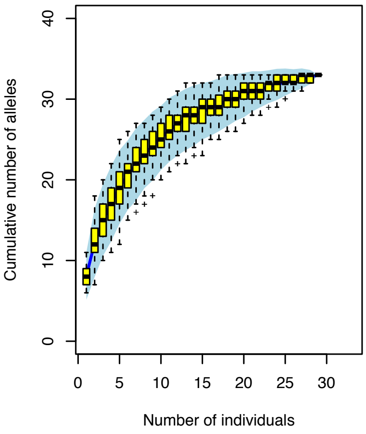
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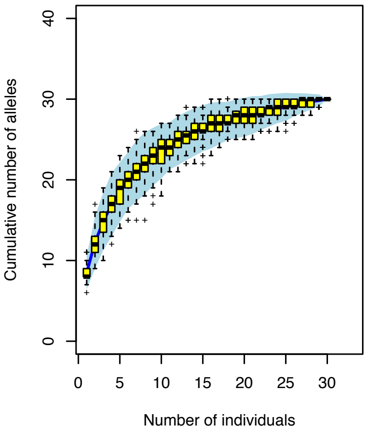
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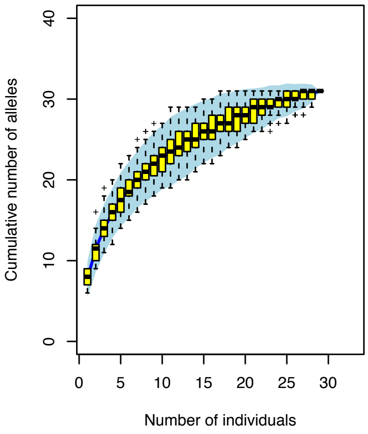
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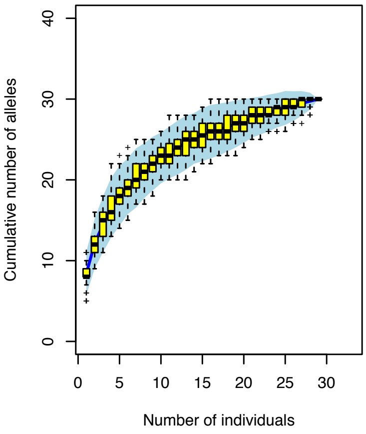
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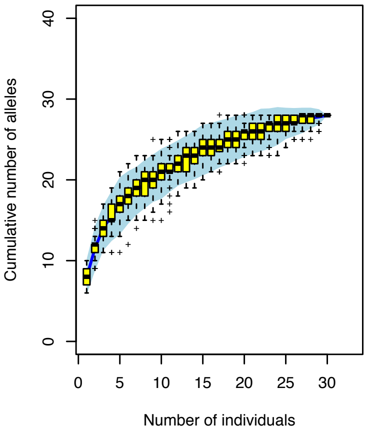
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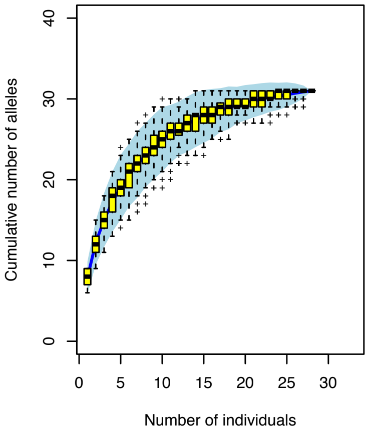
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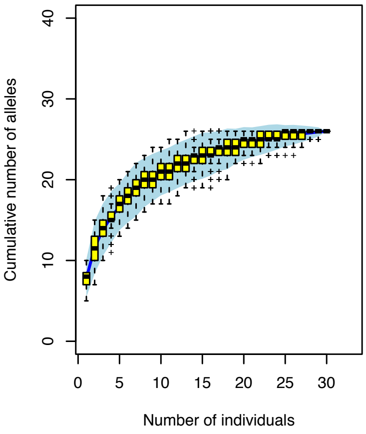
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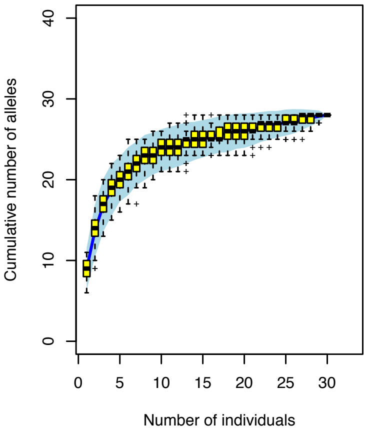
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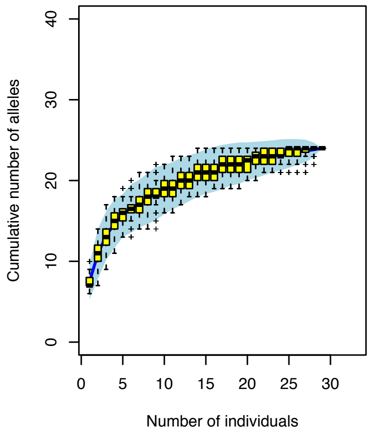

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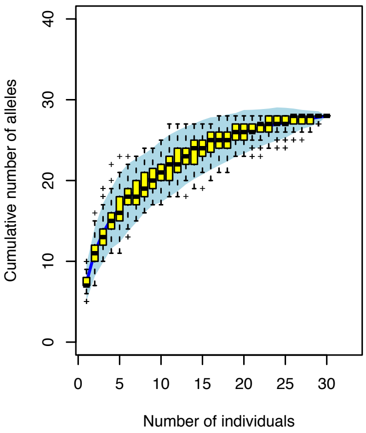

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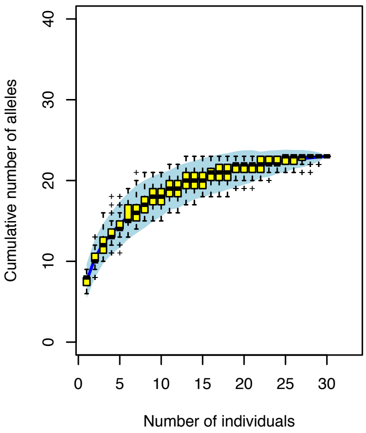

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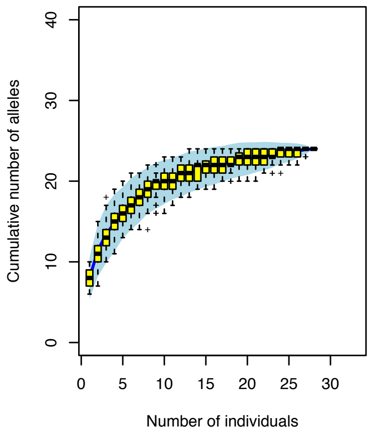

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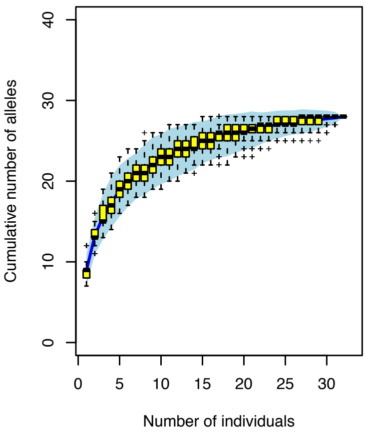

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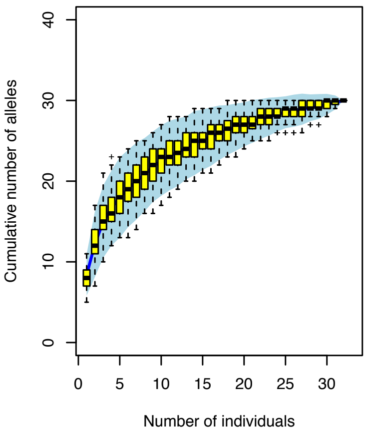

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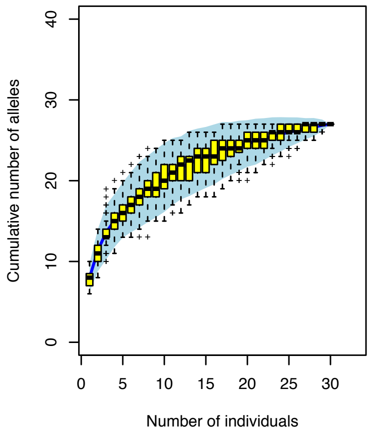

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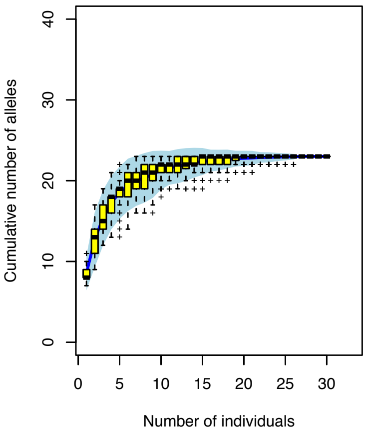

