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Ecología y evolución de los cedros macaronésicos

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Nothing in biology makes sense except
in the light of evolution

Dobzhansky

Nothing in evolutionary biology makes
sense except in the light of ecology

Grant & Grant

How and why species multiply

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Resumen

Durante el desarrollo de esta Tesis Doctoral, se han abordado diferentes aspectos ecológicos y evolutivos de la biología de los cedros macaronésicos, el “cedro canario” *Juniperus cedrus*, presente en las Islas Canarias, y el “cedro do mato” *J. brevifolia*, distribuido en el archipiélago de Azores. A pesar de que el cedro que existe en la isla de Madeira ha sido recientemente propuesto como una nueva especie (*J. maderensis*), al comienzo de este trabajo de investigación, dicho taxón era catalogado como *J. cedrus*, por lo que fue considerado como tal. Además, aunque ha sido extensamente cultivado, la población natural de *J. maderensis* es realmente testimonial (aproximadamente 40 individuos). Las dos especies de cedro objeto de esta Tesis se encuentran amenazadas en distinto grado como consecuencia de las intensas talas sufridas en el pasado. Debido a su calidad, su apreciada madera tuvo multitud de usos (ebanistería, construcción, fuego…), lo que provocó un drástico declive de las poblaciones naturales, que quedaron en su mayoría relegadas a zonas abruptas de difícil acceso en el caso de *J. cedrus*, o incluso llegaron a desaparecer de alguna isla, como ocurrió en Graciosa con *J. brevifolia*, en el archipiélago de Azores. Sin embargo, en este último archipiélago, aún es posible encontrar zonas de vegetación dominadas por *J. brevifolia*. La Tesis Doctoral que aquí se presenta, recopila información que incrementa el conocimiento sobre los procesos ecológicos y evolutivos que ocurren en medios insulares y, además, resulta básica para un manejo apropiado de estas especies amenazadas. Mediante el estudio de diferentes aspectos reproductivos (fenología y principales características de las gálbulas maduras), así como de la efectividad de sus respectivos dispersores de semillas, se ha podido evaluar la complejidad y la robustez del sistema de dispersión de semillas de *J. cedrus* y *J. brevifolia*. Los resultados mostraron un mayor potencial de reclutamiento en el caso de *J. brevifolia*, que presentó valores de viabilidad de semillas superiores a los de *J. cedrus*. En ambos casos, la efectividad de los procesos ornitócoros quedó patente, siendo aves de la familia Turdidae los principales dispersores de semillas tanto a nivel cuantitativo como cualitativo. Sin embargo, pese a la mayor complejidad del sistema de dispersión de *J. cedrus*, su dependencia del

mirlo capiblanco *Turdus torquatus* para la dispersión a larga distancia de sus semillas denotó una mayor fragilidad que en el caso de *J. brevifolia,* donde el mirlo común *T. merula*, una ave nativa muy abundante y ubicua, resultó ser el principal dispersor de sus semillas. Con respecto a los patrones de colonización y diversificación de los cedros macaronésicos en sus respectivos archipiélagos, los análisis filogenéticos de la sección *Juniperus* indicaron historias evolutivas independientes, y apoyaron la propuesta de *J. maderensis* como una especie diferente a *J. cedrus*. El cedro do mato, mantiene altos valores de diversidad genética, cuya distribución indica una diversificación y colonización inter-insular temprana tras la colonización del archipiélago. Por el contrario, la composición genética del cedro canario se encuentra mucho más empobrecida, reflejo de un mayor grado de fragmentación de las poblaciones naturales. Sin embargo, dentro de este contexto general, la isla de La Gomera albergó los mayores niveles de diversidad y diferenciación genética, situándola en un lugar prioritario para la conservación del cedro canario. El conjunto de investigaciones desarrolladas a lo largo de esta Tesis Doctoral sitúan a *J. brevifolia* en un mejor estado de conservación que *J. cedrus*, aunque se trata de una especie que aún soporta numerosas perturbaciones antrópicas que deben ser paliadas. Por otro lado, la conservación de *J. cedrus* pasa inevitablemente por el mantenimiento a largo plazo de sus interacciones mutualistas de dispersión de semillas. Dichas interacciones resultan imprescindibles para la regeneración natural y para la existencia de un flujo génico efectivo, que asegure niveles de diversidad genética suficientes para la persistencia de las poblaciones.

Abstract

This Doctoral Thesis deals with different ecological and evolutionary aspects of the Macaronesian junipers: ‘cedro canario’ *Juniperus cedrus*, distributed in the Canary Islands and ‘cedro do mato’ *J. brevifolia*, in the Azores. Although the juniper on the island of Madeira has recently been proposed as a new species (*J. maderensis*), at the beginning of this research this taxon was catalogued as *J. cedrus*, therefore it was previously considered as such. Despite being widely planted, the natural population of *J. maderensis* is in fact only testimonial (ca. 40 individuals). The two juniper species focused on in this Thesis are threatened to a different extent as a consequence of intense felling in the past. Owing to the quality of its valuable wood that had a wide range of uses (cabinetmaking, joinery, firewood…), there was a drastic decline in the natural populations. These became restricted to craggy inaccessible areas in the case of *J. cedrus*, or even disappeared from some islands, like *J. brevifolia* on Graciosa (Azores). However, in this latter archipelago is still possible to find vegetation areas dominated by *J. brevifolia.* This Thesis compiles information that widens our knowledge of the ecological and evolutionary processes occurring in island environments and, additionally, is fundamental to appropriate management of these threatened junipers. By studying different reproductive aspects (phenology and main characteristics of the female cones) along with the effectiveness of their respective seed dispersers, it has been possible to evaluate the complexity and robustness of the seed dispersal systems of *J. cedrus* and *J. brevifolia*. Results show the greater recruitment potential of *J. brevifolia*, which presents higher seed viability values than *J. cedrus.* In both cases the effectiveness of the ornithochory processes became evident, birds of the Turdidae family being the main seed dispersers at the quantitative and qualitative levels. However, despite the greater complexity in the seed dispersal system of *J. cedrus*, its dependence on the ring ouzel *Turdus torquatus* for long-distance seed dispersal points to a more fragile situation in comparison with *J. brevifolia*, in which the blackbird *T. merula*, an abundant native and cosmopolitan bird, is the main seed disperser. Concerning the colonization and diversification

patterns of the Macaronesian junipers in their respective archipelagos, the phylogenetic analyses of the *Juniperus* section indicated independent evolutionary histories and supported the proposal of *J. maderensis* as a different species from *J. cedrus*. The distribution and high levels of genetic diversity displayed by the Azorean juniper indicate an early diversification and inter-island dispersal after the colonization of the archipelago. In contrast, the genetic composition of the Canarian juniper is much more impoverished, reflecting the severe degree of fragmentation in the natural populations. Within this general context, the island of La Gomera showed the highest levels of diversity and genetic differentiation, further justification for priority measures in the conservation of the Canarian juniper. The research activities carried out as part of this Doctoral Thesis indicate that *J. brevifolia* has a better conservation status than *J. cedrus*, despite still suffering numerous anthropogenic disturbances, which require further control. The conservation of *J. cedrus* is however inevitably linked to the vigour of its mutualistic interactions involving seed dispersal. These are necessary for natural regeneration and the maintenance of effective gene flow, in order to provide sufficient levels of genetic diversity to ensure the persistence of natural populations.

Introducción General

Introducción general

Dispersión de plantas: el reto del movimiento

Entre los organismos sésiles, los estadios que permiten el movimiento (huevos, larvas, esporas, semillas) resultan cruciales para su persistencia y éxito evolutivo. Desde hace siglos, la variedad de mecanismos de dispersión[1] de polen y semillas que presentan las plantas ha despertado el interés de numerosos naturalistas. Así, a lo largo de los siglos XIX y XX ya es posible encontrar documentos que describen formas florales, polinizadores, tipos de frutos o métodos de propagación, en plantas de muy diversas partes del mundo (Ridley 1930, van der Pijl 1982). Estos temas continúan en la actualidad siendo el foco de atención de numerosos estudios biológicos que, además de la mera descripción de los síndromes de dispersión[2], estudian las causas y las consecuencias ecológicas y evolutivas de los mismos.