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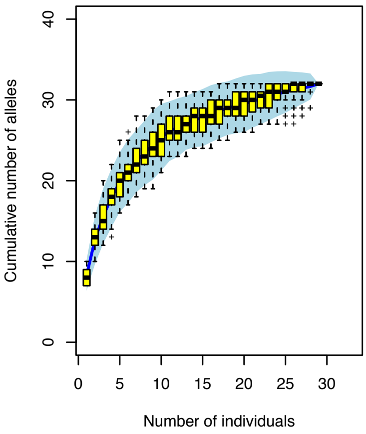

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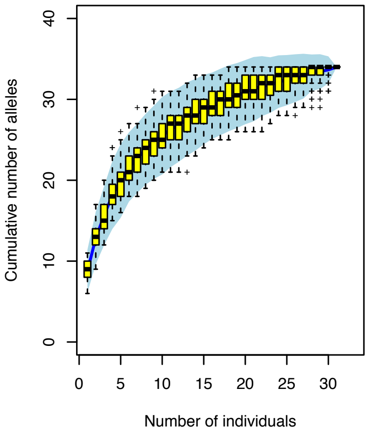

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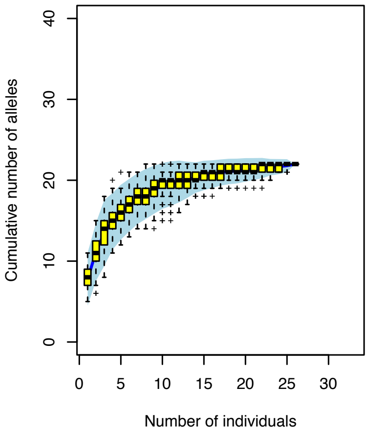

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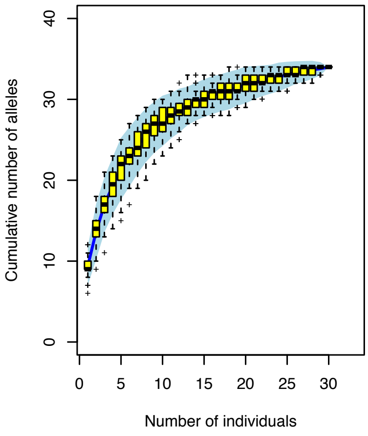

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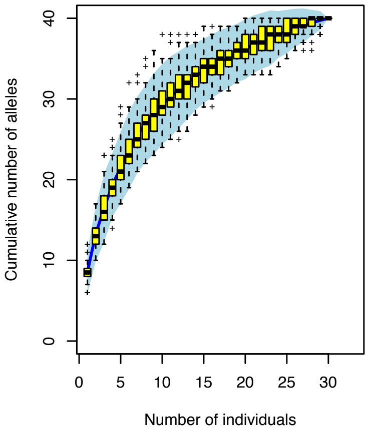

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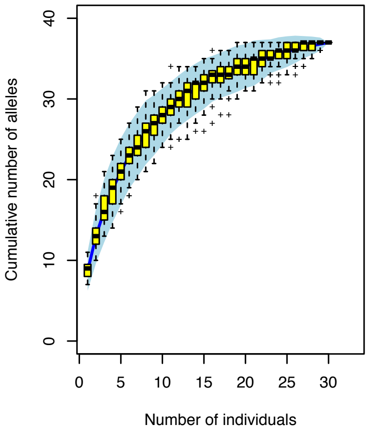

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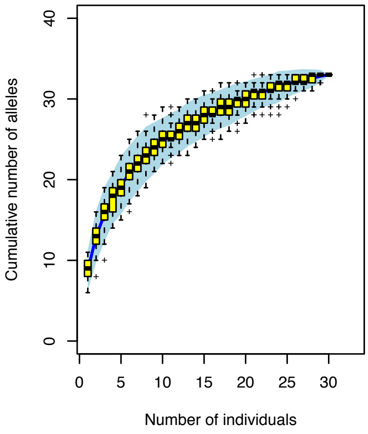

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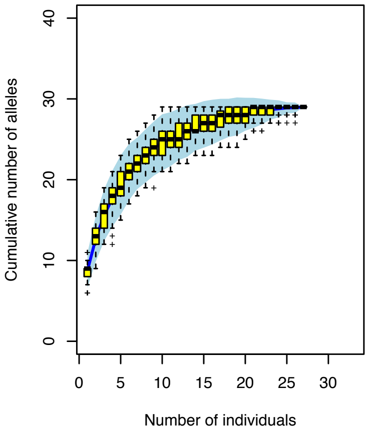

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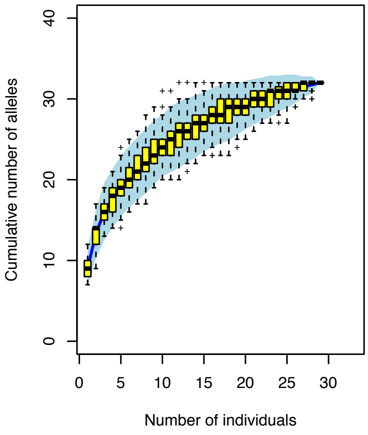

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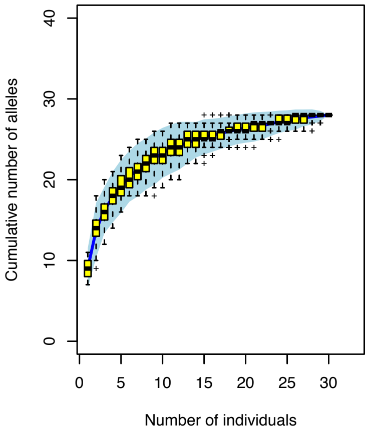

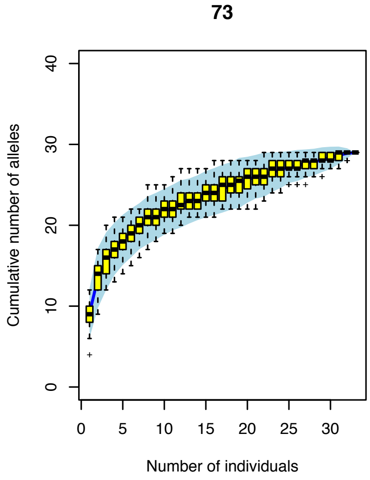
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Cumulative number of alleles
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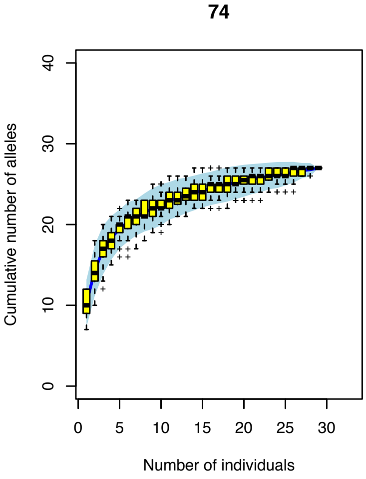
10
20
30
40
0
5
10
15
20
25
30
Number of individuals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Cumulative number of alleles
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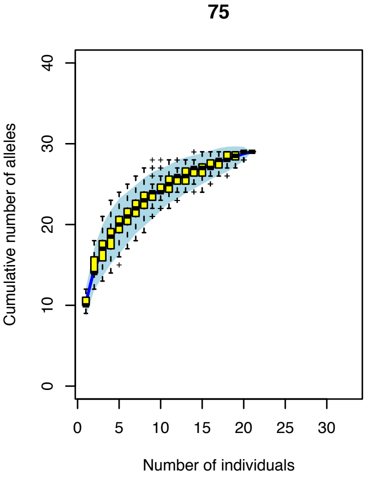
20
25
30
Number of individuals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Cumulative number of alleles
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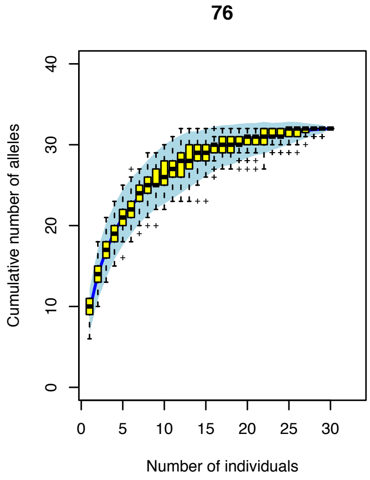
30
40
0
5
10
15
20
25
30
Number of individuals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Cumulative number of alleles
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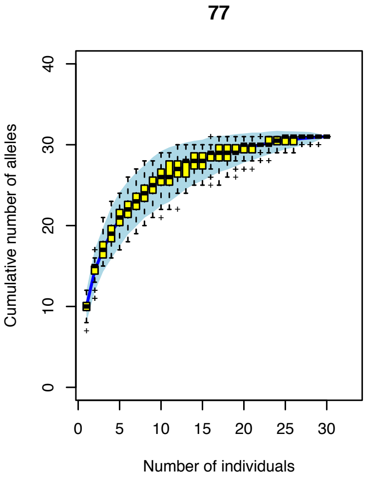
Number of individuals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Cumulative number of alleles
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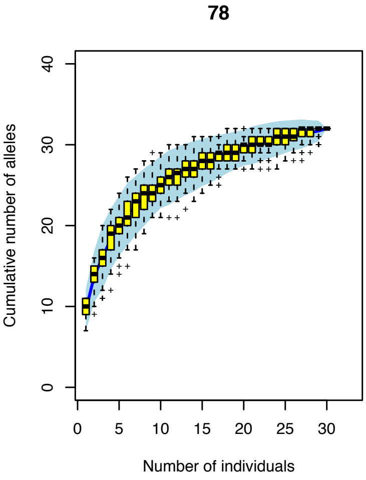
Number of individuals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Cumulative number of alleles
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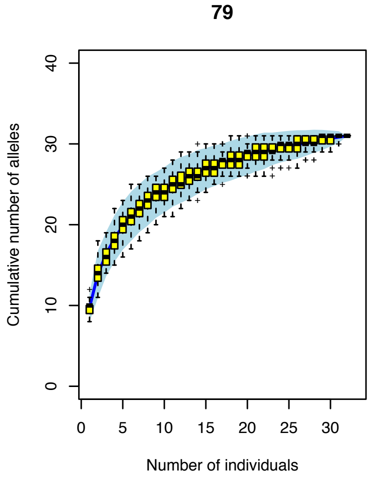
Number of individuals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Cumulative number of alleles
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5
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15
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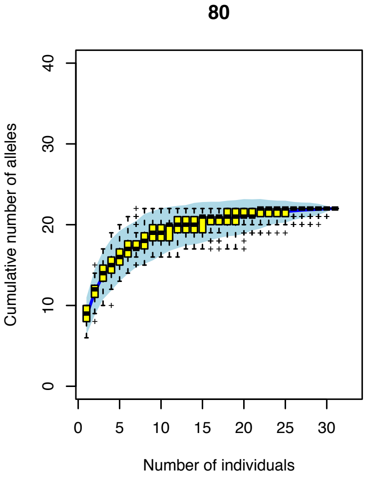
25
30
Number of individuals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Cumulative number of alleles
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10
20
30
40
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5
10
15
20
25
30
Number of individuals

FIG. 4.S3. POPULATION NETWORK PARAMETERS

Values for population network parameters on each island for *Neochamaelea pulverulenta* (Rutaceae). The mean and standard deviation (± 1SD, vertical lines) of the parameter is plotted against increasing network size (n, number of populations or nodes in the network). Population networks were resampled 999 times, and the parameters estimated (mean ± SD), for each network size (from *n*= 1 to *n*= 30 in Gran Canaria; from *n*= 1 to *n*= 28 in Tenerife; from *n*=1 to *n*= 22 in La Gomera). Lizard silhouettes are scaled to indicate relative body sizes (largest silhouette = 280 mm SVL, *G. stehlini* from Gran Canaria).

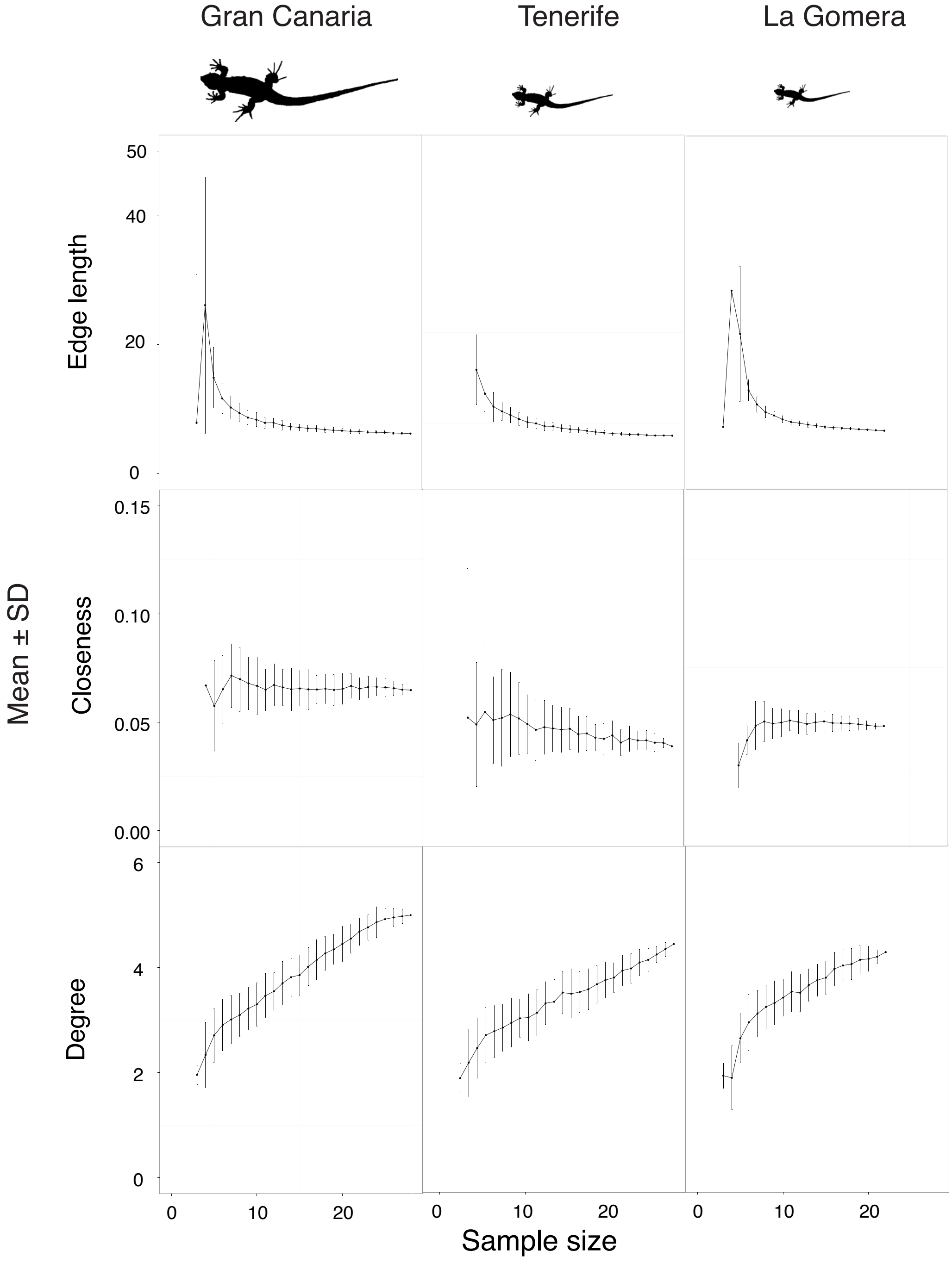


FIG. 4.S4. PHYLOGRAM OF THE UPGMA

Phylogram of the UPGMA based on Euclidean genetic distances (Nei´s distances) among populations of *Neochamaelea pulverulenta* (Rutaceae) in the three islands. * indicates bootstrap values larger than 50% based on 1000 permutations. Population codes are indicated in the right side of the phylogram (see Fig. 4.1 and 4.S1 and Table 4.S1 for geographic locations of each population). Population codes 1-30 correspond to Gran Canaria (orange lines); 31-58 to Tenerife (red lines); 59-80 to La Gomera (blue lines).

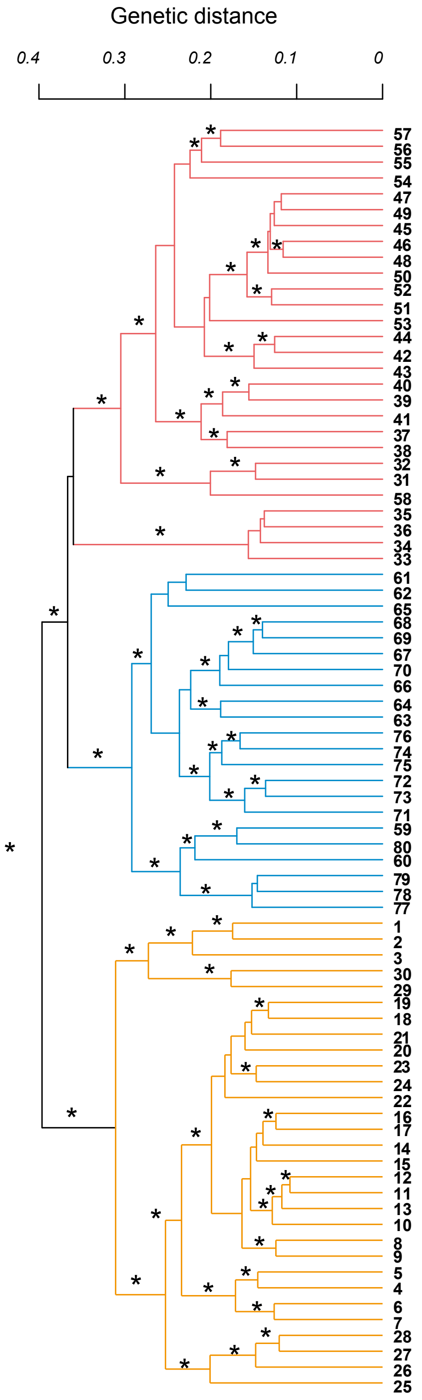


FIG. 4.S5. OPTIMAL NUMBER OF GENETIC CLUSTERS

Values of Delta K (mean (|L''(K)|)/sd(L(K))) (Evanno *et al.* 2005) as a function of the number of clusters (K) after applying a Bayesian clustering analysis with STRUCTURE software (see Fig.4.3; Pritchard *et al.* 2000). Posterior probability of K was estimated with the HARVESTER software (Earl & vonHoldt 2012). Lizard silhouettes are scaled to indicate relative body sizes (largest silhouette = 280 mm SVL, *G. stehlini* from Gran Canaria).