Existen diferentes aproximaciones al estudio de los procesos dispersivos de las plantas. A partir de los trabajos pioneros de John L. Harper (1967), Daniel H. Janzen (1970) y Joseph Connell (1971), la dispersión comienza a abordarse desde dos perspectivas: una ecológica y otra evolutiva. La aproximación ecológica identifica los factores bióticos y abióticos que determinan los procesos de dispersión, así como las consecuencias dinámicas para la especie y la comunidad (cuál es el efecto de la dispersión en el ciclo de reclutamiento[3] de una especie y en el ensamblaje de las especies que componen la comunidad vegetal). La aproximación evolutiva, en cambio, trata de determinar y cuantificar las ventajas de dispersar el polen y las semillas (en qué medida las plantas que dispersan su polen y/o sus semillas incrementan su eficacia biológica). Janzen (1970) y Connell (1971) ya consideran la dispersión como un proceso central en el ciclo de reclutamiento de los organismos, y proponen un modelo bajo el cual el reclutamiento de las plantas depende de la curva de densidad de semillas (que disminuye con la distancia a medida que se aleja de la planta madre) y la curva de supervivencia de las plántulas (que aumenta con la distancia a la planta madre debido a la disminución de la actividad patógena y depredadora). La interacción de ambas curvas, a cierta distancia de la planta madre, indicaría la zona en la cual la probabilidad de reclutamiento alcanza su valor máximo (Fig. 1). Tras estos trabajos, los beneficios de la dispersión de semillas

Cuadro 1 Glosario

[1]**Dispersión/diseminación**: traslado de las unidades biológicas que contienen a la semilla cuando está madura, desde la planta originaria hasta el lugar de germinación.

[2]**Síndromes de dispersión**: conjunto de caracteres morfológicos de los propágulos que se correlacionan con vectores de dispersión particulares.

[3]**Reclutamiento**: proceso por el cual los organismos juveniles sobreviven a las fases tempranas de su desarrollo y pasan a formar parte de la población. El término se usa generalmente para referirse a un estado en el que los organismos se han establecido y pueden ser detectados por un observador.

[4]**Flujo génico**: incorporación al acerbo genético de una población, de genes procedentes de otra u otras poblaciones. En plantas, con algunas excepciones de especies cuyos individuos se dispersan enteros, este traslado se produce mediante la dispersión del polen o las semillas.

[5]**Diversidad genética**: estimación, mediante diferentes parámetros, del número de variantes genéticas (alelos) que contiene una especie. En líneas generales, a mayor diversidad, mayor probabilidad de que algunos individuos posean los alelos adecuados para sobrevivir a cambios ambientales que no impliquen su destrucción física.

[6]**Presión selectiva**: cualquier causa que, en cierta proporción, reduzca el éxito reproductivo de una población. Con suficiente presión, caracteres heredados que mitiguen sus efectos, incluso siendo deletéreos en otras circunstancias, pueden llegar a ser ampliamente propagados en la población sobreviviente.

[7]**Endozoocoria**: dispersión de semillas por vertebrados que ingieren frutos y regurgitan o defecan las semillas que contienen sin dañarlas durante el proceso.

[8]**Agente dispersante**: vector transportador de semillas u otras unidades de dispersión. Los agentes dispersantes pueden incluir factores bióticos, como animales, o factores abióticos como el viento o el agua.

[9]**Frugivoría**: tipo de alimentación basada total o parcialmente en la ingesta de frutos. A diferencia de lo que ocurre en el trópico, en latitudes templadas este tipo de alimentación suele ser estacional.

[10]**Diplocoria**: dispersión de semillas por la secuencia de dos o más fases, cada una de las cuales está mediada por un agente dispersante diferente.

[11]**Marcador molecular**: segmento de ADN con una ubicación física identificable dentro del genoma, y cuya herencia se puede rastrear.

[12]**Sombra/lluvia de semillas**: distribución espacial de las semillas dispersadas con respecto a su planta madre y el resto de individuos conespecíficos.

[13]**Coevolución**: fenómeno de adaptación evolutiva mutua producida entre dos o varias especies de seres vivos, como resultado de relaciones mutualistas.

[14]**Generalismo**: capacidad de una especie para prosperar en una amplia variedad de condiciones ambientales o hacer uso de gran variedad de recursos diferentes.

[15]**Mutualismo**: interacción entre individuos de diferentes especies, de la cual ambos se benefician y mejoran su aptitud biológica.

[16]**Metapoblación**: conjunto de poblaciones locales de una misma especie, entre las cuales podría haber flujo génico, extinción y colonización.

han sido ampliamente abordados en la literatura científica. En resumen, las principales ventajas ecológicas de la dispersión de semillas incluyen el eludir enemigos naturales (patógenos) que pueden concentrarse en la cercanía de los parentales, evitar procesos de competencia con los propios parentales u otros individuos conespecíficos, o incrementar la probabilidad de encontrar un lugar adecuado para el establecimiento (Howe & Smallwood 1982, Willson & Traveset 2000). Desde un punto de vista evolutivo, el flujo génico[4] –movimiento de genes de una población a otra (Slatkin 1985)– ya sea vía polen o semilla, contribuye al mantenimiento de la diversidad genética[5] de las especies, que tendrán mayor capacidad de respuesta a las presiones selectivas[6] de un ambiente cambiante y, por tanto, ofrecerán mayor resistencia a los procesos de extinción (Ellstrand 1992, Frankel *et al.* 1995, Freeman & Herron 1995).

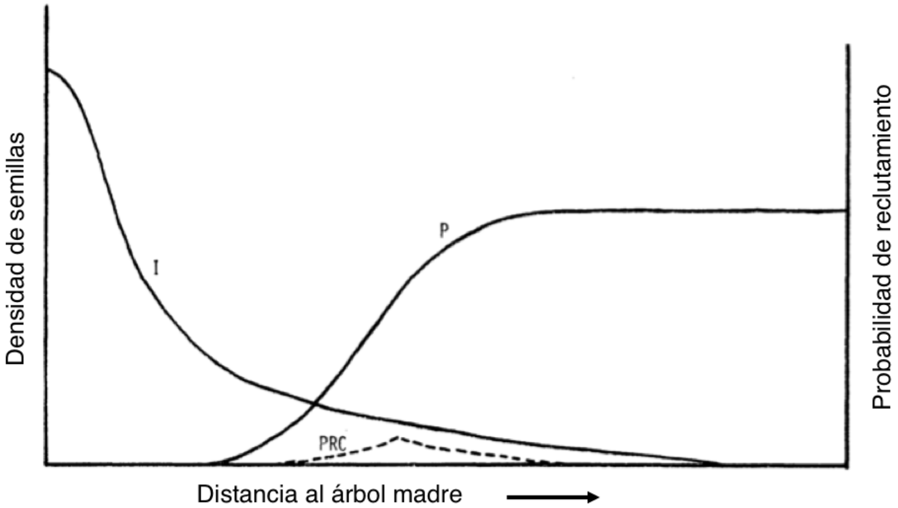


Fig. 1 Modelo propuesto por Janzen (1970), en el que la probabilidad de reclutamiento está sujeta a (1) el tamaño de la cosecha, (2) el tipo de agente dispersante, (3) la distancia al árbol madre, y (4) la actividad de los predadores de las semillas o plántulas. La densidad de semillas dispersadas disminuye exponencialmente con la distancia al árbol madre (I) mientras la actividad de los patógenos específicos que atacan las semillas dispersadas depende positivamente de la densidad. Por tanto, la probabilidad de reclutamiento de una semilla (P) es mayor al alejarse del árbol madre. Esto resulta en una mayor probabilidad de germinación exitosa (PRC), que es máxima a cierta distancia del árbol madre. (Modificado de Janzen 1970).

Los patrones de dispersión de semillas, por tanto, establecen el área potencial de reclutamiento (Schupp & Fuentes 1995). Sin embargo, que la dispersión de semillas sea un proceso dinámico en una determinada especie de planta, no implica necesariamente que también sea efectivo. La efectividad del proceso dispersivo (SDE = '*seed dispersal effectiveness*'), es decir, el éxito en el establecimiento de nuevos individuos, puede cuantificarse mediante el producto del número de semillas dispersadas y la probabilidad de que la semilla dispersada origine un individuo adulto: SDE = Cantidad * Calidad (Schupp 1993, Schupp *et al.* 2010). Según esta fórmula, en los procesos endozoócoros[7], la "Cantidad" es un factor que depende del número de visitas del agente dispersante[8] y del número de semillas dispersadas en cada visita, mientras que la "Calidad" depende de la probabilidad de supervivencia de la semilla tras la manipulación y el tratamiento digestivo del agente dispersante, así como de la probabilidad de que una vez dispersada, la semilla que continúa siendo viable sea capaz de germinar y producir un nuevo adulto (Schupp *et al.* 2010) (Fig. 2).

Tras el establecimiento de este marco conceptual, se produce un auge de trabajos sobre frugivoría[9] que resaltan la importancia del tratamiento mecánico/digestivo que los agentes dispersantes realizan sobre las semillas, pudiendo alterar positiva o negativamente la capacidad de las mismas para la germinación (Traveset 1998). También comienzan a realizarse estudios que ponen de manifiesto la complejidad de los eventos dispersivos, en los que el transporte de semillas puede tener lugar en varias fases, debido a la secuencia de agentes dispersantes tanto bióticos como abióticos (Vander Wall & Longland 2004), o incluso variar entre poblaciones de una misma especie (Westcott *et al.* 2005).