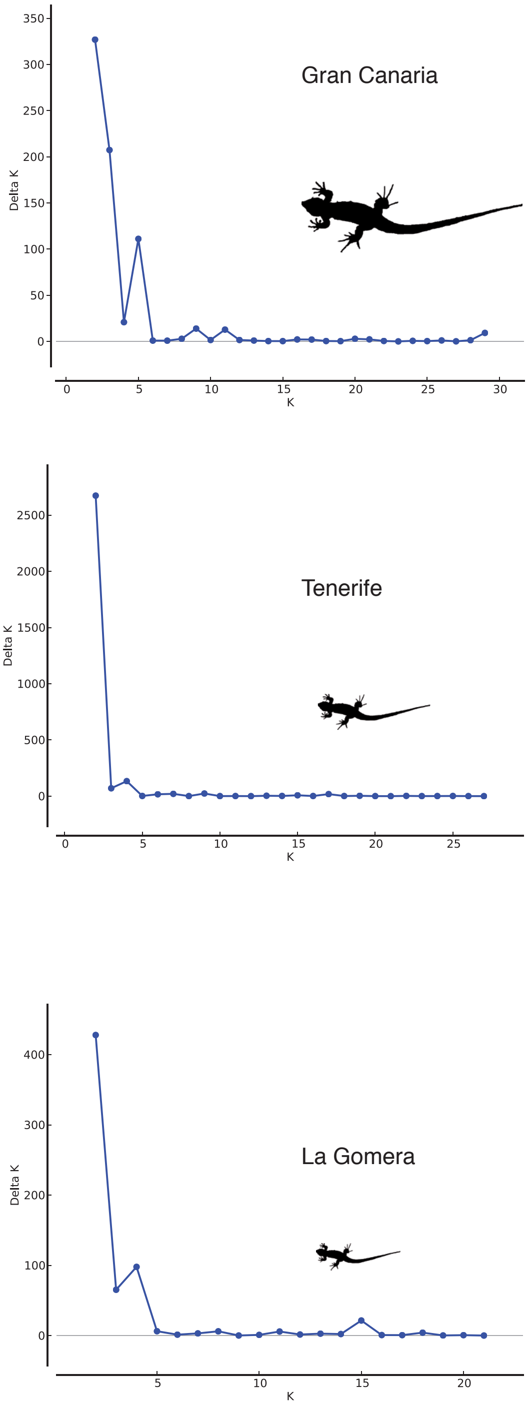
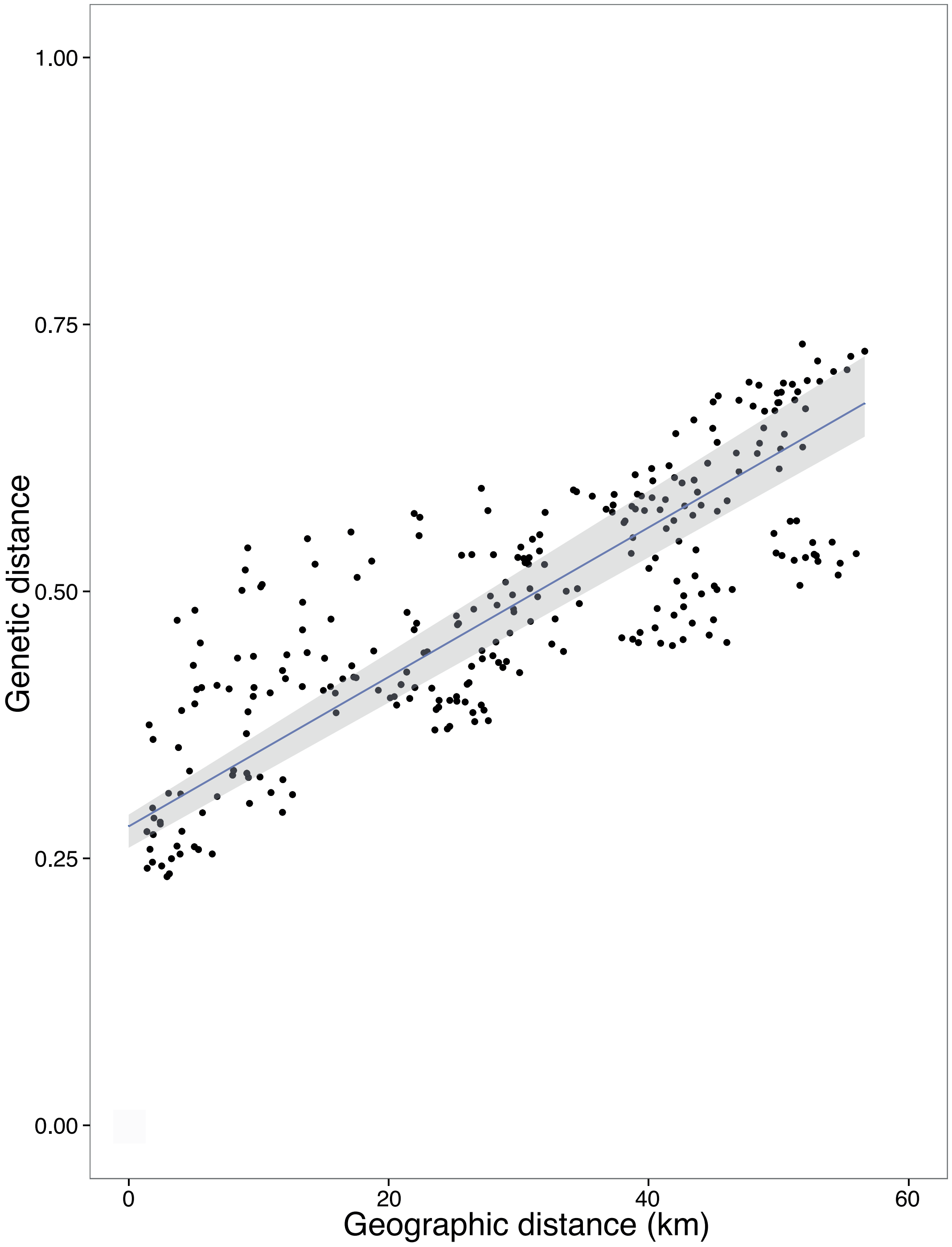


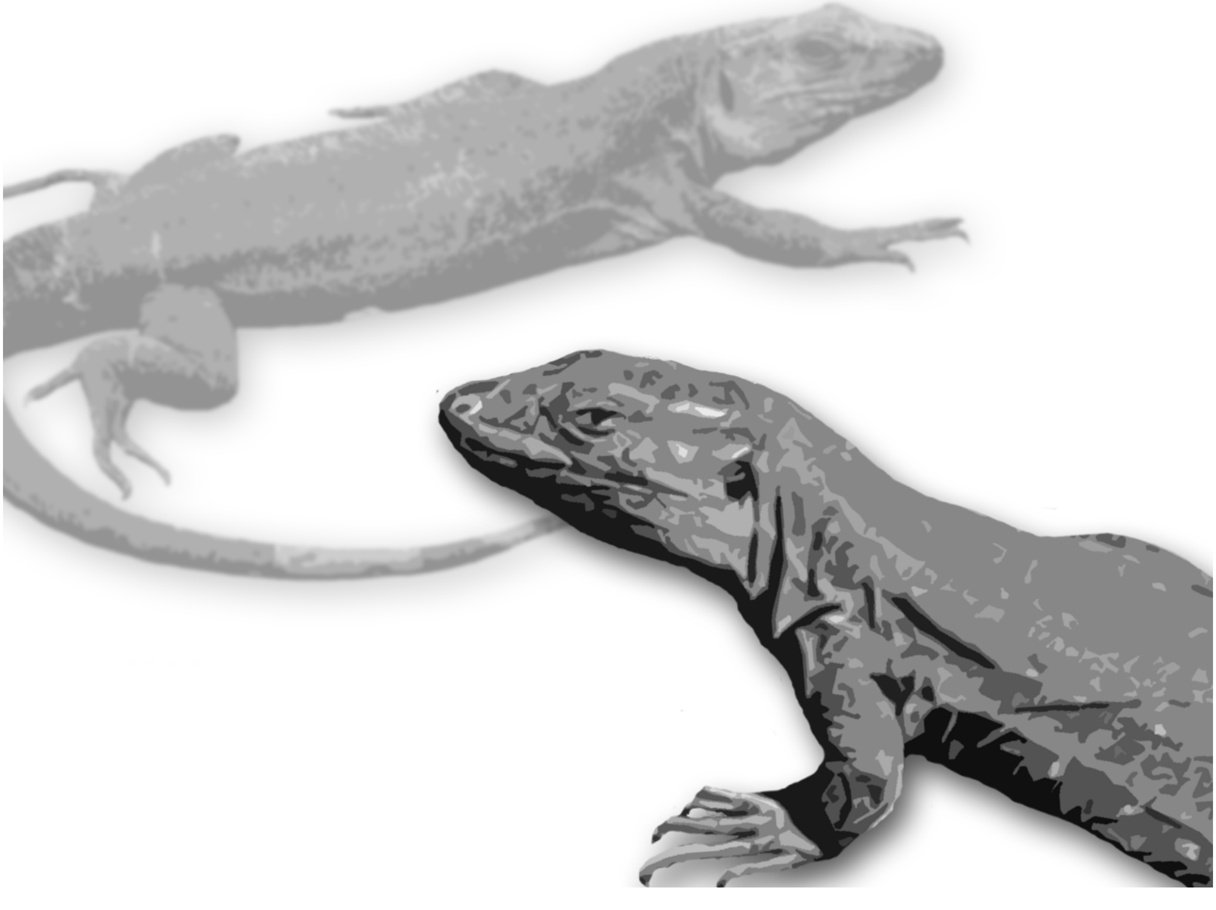
FIG. 4.S6. IBD PATTERN IN TENERIFE

Pattern of isolation by distance (IBD) of *Neochamaelea pulverulenta* (Rutaceae) in Tenerife resulted when we excluded the four populations located in the North of the island (Populations 33-36; see Fig. 4.S1 for their locations). Representation of the Euclidean genetic distance and Euclidean geographic distance (km). The reduced major axis regression line (blue) and the bootstrapped (1000 times) 95% confidence interval (grey area) are shown.



CAPÍTULO 5

Síntesis y Discusión General



La defaunación en el Antropoceno es un fenómeno incesante que está provocando la pérdida no sólo de una multitud de especies de vertebrados frugívoros sino la extirpación de muchas de sus poblaciones y de individuos dentro de las mismas (Dirzo *et al.* 2014; McCauley *et al.* 2015; Malhi *et al.* 2016). Dado que este fenómeno es selectivo hacia las especies (e individuos) de mayor tamaño, uno de los resultados inmediatos es la reducción de talla generalizada en las comunidades y poblaciones de vertebrados (Koch & Barnosky 2006; Hansen & Galetti 2009). Esto podría desencadenar una degradación paralela de las relaciones mutualistas planta-animal y del proceso de dispersión de semillas en que intervienen (Wright *et al.* 2007; Galetti & Dirzo 2013; Dirzo *et al.* 2014). Comprender cuál es la magnitud y las consecuencias últimas de este fenómeno debe ser una prioridad si queremos disponer de información suficiente para diseñar estrategias adecuadas que atajen esta pérdida de biodiversidad en sentido amplio (especies, interacciones y funciones ecosistémicas). Sin embargo, a pesar del enorme esfuerzo de investigación realizado en los últimos años, aún estamos lejos de entender los múltiples efectos de la defaunación sobre la dinámica demográfica y, más aún, sobre las características genéticas de las especies de plantas con las que los vertebrados frugívoros interactúan. En esta tesis se combinan datos ecológicos y genéticos, en un experimento "inducido" con tres situaciones (islas) muy contrastadas desde el punto de vista del tamaño de los dispersores de semillas, para evaluar estos efectos (véase resumen en Fig. 5.1). Los objetivos específicos han sido, demostrar que la reducción de tamaño de los dispersores de semillas tiene consecuencias demográficas negativas, caracterizadas por un reclutamiento temprano empobrecido en poblaciones defaunadas (**capítulo 2**). Además, este fenómeno provoca una reducción paralela del flujo de genes vía semillas (**capítulo 3**), generando un impacto marcado en las características genéticas de las poblaciones a distintas escalas espaciales (**capítulos 3 y 4**). En primer lugar, en la distribución espacial de la variación genética y en el tamaño efectivo poblacional a escala local (dentro de poblaciones) (**capítulo 3**) y, en segundo lugar, en los patrones de conectividad genética y de aislamiento genético por distancia entre poblaciones a escala regional (dentro de islas) en el rango geográfico completo de la especie(**capítulo 4**).

DEFAUNACIÓN Y DEGRADACIÓN DEL PROCESO DE DISPERSIÓN DE SEMILLAS

Los frugívoros de mayor tamaño generalmente son dispersores más efectivos en términos de tamaño de semillas dispersadas, de tasa de consumo de frutos y de distancias de dispersión (Howe & Smallwood 1982; Wheelwright 1985; Jordano *et al.* 2007). Los resultados de esta tesis muestran que la pérdida de tamaño en las comunidades de lagartos frugívoros de las Islas Canarias (*Gallotia* spp.) provoca un empeoramiento significativo de los servicios de dispersión, y eventualmente su colapso, que brindan a *Neochamaelea pulverulenta*. La figura 5.1 A ilustra un esquema de cómo es esta degradación progresiva de las distintas componentes de la dispersión de semillas asociada a la pérdida de tamaño de los lagartos frugívoros.

La mayoría de aspectos señalados en este esquema han sido puestos de manifiesto

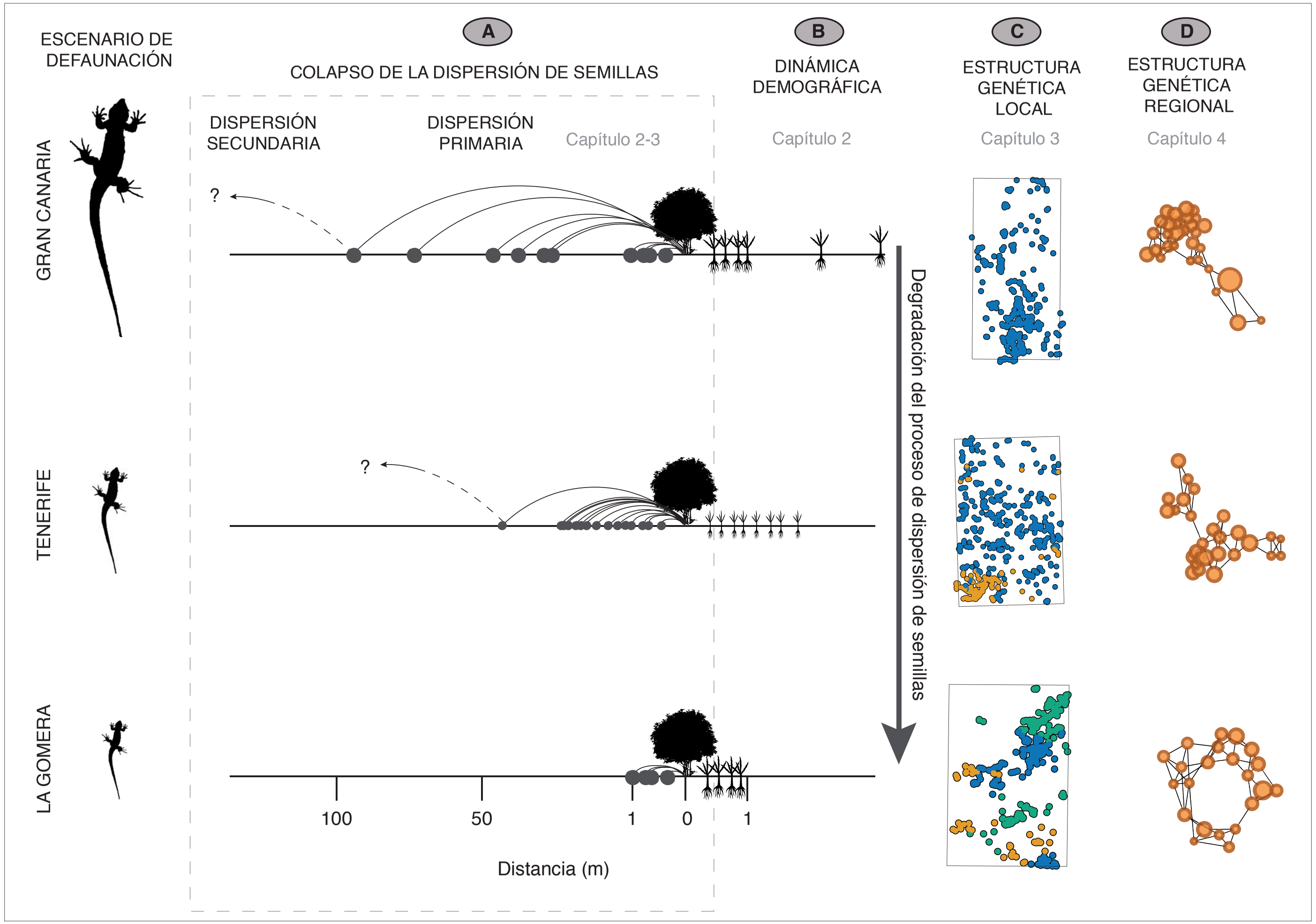


Fig. 5.1. Esquema resumen de los efectos en cascada de la reducción de talla de los lagartos frugívoros (*Gallotia* spp.) en tres escenarios insulares de defaunación. A: Degradación progresiva, y eventual colapso, del proceso de dispersión de semillas de *Neochamaelea pulverulenta* (Rutaceae), en términos de distancias de dispersión. B: Consecuencias demográficas de la defaunación en términos de reclutamiento efectivo (>1 m de planta adulta) y vigor de plántulas establecidas. C: Consecuencias genéticas a escala local (dentro de poblaciones) en términos de generación de vecindarios genéticos (clusters genéticos) de mayor parentesco entre individuos. D: Consecuencias genéticas a escala regional (entre poblaciones dentro de isla) en términos de conectividad genética. Véanse discusión y capítulos respectivos para más detalles.