Los patrones de comportamiento y movimiento de los dispersores juegan un papel central en el ciclo de reclutamiento de las plantas que dispersan. Así, la manipulación de los frutos o incluso de una presa en el caso de procesos de diplocoria[10], puede ser determinante en el mantenimiento de la viabilidad de las semillas tras el evento dispersivo (Padilla & Nogales 2009). El estudio de los patrones de movimiento de los dispersores se ha basado fundamentalmente en observaciones de

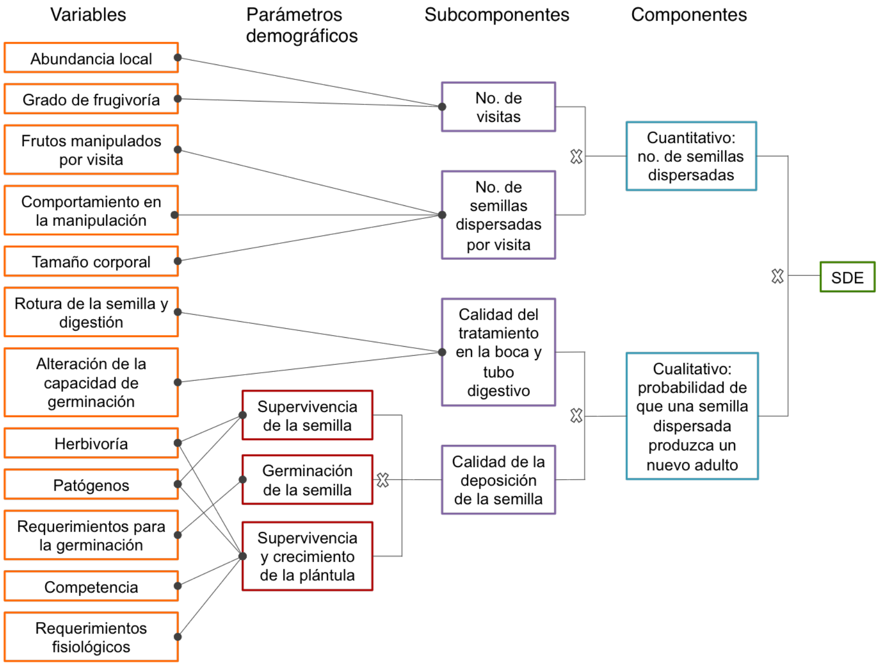


Fig. 2 Diagrama de flujo jerárquico que representa los factores determinantes para la eficacia de la dispersión de semillas (SDE, 'seed dispersal effectiveness') en un modelo endozoócoro. Los *Componentes* y *Subcomponentes* constituyen el marco principal de organización para el desarrollo de estudios y cálculo de SDE. Los *Parámetros demográficos* representan un esquema simplificado para determinar la *Calidad de la deposición*. Las *Variables* son medidas representativas que son relevantes para los estudios de SDE. Los cuadros conectados por líneas en ángulo recto con un símbolo "X" representan factores que son, en principio, multiplicativos (p. ej. n_o de visitas * n_o de semillas dispersadas por visita = n_o de semillas dispersadas). Las líneas conectoras con círculos en los extremos indican que la variable afecta al *Subcomponente* o al *Parámetro demográfico*, pero no de forma multiplicativa. (Modificado de Schupp *et al.* 2010).

campo con distintas técnicas de marcaje (p. ej. anillas, radiotransmisores, GPS). Sin embargo, a raíz del avance de las técnicas moleculares en los últimos años, el auge de trabajos que emplean marcadores moleculares[11] para hacer un seguimiento directo del evento dispersivo (Godoy & Jordano 2001) ha permitido documentar las diferentes contribuciones de los distintos grupos de dispersores y su importancia en la estructura espacial de la sombra de semillas[12] y plántulas (García & Grivet 2011, Garcia *et al.* 2009, Jordano *et al.* 2007). Estos trabajos, a su vez, han puesto de manifiesto que las distintas curvas de supervivencia de las plántulas no solo son el resultado de procesos dependientes de la densidad y la distancia, tal como se predice en el modelo de Janzen y Connell (p. ej. depredación de semillas), sino que también se encuentran influenciadas por las preferencias de los frugívoros por ciertos microhábitats (Garcia *et al.* 2009) y por el comportamiento social de los dispersores (Karubian *et al.* 2010), que dan lugar a una distribución no azarosa de las progenies maternas en la lluvia de semillas[12] (Fig. 3).

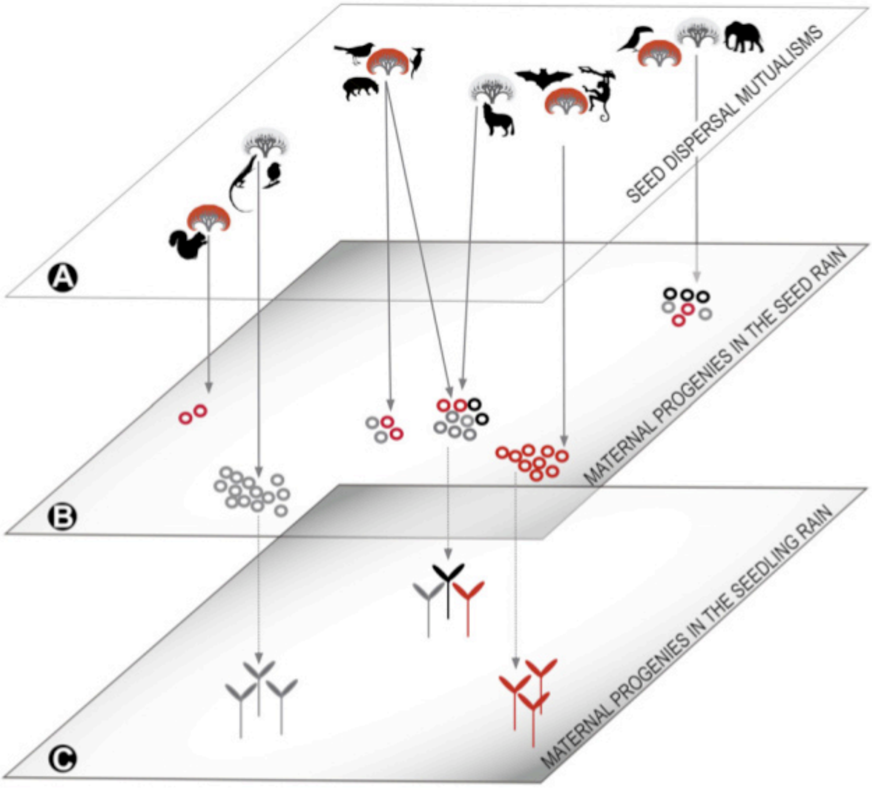


Fig. 3 Los vertebrados frugívoros movilizan una gran cantidad de semillas a lo largo del paisaje (A). Su talla corporal, el tipo de alimentación y su comportamiento reproductor, determinan la distribución espacial de la progenie materna en la lluvia de semillas (B). De este modo, los vertebrados de pequeña talla (pequeños paseriformes, roedores o lagartos) tenderían a dispersar grupos de propágulos emparentados cerca de la planta-madre. Los vertebrados de mediana y gran talla podrían crear lluvias de semillas complejas donde los grupos de propágulos emparentados se combinan con grupos mayores de semillas procedentes de diferentes plantas-madre. Las plantas-madre de estos grupos de semillas podrían estar localizadas dentro de la población focal (progenie en color rojo y gris), o a cierta distancia de ella (progenie en color negro) como resultado de eventos de dispersión a larga distancia. La variabilidad de la composición genética de la lluvia de semillas representa una de las principales consecuencias de los mutualismos dispersivos con potencial para influenciar la dinámica de regeneración, y podría explicar diferentes éxitos de reclutamiento a lo largo del paisaje (C). (Extraído de García & Grivet 2011).

Ya desde la década de los 70, la dispersión de semillas comenzó a verse como un proceso de beneficio mutuo entre las plantas –con estrategias dirigidas a la atracción de dispersores eficientes– y los frugívoros –con estrategias dirigidas a maximizar la obtención de nutrientes–, lo que promovió la aplicación de modelos de coevolución[13] al estudio de la dispersión de semillas (McKey 1975). Así, las interacciones con animales frugívoros han sido determinantes en la diversificación de estructuras reproductoras y mecanismos de dispersión (Bascompte & Jordano 2007). Dependiendo del grado de generalismo[14] de las plantas, éstas pueden ser visitadas por un mayor o menor número de agentes dispersantes pertenecientes a diferentes taxones. De este modo, el sistema de dispersión de semillas de una planta concreta estará formado por una red más o menos compleja de interacciones, cuya estructura y estabilidad está directamente relacionada con el mantenimiento de las poblaciones a largo plazo (Bascompte & Jordano 2007, Bascompte *et al.* 2003, 2006). No son pocos los casos de patrones de interacción altamente específicos, en los que la extinción del agente dispersante provoca un impacto muy negativo en las poblaciones vegetales que dependían del mismo (p. ej. Meehan *et al.* 2002, Temple 1977). Por todo ello, el conocimiento de los patrones ecológicos y evolutivos de las interacciones mutualistas[15] (entre las que se encuentra la dispersión de semillas), cobra especial relevancia para el mantenimiento de las comunidades vegetales y, en general, del conjunto de la biodiversidad (Howe & Smallwood 1982).

En la actualidad, la fuerte fragmentación de las comunidades vegetales hace que muchas poblaciones que ocuparon grandes extensiones, se encuentren formando parches aislados entre sí. La fragmentación y el aislamiento de las poblaciones constituyen pasos decisivos en el proceso de especiación (Futuyma 1998). Sin embargo, la rápida fragmentación del paisaje provocada por la acción del hombre, impide que los organismos dispongan del tiempo suficiente para reaccionar ante ella, y por lo general tiene un efecto negativo al reducir la diversidad genética y aumentar la consanguinidad y el riesgo de extinción (Frankham *et al.* 2002). En esta situación, el mantenimiento de niveles adecuados de flujo génico (vía polen o semilla) entre los parches de vegetación se torna imprescindible para contrarrestar el impacto de la fragmentación. Dado que la mayoría de las semillas son dispersadas a corta distancia (desde cero hasta algunas decenas de metros; ver Howe and Smallwood 1982 y referencias allí citadas), los eventos de dispersión a larga distancia, pese a ser menos frecuentes, son de una importancia biológica extrema (Cain *et al.* 2000, Nathan 2006). Aparte de ser determinante en la dinámica ecológico-evolutiva de las metapoblaciones[16], la dispersión a larga distancia también permite la colonización de nuevos hábitats disponibles, cuyo exponente podemos encontrarlo en islas oceánicas remotas que distan varios cientos de kilómetros de las masas continentales más cercanas (Carlquist 1965, 1966, 1967, Sorensen 1986).

Colonización insular: características biológicas y genéticas de las plantas

A diferencia de las islas de origen continental, que una vez formaron parte del continente, las islas oceánicas son el resultado de la actividad volcánica del lecho marino y generalmente se encuentran situadas lejos de los continentes y separados de éstos por grandes profundidades (Whittaker & Fernández-Palacios 2007). Surgen, por tanto, desprovistas de vida, y van siendo pobladas paulatinamente por una biota generalmente caracterizada por su poder de dispersión a larga distancia, aunque muchas veces carecen de síndromes específicos (Higgins *et al.* 2003, Vargas *et al.* 2011). Las plantas que consiguen sortear la barrera marina y establecerse en islas remotas, lo hacen muchas veces favorecidas por adaptaciones morfológicas de las semillas, como es el caso de estructuras que facilitan la dispersión por el viento (anemocoria), el anclaje a los animales (ectozoocoria), la flotabilidad (hidrocoria) o los procesos endozoócoros (Chambers & MacMahon 1994, Darwin 1859, Gillespie *et al.* in press, Nogales *et al.* 2001, Renner 2004, Sorensen 1986, van der Pijl 1982) (Fig. 4). Numerosos estudios señalan a las aves como los responsables más comunes de la llegada de propágulos[1] a los ecosistemas insulares (ver Crawford *et al.* 2011 y referencias allí citadas). En el caso particular de las islas macaronésicas, las plantas con síndromes de dispersión endozoócoros que han sido estudiadas bajo un punto de vista filogenético[2] se encuentran asociadas a múltiples eventos de colonización a los archipiélagos (Vargas 2007).

Cuadro 2 Glosario

[1] **Propágulo**: parte de una planta capaz de originar vegetativamente otro individuo.

[2] **Filogenia**: determinación de la historia evolutiva de los organismos a partir de sus ancestros comunes, incluyendo la secuencia de diversificación (inferida a partir de la posición de las ramas en el árbol filogenético) y, a veces, las edades absolutas de divergencia.

[3] **Anemofilia**: polinización por medio del viento.

[4] **Establecimiento**: fase posterior a la dispersión de las semillas para la colonización de nuevas áreas en las cuales un individuo o unos pocos individuos echan raíces y germinan, reclutando nuevos individuos para la ocupación efectiva.

[5] **Dioecia**: característica por la que las gónadas, y por tanto los gametos masculinos y femeninos, son portados por individuos distintos de la misma especie.

[6] **Autocompatibilidad**: capacidad de una planta hermafrodita de lograr la fecundación de sus óvulos por efecto de su propio polen y, como consecuencia, producir sus propias semillas.

[7] **Autoincompatibilidad**: incapacidad de una planta hermafrodita para producir semillas por autopolinización aunque presente gametos viables.

[8] **Variabilidad/variación genética**: medida de la variación debida a diferencias genéticas entre individuos de una población. Constituye la materia prima para el cambio evolutivo, permitiendo a las poblaciones evolucionar en respuesta a la heterogeneidad ambiental.

[9] **Radiación evolutiva**: patrón de divergencia en un intervalo de tiempo geológico relativamente corto, de miembros de una misma línea filogenética en una variedad de formas. Se diferencia de la "Radiación adaptativa", que atribuye las diferencias entre formas a cambios adaptativos, como por ejemplo por el uso diferencial de recursos o hábitats.

[10] **Selección**: supervivencia o reproducción diferencial no aleatoria de organismos fenotípicamente diferentes.

[11] **Especiación**: evolución del aislamiento reproductivo dentro de una especie ancestral, resultando en dos o más especies descendientes.

[12] **Grupo monofilético**: grupo de organismos que han evolucionado a partir de un ancestro común, y donde todos los descendientes de ese ancestro están incluidos en el grupo. **Grupo parafilético**: grupo taxonómico que contiene algunos pero no todos los descendientes del ancestro común más reciente.

Grupo polifilético: grupo taxonómico compuesto por organismos que no comparten un único ancestro común.

[13] **Efecto fundador**: pérdida de variación genética que se produce cuando una nueva población se forma a partir de un número pequeño de individuos que portan sólo una fracción de la variación genética presente en la población de origen.

[14] **Deriva genética**: cambios al azar en la frecuencia de dos o más alelos o genotipos dentro de una población.

[15] **Cuello de botella**: reducción severa y temporal en el tamaño poblacional que provoca la variación aleatoria de las frecuencias alélicas.

[16] **Depresión por consanguinidad**: reducción, en individuos consanguíneos, del valor medio de una carácter generalmente correlacionado con la aptitud biológica.

[17] **Microsatélites (SSR, Short Sequence Repeat)**: secuencias de ADN en las que un fragmento variable de 1-6 nucleótidos se repite en tándem. Generalmente se encuentran en zonas no codificantes y poseen una alta tasa de mutación, lo que los hace muy polimórficos.

[18] **Secuenciación masiva (pirosecuenciación)**: tecnología de determinación de la secuencia del ADN a gran escala mediante luminiscencia, aplicable a genomas completos.

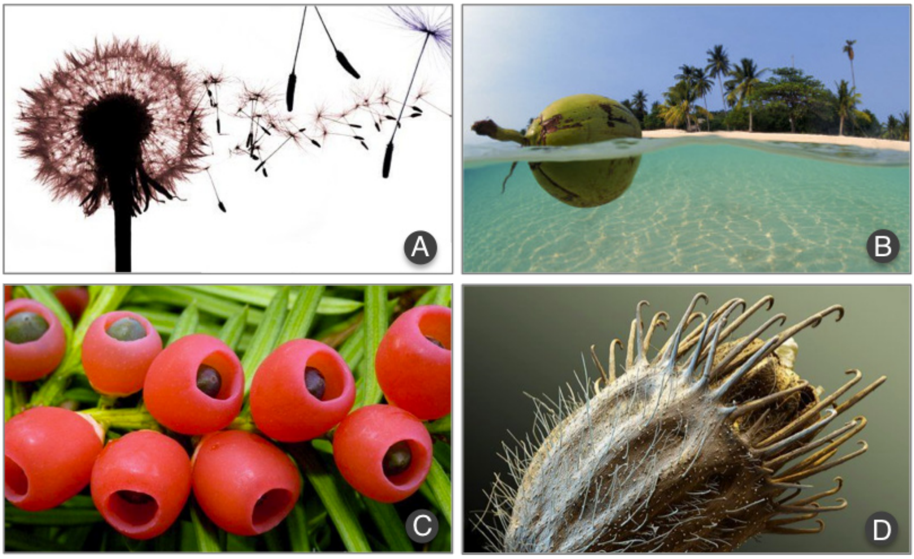


Fig. 4 Algunas adaptaciones morfológicas de las semillas que facilitan su dispersión a larga distancia mediante diferentes vectores. (A) Anemocoria: las semillas suelen estar dotadas de estructuras que aumentan su superficie y su rozamiento con el aire; (B) Hidrocoria: las semillas poseen membranas que garantizan la impermeabilidad, así como cámaras de aire o aceite que permiten la flotación; (C) Endozoocoria: las semillas son tragadas por determinados animales atraídos por una cubierta carnosa, nutritiva, aromática o con colores vistosos; y (D) Ectozoocoria: las semillas o frutos se adhieren a la superficie de los animales por medio de sustancias adhesivas o de estructuras mecánicas que favorecen la fijación, tales como ganchos o arpones. Fotos: A, C y D, SCIENCEphotoLIBRARY (www.sciencephoto.com); B, Jurgen Freund.