separadamente, y de forma más o menos explícita, en estudios previos sobre la pérdida de interacciones ecológicas con frugívoros (p. ej. Pacheco & Simonetti 2000; Cordeiro & Howe 2003; Traveset & Riera 2005; Traveset *et al.* 2012; Calviño-Cancela *et al.* 2012). En este trabajo abordamos los efectos de defaunación desde una perspectiva innovadora. Hemos aunado el análisis de las consecuencias demográficas y genéticas de la pérdida de interacciones mutualistas a escalas espaciales no estudiadas con anterioridad. Como se demuestra en esta tesis la alteración de todos estos elementos del proceso de dispersión de semillas va a tener consecuencias negativas muy marcadas en la dinámica demográfica y en la composición genética de las poblaciones de *N. pulverulenta* a diferentes escalas espaciales.

EFECTOS DEMOGRÁFICOS DE LA DEFAUNACIÓN

La degradación del proceso de dispersión de semillas asociado a la pérdida de tamaño de los dispersores de semillas debería ocasionar impactos negativos en la dinámica demográfica en las poblaciones plantas (Markl *et al.* 2012). Los resultados obtenidos en esta tesis (**capítulo 2**), demuestran que, efectivamente, la regeneración de *Neochamaelea pulverulenta* se ve fuertemente afectada en los escenarios que sólo albergan lagartos de tamaño mediano (Tenerife) y pequeño (La Gomera) (Fig.5.1 B). Sin embargo, estos efectos sólo se evidencian cuando analizamos ambas componentes de la efectividad de la dispersión de semillas, la *cuantitativa* y la *cualitativa* (Schupp *et al.* 2010). En este sentido, la *componente cuantitativa* analizada en este estudio, es decir, la proporción de plántulas y la estructura de edad de las poblaciones, fue muy similar en los tres escenarios. La presencia de esta dinámica de regeneración, contrasta altamente con las poblaciones envejecidas detectadas en otros sistemas defaunados, especialmente tropicales (p. ej. Cordeiro & Howe 2003). La alta mortalidad de semillas (depredadores o patógenos) y plántulas (herbivoría o patógenos) debajo de individuos adultos, debido a un efecto de denso-dependencia (Efecto Janzen-Connell; Janzen 1970; Connell 1971), sesga la estructura de edad hacia los adultos. En nuestro sistema insular, sin embargo, parece que este efecto es menos evidente. A pesar de que una fracción de la cosecha de frutos que cae bajo las plantas adultas sin consumir puede ser depredado por ratas, otra fracción es manipulada de forma esporádica por pequeños lagartos que no dispersan legítimamente las semillas, pero sí las despulpan parcial o totalmente, de manera que permiten su germinación. Además, tras visitar un gran número de poblaciones de *N. pulverulenta* durante el desarrollo de esta tesis nunca se detectaron signos de herbivoría en plántulas.

Cuando se tiene en cuenta la *componente cualitativa* del reclutamiento (reclutamiento efectivo y vigor de las plántulas*)* los efectos de la defaunación se hacen evidentes. En los dos escenarios donde aún se preserva la interacción mutualista (Gran Canaria y Tenerife) existe una proporción alta de plántulas establecidas fuera del vecindario materno (reclutamiento efectivo; > 1 m), donde las probabilidades de sobrevivir suelen ser mayores. El mayor reclutamiento efectivo detectado en Tenerife respecto a Gran Canaria es un resultado inesperado, ya que la eficiencia de *G. galloti* como dispersor de semillas probablemente es menor que la de *G. stehlini* en Gran Canaria. Sin embargo, se sabe que la abundancia de lagartos está íntimamente relacionada con su tamaño (Buckley & Jetz 2007; Buckley *et al.* 2008). En este sentido, los resultados de conteo de excrementos mostraron que *G. galloti* presenta una densidad seis veces mayor en Tenerife que *G. stehlini* en Gran Canaria (**capítulo 2**). Esto sugiere que la menor eficiencia de los lagartos medianos en cuanto a la tasa de consumo y dispersión de semillas puede ser compensada por un incremento en su abundancia (Vázquez *et al.* 2005).

En el escenario más defaunado (La Gomera), la regeneración fuera del entorno materno es prácticamente nula (**capítulo 2**), debido al colapso del proceso de dispersión de semillas (**capítulo 3**) en esta isla. Esto ocurre porque *G. caesaris,* el único lagarto abundante en esta isla tras la extinción de los lagartos gigantes, no presenta un tamaño suficiente para consumir frutos de *N. pulverulenta*. Se produce, por tanto, un desajuste morfológico entre el tamaño de frutos y el ancho de la comisura bucal de los lagartos, impidiendo así la dispersión legítima de semillas. Aún así, una pequeña proporción de plántulas es capaz de establecerse fuera del vecindario materno (>1 m), probablemente como resultado del manejo de los frutos por parte de consumidores de pulpa (dispersores ilegítimos) como *G. caesaris* o de depredadores de semillas como ratas (*Rattus rattus)*. Además, a menudo las poblaciones se encuentran en laderas de barrancos de pendientes pronunciadas por lo tanto una proporción de estas semillas pueden dispersarse por acción de la gravedad o de la lluvia. Otros sistemas defaunados también conservan esta dispersión de semillas residual, normalmente mediada por vectores secundarios poco eficientes (Traveset & Riera 2005; Guimarães *et al.* 2008).

La evidencia de un aumento de la calidad del reclutamiento en el escenario con lagartos grandes (Gran Canaria) frente al escenario con lagartos medianos (Tenerife) sólo surge cuando se analiza el vigor de las plántulas establecidas (**capítulo 2**; Fig. 5.1B). En este sentido, se detectaron valores en Gran Canaria que prácticamente duplican los de Tenerife. Este resultado se explica porque este rasgo está íntimamente relacionado con el tamaño de semillas del que provienen (Howe & Richter 1982). Como se ha documentado previamente (Valido 1999), la reducción de talla de los dispersores de semillas conlleva una disminución del rango de tamaño de semillas dispersado. Este rango es mayor en Gran Canaria (diámetro medio= 8.7 mm ; *d.t.*= 1.1), donde aún se conservan lagartos grandes similares a la época pre-humana (*G. stehlini)*. Esto ocurre porque el tamaño de las semillas no supone ninguna limitación morfológica (el tamaño de fruto se mantiene menor que la anchura de la comisura bucal) para el consumo por parte de *G. stehlini*. En cambio, en Tenerife sólo se dispersa un rango de semillas más pequeño (diámetro medio= 8.2 mm ; *d.t.*= 1.0), dada la clara limitación de tamaño de *G. galloti* para el consumo de frutos con las semillas más grandes (Fig. 5.1 A). La reducción del vigor de las plántulas en Tenerife, que normalmente se relaciona con una peor tolerancia al estrés hídrico (Leishman & Westoby 1994), podría comprometer la regeneración de *Neochamaelea pulverulenta* ante las proyecciones de cambio climático estimadas para Canarias, donde se espera un aumento de la desertización en las próximas décadas (Petit & Prudent 2008).

La mayoría de estudios hasta la fecha que evalúan el impacto de la defaunación en la regeneración de plantas, se han centrado en analizar de forma aislada o incluyendo sólo algunas de las sub-componentes de la dispersión (p. ej. Chapman & Chapman 1995; Bleher & Böhning-Gaese 2001; Cordeiro & Howe 2003; Traveset *et al.* 2012). Sin embargo, estos resultados ponen de manifiesto la variedad de formas en que la defaunación puede afectar a este proceso, dependiendo de las características propias del sistema analizado. La necesidad de analizar las diferentes componentes de forma conjunta se hace, por tanto, necesaria en este tipo de estudios. De otra forma, pueden pasar inadvertidos algunos efectos poco evidentes, pero de vital importancia para el resultado final del reclutamiento.