Por otro lado, la elevada incidencia de plantas anemófilas[3] en algunas islas oceánicas fomentó que ciertos autores postularan hipótesis sobre los beneficios de la polinización por viento en islas (Carlquist 1974, Ehrendorfer 1979). Estos beneficios implicarían que: (1) las plantas anemófilas estarían más favorecidas que las plantas entomófilas durante el proceso de establecimiento[4], dada su independencia de los polinizadores, (2) las inclemencias atmosféricas beneficiarían la polinización, y (3) la polinización por viento promovería una dispersión de polen más efectiva que la polinización por animales, con los consiguientes beneficios derivados del cruzamiento. Sin embargo, estas hipótesis han perdido fuerza a medida que otros estudios han puesto de manifiesto la escasez de especies anemófilas en determinados archipiélagos (p ej. Galápagos, McMullen 1987), o la asociación de la anemofilia con formas de vida como la leñosidad o la dioecia[5] (Lloyd 1985, Sakai *et al.* 1995).

Otro de los atributos más importantes que caracteriza a los ancestros de las plantas insulares es el sistema de cruzamiento. La “regla de Baker” propone que, durante la fase de establecimiento que sigue al evento dispersivo, las especies auto-compatibles[6] estarían en una posición ventajosa frente a las auto-incompatibles[7] (Baker 1955). Esto se debe a que un solo propágulo de una planta auto-compatible podría iniciar una población sexualmente reproductora, mientras que en el caso de las plantas auto-incompatibles, se necesitarían dos o más propágulos. Carlquist (1974), por su parte, apuntó que los propágulos colonizadores auto-compatibles no generarían suficiente variación genética[8] para sustentar la radiación[9] y la diversificación de su progenie. Sin embargo, los datos existentes apoyan la predicción general de un dominio de las especies auto-compatibles en los sistemas insulares (Anderson *et al.* 2001, Barrett 1996, Bernardello *et al.* 2001, Ehrendorfer 1979, McMullen 1987, Webb & Kelly 1993).

La efectividad de la colonización insular pasa por el éxito en el establecimiento de los propágulos, así como por la radiación y diversificación de la progenie en el nuevo lugar de establecimiento. Ya en la década de 1970, Carlquist (1974) sugirió que la probabilidad de dispersión constituía un obstáculo menor que el establecimiento en sí, sobre el cual actúan diferentes procesos selectivos[10] que determinan el éxito de la colonización. La idoneidad del sitio de arribada, el nivel de competencia, la probabilidad de supervivencia, los niveles de fecundidad, etc., son algunos de los factores implicados en la efectividad del proceso dispersivo, es decir, del establecimiento exitoso de individuos reproductores en las islas.

Tras la colonización, el aislamiento que de forma natural ofrecen los ecosistemas se torna crucial para la evolución de las plantas que han logrado establecerse. Por esta razón, los archipiélagos oceánicos ofrecen un marco espacio-temporal ideal para el estudio de patrones de colonización y variación genética asociados a la especiación[11] (Emerson 2002). Por lo general, aunque con algunas excepciones, los estudios filogenéticos indican que, en la mayoría de los casos, las áreas continentales más cercanas constituyen la fuente de origen de los ancestros que colonizaron las islas (p. ej. ver la compilación de Carine *et al.* 2004 para las Islas Canarias). En otras ocasiones, las islas pueden actuar también como fuentes de propágulos que se dispersan hacia el continente u otros archipiélagos (Carine *et al.* 2004, Harbaugh & Baldwin 2007, Harbaugh *et al.* 2009). Hoy en día existen numerosas filogenias moleculares que han clarificado la historia evolutiva de muchos grupos de plantas confinadas a islas y que, en general, indican que la mayoría de ellas tienen un origen monofilético[12] y por lo tanto deben haber evolucionado a partir de un solo evento colonizador en el archipiélago donde se encuentran (ver Silvertown 2004 y referencias allí citadas).

Como resultado del efecto fundador[13] y, por tanto, de la deriva genética[14] asociada al tamaño poblacional reducido, la arribada de una nueva especie a las islas conlleva por lo general una pérdida de variación genética respecto de la existente en las poblaciones suministradoras. Así, para la mayoría de grupos de plantas presentes en islas remotas, los cuellos de botella[15] acusados han marcado su historia evolutiva (Fig. 5), de modo que, con algunas excepciones (Fernández-Mazuecos & Vargas 2011), las poblaciones insulares presentan menores niveles de diversidad genética que sus congéneres continentales, y son más propensas a sufrir los efectos negativos de la deriva genética y la depresión por consanguinidad[16] (Ellstrand & Elam 1993, Frankham 1997). Por otro lado, también puede ocurrir que múltiples introducciones aumenten los niveles de heterogeneidad genética y que las islas alberguen genotipos únicos dentro de la especie, convirtiéndose así en zonas genéticamente muy particulares. De hecho, algunos autores sugieren la posibilidad de que en algunos linajes con topologías monofiléticas inferidas a través de técnicas moleculares, hayan ocurrido múltiples introducciones y eventos de hibridación antes de que haya tenido lugar la radiación (ver Caujapé-Castells 2011 y referencias allí citadas).

En conjunto, el tamaño poblacional y el rango de distribución reducido de los organismos insulares, junto con las características singulares derivadas de largos periodos evolutivos en condiciones de aislamiento, los sitúa ante un riesgo genético superior al que se enfrentan los organismos continentales y, en definitiva, los hace más susceptibles a los procesos de extinción (ver Frankham 1998 y referencias allí citadas). Además de esta situación de vulnerabilidad genética, al estar menos afectadas por cambios climáticos del Plioceno y Pleistoceno (incremento de la aridez primero y ciclos de glaciaciones después), las islas pueden conservar una parte de la variabilidad genética que existía en las poblaciones continentales fundadoras que se extinguieron durante esos periodos. Por estos motivos, e independientemente del valor cuantitativo de la variabilidad genética residente en ellas, las islas deben ser prioritarias desde el punto de vista de la conservación, ya que capturan elementos de la historia evolutiva de especies que han desaparecido del resto de su rango de distribución ancestral (Sechrest *et al.* 2002).

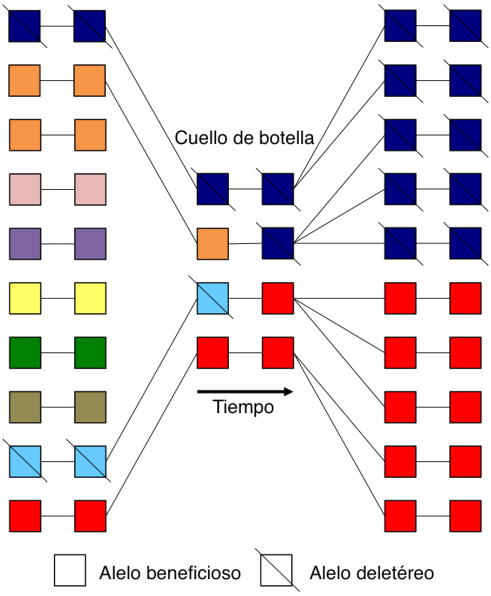


Fig. 5 Las poblaciones que pasan por un cuello de botella son aquellas que ven reducido su número de individuos, y posteriormente se recuperan. La variación genética se ve típicamente reducida durante el proceso y, a menudo, las variantes deletéreas que se encontraban a baja frecuencia en la población de origen se vuelven más comunes, reduciendo la capacidad adaptativa de la población recuperada. (Modificado de Lewin 1999).

Actualmente, numerosas herramientas moleculares –desde aproximaciones filogeográficas basadas en la variación del ADN de orgánulos celulares (Avise 2000, Petit & Vendramin 2007), hasta el uso de marcadores hipervariables como los microsatélites[17] (SSRs, por '*simple* *sequence repeats'*), o incluso la secuenciación masiva[18]– permiten la evaluación de la variación genética a nivel intraespecífico, imprescindible para inferir la historia evolutiva que existe tras la situación actual de los diferentes taxones insulares.

La actividad humana ha tenido un impacto especialmente severo en islas de todo el mundo. La pérdida de interacciones mutualistas, la destrucción y alteración del hábitat, la introducción de especies invasoras, así como el cambio climático y la polución, son algunas de las principales amenazas para la flora vascular oceánica (Caujapé-Castells *et al.* 2010, Heywood & Iriondo 2003). Mientras la población humana siga incrementándose, también lo hará este tipo de factores asociados, por lo que los esfuerzos dirigidos a ampliar el conocimiento ecológico y evolutivo de las especies, así como a estrechar lazos entre el ámbito científico, la gestión y la educación medioambiental, son de absoluta prioridad para la conservación de la biodiversidad.

Los cedros endémicos de las islas macaronésicas

Situadas en el océano atlántico, las islas macaronésicas conforman los archipiélagos de Azores, Madeira, Salvajes, Canarias y Cabo Verde (Fig. 6). Estos archipiélagos, de origen volcánico, difieren en latitud, altitud, área, aislamiento y edad (Tabla 1), lo que les confiere una serie de características propias determinantes para la biota que albergan.

El conjunto de islas macaronésicas constituye un punto caliente de biodiversidad. En ellas se han identificado alrededor de 3.100 especies de fanerógamas, de las cuales un 20% constituye el elemento endémico (Hansen & Sunding 1993). Por otra parte, el efecto atemperador del océano, ha permitido que durante las glaciaciones acaecidas en el Terciario tardío y el Cuaternario, los archipiélagos macaronésicos actuaran como un refugio para la biota subtropical que no sobreviviría al frío y a la sequedad de los ecosistemas continentales (Hewitt 2000).

Con excepción de los archipiélagos de Cabo Verde y Salvajes, las islas albergan distintas especies pertenecientes al género *Juniperus* (Cupressaceae), uno de los más diversos dentro de las coníferas. Con unas

Fig. 6 Situación de los archipiélagos macaronésicos.

Tabla 1 Algunas características geográficas de los archipiélagos macaronésicos (extraído de Fernández-Palacios & Dias 2002).

	Azores	Madeira	Salvajes	Canarias	Cabo Verde
N° islas (> 1 km^2)	9	4	2	11	13
Latitud (°N)	37-40	32-33	30	28-29	15-17
Área (km^2)	2388	815	4	7447	3580
Altitud máxima (m)	2351	1846	154	3718	2835
Aislamiento (km)	1370	630	388	96	570
Edad (Ma)	8,1	15	24-27	20,5	10,3

67 especies, el género puede dividirse en tres secciones: *Caryocedrus*, con una sola especie (*J. drupacea*) confinada a la región de Grecia y Turquía; *Juniperus*, con 11 especies; y *Sabina*, con 55 especies (Adams 2011). A excepción de *J. procera*, que habita las montañas del Rift africano, todos estos taxones se distribuyen en el hemisferio norte, lo que sugiere que el género ya existía cuando ocurrió la separación de Laurasia, hace aproximadamente 65 Ma (Adams 2011, Mao *et al.* 2010). Siguiendo la clasificación propuesta por Adams (2011), los archipiélagos albergan, además de una especie de la sección *Sabina* (*J. phoenicea* L. var. *turbinata* (Guss.) Parl.) en las Islas Canarias y Madeira, tres especies endémicas pertenecientes a la sección *Juniperus*: (1) el "cedro do mato" *J. brevifolia* (Seub.) Antoine, circunscrito al archipiélago de Azores y distribuido en

todas las islas excepto en Graciosa (Elias 2007); (2) el "cedro canario" *J. cedrus* (Webb & Berth.), presente en las Islas Canarias, concretamente en Gran Canaria, Tenerife, La Gomera y La Palma (Acebes-Ginovés *et al.* 2004); y (3) el "cedro de Madeira" *J. maderensis* (Menezes, R. P. Adams), endémico de la isla de Madeira. En base a datos moleculares y bioquímicos, esta última especie ha sido recientemente propuesta como tal (Adams *et al.* 2010), ya que por mucho tiempo fue considerada como la misma especie que se encuentra en el archipiélago canario.

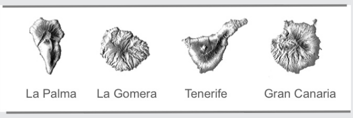
Tras la conquista europea de las islas macaronésicas en el siglo XV, los bosques fueron sometidos a un intenso aprovechamiento, que tuvo como consecuencia la práctica desaparición de algunas de las formaciones boscosas más diversas. Tal es el caso de los bosques termófilos de las Islas Canarias, situados en áreas de medianías donde tuvo lugar el mayor asentamiento de la población humana (Fernández-Palacios *et al.* 2008). Los cedros macaronésicos, cuya madera tradicionalmente ha sido muy apreciada para la ebanistería, la reparación y fabricación de barcos, la industria del vino y el tabaco (confección de barriles y cajas), la fabricación de instrumentos musicales, o simplemente para hacer fuego, tampoco se salvaron de las intensas talas, que diezmaron las poblaciones en todo el rango de distribución.

En el archipiélago de Azores, el cedro do mato fue extinguido de la isla de Graciosa y llevado al borde de la extinción en la isla de Santa Maria (Elias 2007). Sin embargo, aunque su distribución actual dista mucho de la potencial, en otras islas como por ejemplo Terceira y Flores, aún es posible encontrar grandes áreas vegetales ocupadas por comunidades dominadas por *J. brevifolia*, principalmente por encima de los 500 m de altitud (Dias *et al.* 2007, Elias 2007, Elias & Dias 2009). En el caso del cedro de Madeira, se estima que la población natural, restringida a zonas abruptas e inaccesibles, no supera los 40 individuos (IUCN 2011). Afortunadamente, pese a la crítica situación de este cedro en estado natural, sus semillas han sido colectadas y la especie ha sido extensamente cultivada en la isla. En las Islas Canarias, existen algunas referencias que indican una amplia distribución de *J. cedrus* en el pasado, sobre todo en aquellas islas de mayor área y heterogeneidad geográfica como Gran Canaria y Tenerife (Francisco-Ortega *et al.* 2009, Hollermann 1978, Leuschner 1996). Sin embargo, la intensidad de las talas en las islas fue tal, que las poblaciones quedaron reducidas a unos pocos individuos distribuidos principalmente en zonas escarpadas y de difícil acceso. Actualmente, las poblaciones de *J. cedrus* están ubicadas en áreas protegidas, lo que ha facilitado una leve recuperación de las mismas. Sin embargo, el grado de fragmentación severo que aún presentan, el bajo número de individuos sexualmente maduros (IUCN 2011), o la intolerancia al fuego (B.R., obs. pers.), son factores que aún sitúan al cedro canario en un delicado estatus de conservación.

Los cedros macaronésicos se caracterizan por mostrar una gran amplitud ecológica. El cedro do mato puede crecer desde el nivel del mar (aunque con niveles bajos de cobertura), hasta los 1.500 m de altitud. Por su parte, el hábitat natural de *J. cedrus* se extendía desde los bosques termófilos (~ 500 m s.n.m.) hasta la alta montaña (~ 2.400 m s.n.m.). Actualmente, es posible encontrar poblaciones coexistiendo tanto con vegetación eminentemente termófila –como es el caso de La Montaña del cedro en Gran Canaria–, como con elementos de la Laurisilva en la isla de La Gomera, o formando parte de la vegetación de la alta montaña de las islas de Tenerife y La Palma, donde constituye la única especie arbórea de este piso de vegetación canaria. La elevada variedad morfológica de ambas especies es una característica indicadora del grado de adaptación a las diferentes condiciones ambientales de las islas. Así, las poblaciones costeras de *J. brevifolia* exhiben características xeromórficas como resultado de las elevadas temperaturas y escasa precipitación, mientras que las poblaciones de montaña presentan individuos de pequeñas dimensiones, postrados, y con adaptaciones foliares a la elevada exposición y precipitación, temperaturas bajas y deficiencia de nutrientes (Elias 2007). En el caso de *J. cedrus*, aunque no existen estudios específicos al respecto, llama la atención la diferencia de porte que puede apreciarse entre los ejemplares que crecen en zonas boscosas como el Monteverde, con troncos rectos que pueden alcanzar los 15 m de altura, y algunos individuos de la alta montaña, con troncos retorcidos y porte achaparrado.