EFECTOS GENÉTICOS DE LA DEFAUNACIÓN

La variación genética de las poblaciones de plantas y, su distribución espacial, viene determinada por la acción conjunta del flujo de genes dentro y entre poblaciones, así como otros procesos como la selección, la deriva génica y la tasa de mutación (White *et al.* 2002; England *et al.* 2003). Por tanto, dado que los animales de mayor tamaño tienden a moverse mayores distancias (Harestad & Bunnell 1979), la reducción de talla de los vertebrados frugívoros debería ocasionar una serie de efectos en cascada, caracterizados por una disminución del flujo de genes vía semillas y una alteración de las características genéticas de las poblaciones de plantas afectadas (Hamrick *et al.* 1993). Efectivamente, los resultados obtenidos en esta tesis muestran una reducción drástica de las distancias de dispersión de semillas que refleja el gradiente de defaunación. En la parcela principal de estudio de Gran Canaria se detectaron las mayores distancias de dispersión de semillas (distancia máxima = 94 m), prácticamente el doble que las distancias máximas detectadas en la de Tenerife (distancia máxima = 46 m), la isla que alberga lagartos de tamaño mediano (*G. galloti*). Por su parte, prácticamente todas las semillas analizadas en la parcela de la isla más defaunada (La Gomera) no se dispersaron más allá del vecindario materno (<1m) (**capítulo 3**; Fig. 5.1 A). Aún así se detectó una pequeña proporción de semillas que alcanzaron distancias sutilmente superiores (distancia máxima= 4.5 m), muy probablemente dispersadas, como se discutió previamente, por consumidores de pulpa poco eficientes, por acción de la gravedad o por la lluvia. Estos resultados son extrapolables a escala insular, dado que los resultados obtenidos de las réplicas en micro-parcelas muestran unos patrones muy similares. Estas distancias de dispersión de semillas fueron, además, consistentes con las distancias de reclutamiento efectivo, es decir las distancias entre las plantas madres y el lugar de establecimiento de plántulas, brinzales y juveniles (**capítulo 3**). Todas las parcelas de estudio se sitúan dentro de poblaciones extensas de *N. pulverulenta* bien conservadas y que presentan condiciones abióticas (precipitación, temperatura, tipo de suelo) y bióticas (comunidades de polinizadores, estructura de la vegetación) muy similares. Debido a ello descartamos que las diferencias ambientales entre parcelas expliquen el patrón encontrado. Por el contrario, la explicación más plausible es que esta variación en las distancias de dispersión refleja, en gran medida, los patrones de movimiento asociados al tamaño de los lagartos frugívoros. En este sentido, el tamaño de las áreas de campeo co-varía estrechamente con el tamaño de los lagartos

(Turner *et al.* 1969; Perry & Garland 2002). Además nuestros datos de radio seguimiento de estas especies (n= 42 individuos) muestran unas áreas de campeo bastante superiores en *G. stehlini* que en *G. galloti* (datos no publicados). El colapso de la dispersión primaria en La Gomera, sin embargo, tiene que ver más con el desajuste morfológico que ya se ha discutido. La disrupción de la interacción mutualista en La Gomera conlleva no sólo la pérdida del proceso de dispersión primario mediado por lagartos, sino que imposibilita además la dispersión secundaria mediada por aves depredadoras (*Falco tinnunculus;* Padilla *et al.* 2012) (Fig. 5.1 A). Aunque no se sabe prácticamente nada sobre este proceso, y además queda fuera del alcance de esta tesis doctoral, cabe esperar una pérdida de la capacidad de dispersión a larga distancia en esta isla.

El colapso de la dispersión de semillas detectado en las islas más defaunadas (**capítulo 2 y 3**) debería ocasionar una pérdida de la variación genética intra-poblacional debido a un incremento del aislamiento genético y de la deriva génica. Por el contrario, se detectó una mayor diversidad en la isla más defaunada (La Gomera) que en los escenarios donde aún se preserva la interacción mutualista (Gran Canaria y Tenerife) (**capítulo 3 y 4**). Estos resultados sugieren que otros factores, como la historia biogeográfica de la especie (p. ej. patrón de colonización de islas) o una menor presión antropogénica (p. ej. menor fragmentación del hábitat) en La Gomera, han podido jugar una papel más importante determinando estos niveles de variación, que la extinción de los frugívoros legítimos y la disrupción del proceso de dispersión. Ello indica un efecto retardado de la defaunación respecto a otras presiones antropogénicas como la fragmentación del hábitat, que normalmente va acompañado de una pérdida repentina de individuos y de variación genética asociada (Young *et al.* 1996).

El efecto de la defaunación surge, sin embargo, cuando se analiza la distribución espacial de los acervos genéticos a distintas escalas espaciales (**capítulos 3 y 4**). A escala local, a medida que las distancias de dispersión de semillas de *N. pulverulenta* se acortan, se reduce el solapamiento de la sombra de semillas de los individuos reproductivos (García & Grivet 2011). Se generan de esta manera vecindarios de individuos muy emparentados genéticamente. El resultado es un incremento del grado de estructuración genética espacial que refleja el gradiente de pérdida de tamaño de los lagartos frugívoros en los tres escenarios (La Gomera > Tenerife > Gran Canaria) (**capítulo 3**) (Fig. 5.1 C). El incremento de la agrupación espacial de los acervos genéticos dentro de las poblaciones podría desencadenar una pérdida rápida de variación genética ante eventos de perturbación del hábitat, como, por ejemplo, incendios o cambios en el uso del suelo (Young *et al.* 1996). Además, el efecto combinado de esta alta estructuración genética y la acción de los polinizadores, que suelen promover cruces entre individuos cercanos dentro de los vecindarios genéticos (García *et al.* 2005), podría estar relacionados con la reducción del tamaño efectivo poblacional (N_e) (Neel *et al.* 2013) detectado en las poblaciones más defaunadas (**capítulo 3**).

Los resultados de esta tesis muestran que las consecuencias genéticas de la reducción de flujo génico vía semillas se extienden más allá de esta escala local (**capítulo 4**). La topología de las redes genéticas a nivel insular varían ostensiblemente entre los tres escenarios analizados (Fig. 5.1 D). Mientras que la red de Gran Canaria preserva una mayor conectividad genética entre poblaciones, en Tenerife se observan signos de una ligera reducción de la misma. Este efecto es mucho más marcado cuando el proceso de dispersión de semillas colapsa, es decir en el escenario de La Gomera, donde la conectividad genética entre poblaciones desciende bruscamente a partir de los pocos kilómetros (~4 km). Consistentemente, el aislamiento genético por distancia en esta isla fue mucho más acentuado que en los otros dos escenarios. El impacto marcado de la defaunación sobre la conectividad genética y el aislamiento genético por distancia en la Gomera puede estar influenciado no sólo por la extinción de los únicos consumidores de frutos de *N. pulverulenta* en esta isla sino también por la disrupción del proceso secundario de dispersión mediado por rapaces (Padilla & Nogales 2009; Padilla *et al.* 2012). Aún no se conocen cuáles son las consecuencias de este fenómeno de disrupciones en cadena, puesto que no existen datos sobre la frecuencia ni la magnitud de este proceso de dispersión secundaria. Sin embargo, cabe esperar que la poca conectividad genética encontrada en La Gomera, sea en parte provocada por esta disrupción doble del proceso de dispersión primaria y secundaria. Frecuentemente se examina la estructura de redes genéticas espaciales en relación a procesos filogeográficos asociados a estructura de paisaje y efectos históricos (Dyer & Nason 2004; Sork *et al.* 2010; Herrera-Arroyo *et al.* 2013; Dyer 2015). En nuestro trabajo encontramos una señal inequivoca de la extición de especies mutualistas sobre la conectividad y estructuración genética a gran escala.

IMPLICACIONES DE LA DEFAUNACIÓN PARA LA VIABILIDAD DE LAS POBLACIONES DE PLANTAS

El escenario de La Gomera representa una situación de colapso absoluto de la interacción mutualista entre *Neochamaelea pulverulenta* y los lagartos frugívoros y, consecuentemente, del proceso de dispersión de semillas. El impacto de este fenómeno a escala poblacional es una regeneración demográfica muy limitada al entorno materno, donde se forman vecindarios de individuos muy emparentados genéticamente, y una disminución del tamaño efectivo poblacional. Esto podría acarrear problemas de depresión por endogamia y una disminución del *fitness* de la progenie y de sus posibilidades de supervivencia. A escala regional, el colapso del flujo de genes vía semillas limita drásticamente la conectividad genética de las poblaciones y genera un aislamiento genético por distancia muy marcado. Esto podría ocasionar una pérdida de adaptabilidad ante escenarios de cambio global (Kremer *et al.* 2012), pero además una pérdida de la capacidad de colonización de nuevos hábitats (Howe & Smallwood 1982).