Cuadro 3 Características principales de *Juniperus cedrus*

Juniperus cedrus



Nombre común: cedro canario

Distribución: endemismo canario (La Palma, La Gomera, Tenerife y Gran Canaria)

Caract. reproductivas destacables:
- Dioecia
- Anemofilia (liberación de polen en otoño)
- Endozoocoria

Estado de conservación:
- UICN: en peligro
- Catálogo nacional: vulnerable
- Catálogo regional: desprotegido

Cuadro 4 Características principales de *Juniperus brevifolia*

Juniperus brevifolia

Nombre común: cedro do mato

Distribución: endemismo de Azores (todas las islas excepto Graciosa)

Caract. reproductivas destacables:
- Dioecia
- Anemofilia (liberación de polen en primavera)
- Endozoocoria

Estado de conservación:
- UICN: en peligro

Como la gran mayoría de especies del género *Juniperus*, los cedros macaronésicos son dioicos, es decir, con sexos en distintos pies de planta. Las plantas masculinas producen unos conos escuamiformes (conos masculinos) que portan los sacos polínicos. Una vez liberado el polen, el viento constituye el vector de dispersión responsable de la polinización. Pese a su condición de gimnospermas, las especies pertenecientes al género *Juniperus* han desarrollado unos conos femeninos carnosos conocidos como “gálbulas”. Estas estructuras reproductoras son frecuentemente consumidas por aves y pequeños mamíferos (Adams 2011). De hecho, en los ambientes continentales, las aves son los principales agentes dispersantes para las especies de este género (García 2001, Jordano 1993, Livingston 1972, Zamora 1990). La dispersión a larga distancia, que supone el consumo de semillas por parte de las aves, queda patente en el establecimiento de *Juniperus* en islas atlánticas como Azores, Madeira, Bermuda, el Caribe y las Islas Canarias (Adams 2011).

A pesar de tratarse de especies emblemáticas y en un delicado estado de conservación, la información sobre aspectos ecológicos y evolutivos de los cedros macaronésicos era inexistente en algunos casos y muy insuficiente en otros, previo a la elaboración de esta Tesis Doctoral. Como ya ha sido destacado en apartados anteriores, los frugívoros determinan el patrón de la sombra de semillas y, por tanto, ejercen un efecto determinante tanto en la demografía como en la composición genética de las plantas que dispersan (Jordano & Godoy 2002). Sólo algunos trabajos previos señalaban puntualmente la interacción del cedro canario con los cuervos *Corvus corax* (Nogales *et al.* 1999) –en drástico declive en las islas– o con los lagartos nativos de la isla de Tenerife *Gallotia galloti* (Valido 1999). En el caso del cedro do mato, una cita puntual sobre la presencia de semillas de *J. brevifolia* en el tracto digestivo de una curruca capirotada *Sylvia atricapilla* (Neves *et al.* 2004), así como algunas observaciones de campo de mirlos comunes *Turdus merula* consumiendo gálbulas (R.B.E., com. pers.), apuntaban a estas aves de pequeño y mediano tamaño como los posibles dispersores de semillas de *J. brevifolia* en Azores.

Con respecto a la historia evolutiva de los cedros macaronésicos, los datos previos eran muy escasos. Basándose en datos bioquímicos y genéticos (RAPDs, de *Randomly Amplified Polymorphic DNA*), Adams (2000) señaló la proximidad genética entre *J. brevifolia* y *J. cedrus*. Sin embargo, este mismo autor, en su reciente libro ‘Junipers of the World: The genus *Juniperus*’ (Adams 2011) hace referencia a una filogenia no publicada de todo el género (Schwarzbach *et al.* in prep.) basada en secuencias del ADN nuclear y cloroplástico, en la que se incluye a las especies macaronésicas, quedando esta vez en una posición más alejada filogenéticamente. Excepto estos trabajos, ningún otro había prestado atención a las relaciones filogenéticas existentes entre ellos y menos aún a los patrones filogeográficos que presentan en sus respectivos archipiélagos.

Justificación y estructura de la Tesis Doctoral

Tal y como se ha expuesto en los apartados anteriores, determinados aspectos ecológicos del la vida de las plantas, como los procesos de dispersión de semillas, tienen un efecto fundamental en la dinámica y evolución de las poblaciones (Jordano & Godoy 2002). Conocer dichos procesos se torna fundamental para poder sentar las bases de estrategias de manejo adecuadas, sobre todo en el caso de especies con cierto grado de amenaza como ocurre con los cedros macaronésicos. Por otro lado, el desarrollo de las técnicas moleculares, cada vez más potentes y asequibles, se ha convertido en una herramienta imprescindible para lograr un mejor entendimiento de los procesos ecológicos. De este modo, la conservación de las especies en sí queda estrechamente ligada a la conservación de sus interacciones ecológicas y de su diversidad genética, absolutamente indispensable para persistir en un medio ambiente cada vez más cambiante.

Dado el carácter insular y endémico de los cedros macaronésicos, así como su delicado estado de conservación y la escasa información existente sobre aspectos básicos de su biología, el objetivo general de esta tesis era el de incrementar el conocimiento ecológico y evolutivo disponible sobre ellos. Evidentemente, “ecología” y “evolución” son términos amplísimos dentro del campo de la biología y, por tanto, el presente trabajo simplemente aborda algunos de los muchos aspectos integrados en estas disciplinas. A través de los cinco estudios recogidos en esta tesis (los cuales se referirán en el texto mediante números romanos I–V), se ha tratado de recabar información sobre el sistema de dispersión de semillas de *Juniperus cedrus* y *J. brevifolia*, así como sobre sus diferencias y similitudes, tanto entre ambas especies como en relación a sus congéneres continentales. También se ha intentado arrojar luz sobre la historia evolutiva que existe tras estos endemismos insulares, sobre cuáles han sido sus patrones de colonización dentro de los archipiélagos, de qué modo estos patrones pueden estar condicionados por el modo de dispersión, y qué niveles de diferenciación genética presentan.

Específicamente, esta tesis contempla los siguientes trabajos:

I. Rumeu B, Nogales M, Elias RB, Padilla DP, Resendes T, Rodríguez A, Valdés F, Dias E (2009) Contrasting phenology and female cone characteristics of the two Macaronesian island endemic cedars (*Juniperus cedrus* and *J. brevifolia*). *European Journal of Forest Research* **128**, 567–574.

Este estudio se centró en obtener información acerca de aspectos reproductivos básicos de los cedros macaronésicos, que se encuentran en íntima relación con el sistema de dispersión de semillas de cada uno de ellos. Uno de estos aspectos consistió en el seguimiento fenológico de las gálbulas, fundamental para conocer la disponibilidad para los distintos agentes dispersantes. También se estudiaron otras características de los conos femeninos, tales como el tamaño, contenido hídrico de la pulpa, número de semillas, o viabilidad de las mismas.

Esta información inicial constituyó una base fundamental para la correcta interpretación de los datos que se fueron obteniendo con posterioridad sobre el sistema de dispersión de semillas de *J. cedrus* y *J. brevifolia*.

II. Rumeu B, Padilla DP, Nogales M (2009) The key role of a ring ouzel *Turdus torquatus* wintering population in seed dispersal of the endangered endemic *Juniperus cedrus* in an insular environment. *Acta Ornithologica* **44**, 199–204.

Tras detectar durante varios años consecutivos la presencia del túrdido invernante *Turdus torquatus* (mirlo capiblanco) en las inmediaciones del área de distribución de *J. cedrus* en la alta montaña de Tenerife, se realizó un análisis de su dieta invernal con el objetivo de evaluar su papel como dispersor de semillas. Este trabajo sirvió para obtener una idea de cuán importante son las gálbulas de *J. cedrus* en la dieta del mirlo capiblanco durante su invernada y, por ende, cuán importante es el papel que este túrdido realiza como dispersor de semillas del cedro canario.

III. Rumeu B, Elias RB, Padilla DP, Melo C, Nogales M (2011) Differential seed dispersal systems of endemic junipers in two oceanic Macaronesian archipelagos: the influence of biogeographic and biological characteristics. *Plant Ecology* **212**, 911–921.

Este estudio aborda los sistemas de dispersión de semillas de *J. cedrus* y *J. brevifolia* de forma global, es decir, identificando a los agentes dispersantes que intervienen en dichos sistemas y poniendo en contexto la legitimidad de cada uno de ellos a través de la contribución cuantitativa y cualitativa que realizan. Teniendo en cuenta la información sobre la fenología de las gálbulas maduras recopilada en el trabajo I y, por tanto, conociendo su disponibilidad temporal para los dispersores, en este estudio también se testó la variación estacional del proceso dispersivo del cedro canario (de las dos especies estudiadas, es la que presenta disponibilidad de gálbulas maduras a lo largo de todo el año).

IV. Rumeu B, Caujapé-Castells J, Blanco-Pastor JL, Jaén-Molina R, Nogales M, Elias RB, Vargas P (2011) The colonization history of *Juniperus brevifolia* (Cupressaceae) in the Azores islands. *PLoS ONE* **6**, e27697.

Mediante aproximaciones filogenéticas y filogeográficas, analizando la variación existente en distintas regiones del ADN plastidial, este trabajo trata de reconstruir la historia colonizadora del cedro do mato en el archipiélago de Azores y estimar su grado de diversificación en las islas. Gracias al conocimiento adquirido sobre la capacidad dispersiva de esta especie endémica, se pudo contar con una base de conocimiento muy útil en la interpretación de los datos obtenidos.