En teoría, la viabilidad de estas poblaciones defaunadas, por tanto, debería haberse visto, en cierta manera, afectadas. Sin embargo, es paradigmático que, igual que ocurre con otras especies tropicales que fueron dispersadas por la Megafauna del Pleistoceno (Janzen & Martin 1982; Guimarães *et al.* 2008), estas poblaciones hayan persistido un largo período de tiempo sin sus dispersores legítimos. En el caso de *N. pulverulenta* en La Gomera, varios factores pueden explicar su persistencia a largo plazo. En primer lugar, la aparente baja mortalidad que existe en el entorno materno y la dispersión esporádica, mediada por dispersores ilegítimos y poco eficientes como *Gallotia caesaris* o roedores introducidos, junto con la dispersión por aguas de escorrentía o por gravedad, puede haber contribuido a mantener una cierta dinámica demográfica en estas poblaciones. En segundo lugar, la actividad de los polinizadores puede haber influido en mantener ciertos niveles de flujo de genes (dispersión de polen; **capítulo 3**) que permitan preservar parte de la conectividad genética dentro y entre poblaciones, evitando la pérdida de diversidad por deriva génica. En tercer lugar, a pesar de que la vegetación del piso basal ha sido duramente afectada por la actividad agrícola y urbanística, el relieve de estas islas volcánicas permite la persistencia de poblaciones en barrancos con laderas muy pronunciadas donde la actividad agrícola y la destrucción del hábitat es reducida.

Las conclusiones de esta tesis provienen del estudio de la interacción específica de *N. pulverulenta* con los lagartos frugívoros del género *Gallotia*. Otro gran número de especies de plantas con frutos carnosos de Canarias dependen de estos lagartos para dispersar sus semillas (Valido & Nogales 1994; Olesen & Valido 2003; Valido *et al.* 2003; Rodríguez *et al.* 2008), aunque muy frecuentemente acompañados por varias especies de aves frugívoras. Sin embargo, la contribución de aves y lagartos a la eficiencia de la dispersión es muy variable dependiendo de cada especie de planta (González-Castro *et al.* 2015). Por tanto, a pesar de que muchas especies de plantas endozoócoras tengan dispersores de semillas alternativos, el impacto de la defaunación de los lagartos podría ocasionar un impacto muy marcado en aquellas que dependan mayoritariamente de sus servicios. Además nuestros resultados pueden extrapolarse a otros sistemas insulares donde se hayan perdido especies de frugívoros de gran tamaño (Meehan *et al.* 2002; McConkey & Drake 2006; Wotton & Kelly 2011; McConkey *et al.* 2015) o incluso a sistemas más diversos, pero caracterizados por una baja redundancia funcional (Janzen 1974; McConkey & Brockelman 2011; Bueno *et al.* 2013). Los resultados de esta tesis ponen de manifiesto, por tanto, la necesidad de conservar a los vertebrados frugívoros de mayor tamaño si queremos mantener los servicios de dispersión que ofrecen a las especies de plantas con frutos carnosos. De otra forma, la defaunación puede generar múltiples efectos negativos en cascada como una alteración drástica de la dinámica demográfica y de las características genéticas de las especies vegetales que se han visto privadas de la interacción mutualista.

CONCLUSIONES

1. La reducción de talla de los lagartos frugívoros del género *Gallotia* (Lacertidae), provoca la degradación del reclutamiento temprano de *Neochamaelea pulverulenta* (Rutaceae). Esta pérdida de tamaño no afecta a la *componente cuantitativa* de la dispersión, es decir a la proporción de plántulas y a la estructura de edad en los tres escenarios analizados. Sin embargo, sí existe un impacto muy marcado en la *componente cualitativa*, es decir en el reclutamiento efectivo (fuera del vecindario materno) y en el de vigor de las plántulas. Estos resultados evidencian la necesidad de caracterizar, de forma conjunta, las distintas componentes de la dispersión de semillas al evaluar el impacto de la defaunación en la dinámica demográfica de las poblaciones de plantas.

2. Los análisis genéticos por asignación materna de semillas dispersadas muestran una reducción significativa de las distancias de dispersión y de reclutamiento efectivo, reflejando el gradiente de defaunación. En el extremo más defaunado del gradiente, el proceso de dispersión colapsa por completo debido a un desajuste morfológico entre el tamaño de semillas y la anchura de la comisura bucal de los lagartos.

3. La diversidad genética de las poblaciones de *Neochamaelea pulverulenta* no parece estar relacionada con el estado de defaunación. El flujo de genes vía polen u otros factores históricos, han podido jugar un papel importante moldeando estos niveles de diversidad genética y amortiguando el efecto de la deriva génica en estas poblaciones defaunadas. Se sugiere así un efecto retardado de la defaunación respecto a otras presiones antrópicas, como la fragmentación del hábitat, que normalmente van acompañadas de una rápida pérdida de individuos y de diversidad genética.

4. La reducción de flujo génico vía semillas asociado a la defaunación parece tener un efecto muy marcado aumentando la estructuración genética espacial y disminuyendo el tamaño efectivo poblacional a escala local. El acortamiento progresivo de las distancias de dispersión de semillas, conlleva una reducción del solapamiento de las sombras de semillas de los individuos reproductivos y un aumento del parentesco genético dentro de los vecindarios locales, generando así un aumento de la estructuración genética espacial. Además, existe una relación clara entre esta limitación del flujo de genes y la reducción del tamaño efectivo poblacional.

5. Las diferencias en la topología de las redes genéticas de conectividad poblacional, muestreadas en todo el área de distribución de *Neochamaelea pulverulenta,* indican que la limitación de flujo génico vía semillas asociado a la defaunación de los lagartos frugívoros se relaciona con una reducción progresiva de la conectividad genética entre poblaciones a escala insular. Este aumento del aislamiento genera una mayor diferenciación genética por distancia, al menos en el escenario más defaunado.

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N*o hay mal que por bien no venga,* dice el refranero popular. Nada más cierto. La situación de desempleo de mi padre tras la grave crisis económica, nos brindó la oportunidad de pasar muchísimo tiempo juntos. Se convirtió en mi compañero inseparable durante mis largas estancias en Canarias. Juntos pasamos largas jornadas, entre barrancos de las islas, en busca de alguna pequeña población de Orijama que permitiera añadir un puntito más al mapa. Qué buenos días, y meses, echamos, de sol a sol, en la *parcelita* de Teno Bajo, marcando y midiendo plantas (*chapita pa´arriba, chapita pa´abajo*), preparando experimentos de polinización, haciendo radio-tracking a los lagartos, eres una máquina. Un bocadillo de sardinas, un *fisco* fruta y una cervecita, no hacía falta más. No tengo cómo agradecerte toda tu ayuda. Gracias *viejito.*

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En estos años además he tenido la suerte de interaccionar con una gran cantidad de gente fuera del ámbito puramente académico. Algunos de ellos se han convertido en familia de adopción. Gracias Pablo, María A., Alberto, Carmen, Airam, Ruth, Laura, Duarte (y Mariana xD), David, María M., Rosa, Grego, Camacho por ser nuestro apoyo constante en Sevilla. Gracias a ustedes, y a nuestros constantes encuentros gastronómicos, mi madre ya no me dice que me faltan unos kilitos. Los partidos de fútbol en Bami, y sobre todo el post-partido, fueron mi bálsamo semanal. Fueron muchas las figuras que pasaron por esa cancha, pero los clásicos (Xim, Riki, Marcello, Joaquin, Roberto, Rubén, Javi G, Javi L, David, Airam, Duarte, Grego, Fran) casi siempre estuvieron. Gracias por la calidad exhibida. Aunque son demasiados para nombrarlos a todos, con los componentes del departamento de Ecología Integrativa, especialmente con los del despacho de invasoras y con el grupo de doctorandos, *cachorros* incluidos, pasé momentos espectaculares e inolvidables. Las copitas fueron míticas, las *ferias,* la estancia en el Palacio, las excursiones pal campito y pa' la playa, y tantas y tantas otras fiestas. ¡Qué mal nos lo *pasemos!*.

Las últimas frases de estos agradecimientos quería reservarlos para ti, Anna. No podía terminar de otra forma. Aún recuerdo, como si fuera hoy, aquella noche de hace ya más de seis años. Aquella noche de persecución en el barrio del Raval, aquella en que nos conocimos. Qué rápido pasa el tiempo. Apenas unos meses después surgiría la oportunidad de venirme a Sevilla. Qué loco estaba. En ese momento lo fácil hubiese sido decirme un "*calabaza calabaza cada uno pa' su casa*". Sin embargo, sólo recibí palabras de ánimo y comprensión. Te lanzaste al vacío y apostaste por nosotros, a pesar de la distancia. Cómo podía esperar tanto de ti. Los primeros años no fueron fáciles, el trabajo de campo absorbía todo el tiempo y apenas nos quedaba una llamada cada tarde y un viaje fugaz de 48 horas de tanto en tanto. *La ilusión mueve el mundo,* así que las ganas de estar pronto juntos en un mismo espacio-tiempo, hacía que esos ratos fueran suficientes. Finalmente, el 8 de Enero de 2014, los astros se alineron. Desde entonces has sido la piedra angular en la que se ha sostenido toda la carga emocional de esta tesis. En ti me he apoyado en los momentos más duros, cuando aparecían las dudas, esas que inundan a culquier doctorando. Tus palabras, siempre cargadas de comprensión y cariño, fueron el mejor bálsamo. Contigo he disfrutado también de todas las alegrías. Entre éstas, la mejor ha sido compartir lo más bonito que nos podía haber ocurrido, ese *cuchuflito* renacuajo que nos llena la vida, *Taoro.* Cuando miro hacia atrás sólo puedo decirte una y mil veces ¡GRACIAS! Les quiero.