V. Rumeu B, Vargas P, Jaén-Molina R, Nogales M, Caujapé-Castells J. Origin and genetic differentiation of the Canarian endangered *Juniperus cedrus* (Cupressaceae). *Journal of Biogeography*, under review.

Basándonos nuevamente en el uso de herramientas moleculares, este estudio combina el uso de marcadores plastidiales y nucleares (AFLPs, *Amplified Fragment Lenght Polymorphisms*) para inferir el origen espacio-temporal de *J. cedrus* en las Islas Canarias, y estimar sus niveles de diversidad genética. Los severos cuellos de botella que han sufrido las poblaciones de esta especie y el grado de fragmentación que aún presentan, han marcado su historia evolutiva. Este trabajo recoge los primeros datos genético-poblacionales sobre los que sentar las bases de un manejo más completo, que también dirija sus esfuerzos en conservar los máximos niveles posibles de diversidad genética, salvaguardando así el potencial de adaptación del cedro canario.

Esta tesis se presenta en la modalidad de "Compendio por Publicaciones" contemplada en el Reglamento de Enseñanzas Oficiales de Máster y Doctorado de la Universidad de La Laguna (B.O.E. nº 31, de 16 de febrero de 2009). De acuerdo con este formato, se incluyen en la memoria esta introducción general, una copia completa de los trabajos publicados, y un resumen global de los objetivos de la investigación, de la metodología aplicada, de los resultados obtenidos, de la discusión de estos resultados y de las conclusiones finales.

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I

Contrasting phenology and female cone characteristics of the two Macaronesian island endemic cedars (*Juniperus cedrus* and *J. brevifolia)*

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Abstract

Phenology and female cone characteristics of the two endemic cedars (*Juniperus cedrus* and *J. brevifolia*) from the Macaronesian islands were studied. Despite their closely taxonomic affinity and their evolution under insular conditions, different trends were recorded. Mature *J. cedrus* female cones were present throughout the year, while those from *J. brevifolia* were only present in summer and autumn. *Juniperus* cedrus female cone size was significantly larger than that of *J. brevifolia*, a trend consistent with the presence of larger vertebrates (lizards and birds) in the Canary Islands. However, water content was four times higher in *J. brevifolia* female cones, which can be related with the higher rainfall existing in the Azores. *Juniperus cedrus* has two or three seeds per cone, whereas *J. brevifolia* frequently had three. Seeds from *J. cedrus* were clearly larger and heavier, coinciding with the female cone size trend. However, tetrazolium tests revealed higher viability values in *J. brevifolia.* The relatively low percentage of filled seeds in *J. cedrus* could be a consequence of the climatic stress and limits to pollination due to fragmented populations as described for other *Juniperus* species. In summary, our results reveal that some environmental factors such as the harsh conditions, high population fragmentation and the dependence on large dispersers have compromised the fitness of *J. cedrus* in the Canary Islands.

Keywords: *Juniperus cedrus* • *Juniperus brevifolia* • Female cones • Seasonality • Island plants • Plant conservation

Introduction

The genus *Juniperus* (Cupressaceae) is the second largest of the conifers, with only *Pinus* L. containing more species (Arista *et al.* 1997). It consists of approximately 67 species and 34 varieties, and is divided into three sections: *Caryocedrus* (one species, *Juniperus drupacea* Labill.); *Juniperus* (= *Oxycedrus*, with 10 species) and *Sabina* (with the remaining, 56 species approximately) (Adams 2008). All these species grow in the northern hemisphere except for *J. procera* Hochst. Ex. Endl., which grows southward along the Rift Mountains in east Africa into the southern hemisphere (Adams *et al.* 1993). *Juniperus cedrus* Webb and Berthel. and *J. brevifolia* (Seub) Antoine are the two species from the *Juniperus* section represented in the Macaronesian islands. Genetic analyses using RAPDs (Adams 2000) show a close proximity of *J. cedrus* and *J. brevifolia*, clearly separated from the other species from the section *Juniperus*. They are both endangered (IUCN 2008), especially *J. cedrus*, due to the drastic past deforestation. This species is endemic to Madeira and Canary Islands, being present in La Palma, La Gomera, Tenerife and Gran Canaria (Izquierdo *et al.* 2004). On the other hand, *J. brevifolia* is endemic to the Azores archipelago, where it is distributed in all the islands except Graciosa (Adams 2008, Elias 2007, Sjögren 2001); these two species are dioecious. As the rest of the species of the genus *Juniperus*, they produce fleshy female cones that act functionally as angiosperm fleshy fruits (Herrera 1992). In the case of *J. cedrus*, these cones are reddish in colour when mature, while in *J. brevifolia* the cones are copper (Adams 2008). Both cedars have evolved in two oceanic archipelagos under rather different ecological conditions, such as geographical location, distance from mainland, geological age, climate, altitudes, soils or types of frugivore interactions. At this respect, female cones of *J. cedrus* have been interacting with large seed dispersal agents, such as the raven *Corvus corax* (Nogales *et al.* 1999) and probably by different species of endemic giant lizards (genus *Gallotia*) present in the Canaries. Nowadays, in this archipelago, *J. cedrus* female cones are consumed by medium-sized lizards such us *G. galloti* (snout vent length: 10.7–14.5 cm; Hernández *et al.* 2000) in Tenerife (Valido 1999), while all the dispersers known in the Azores are presumably smaller (warblers, *Sylvia atricapilla*) and medium-sized birds (blackbirds, *Turdus merula*).

Lastly, despite the delicate conservation status of these cedars (especially *J. cedrus*), little knowledge is available about certain ecological features. Therefore, the main aim of this contribution is to present the first data on some basic aspects of their reproductive biology, such as phenology and female cone characteristics, which may be of particular use for conservation purposes.

Material and Methods

Study sites

The Canary Islands lie between 27°–29°N and 13°–18°W and consist of seven main volcanic islands. This archipelago is close to the northwest African coast, with only 96 km separating Fuerteventura and West–Sahara. The islands are of different ages, Fuerteventura (22 Ma) being the oldest one, and El Hierro (1.2 Ma) the most recent; Tenerife, the largest of the islands, emerged 12 Ma ago and is about 2,058 km^2 in size (Carracedo & Day 2002). The study of *J. cedrus* was carried out in '*Riscos de La Fortaleza*' (2,170 m a.s.l.), a craggy geological formation located in '*El Teide*' National Park in Tenerife. The area is influenced by a typical high-mountain climate, with great thermal oscillations throughout the year (differences of about 10°C between maximum and minimum monthly average temperatures) and a mean annual temperature of 10.7°C. The site receives an annual precipitation of about 367.5 mm, most of which falls during the winter months (Bustos & Delgado 2004). The vegetation consists principally of endemic plants (e.g. *Spartocytisus supranubius*, *Pterocephalus lasiospermus*, *Adenocarpus viscosus*, etc.), many of them found only in this region of Tenerife (Wildpret de la Torre & Martín Osorio 2004). In *Riscos de La Fortaleza*, we can distinguish two different areas or subpopulations where *J. cedrus* grows: (1) smaller cedar plants located at the top of the crag (hereafter '*crag-top*'), which is an area exposed to relatively frequent NW winds and scarce soil development and (2) larger cedar plants placed at the base of the crag (hereafter '*crag-base*'), which is more sheltered from the wind and presents better soil presence. These two areas were selected to evaluate the influence of the habitat conditions in the parameters studied.

The Azores Archipelago is located in the North Atlantic, between 36°–40°N and 24°–32°W. It is made up of nine main islands and some small islets aligned on a WNW–ESE direction. The distance between the Azores (São Miguel) and the mainland is about 1,584 km, calculated from Cabo da Roca (the most westerly point of the European continent). The islands are divided into three groups: (1) western: Corvo and Flores; (2) central: Faial, Pico, Graciosa, São Jorge and Terceira and (3) eastern: São Miguel and Santa Maria, plus the Formigas islets (Borges & Brown 1999). They are all volcanic islands of recent origin; Santa Maria being the oldest island (8 Ma) and Pico the youngest (0.25 Ma). The study of *J. brevifolia* female cones was carried out in Terceira, which has an area of 402.2 km^2 and an estimated geological age of 3.52 Ma (França *et al.* 2003). Although we studied one of the main populations of *J. cedrus* in Tenerife, whose distribution is rather limited, in Terceira it is still possible to find some well-preserved and widely distributed *J. brevifolia* populations (Dias *et al.* 2004, Elias & Dias 2004, 2009). For this reason, the study was carried out at three different sites in the central and western parts of the island. The three populations studied were '*Malha Grande*', '*Pico Alto*' and '*Santa Bárbara*', which are located at 505, 685 and 974 m a.s.l., respectively. At these sites the mean annual temperatures are 14.0, 13.1 and 11.1°C and total annual rainfall is 2,168, 2,387 and 3,078 mm, respectively. The rainfall occurs mainly in autumn and winter (Elias 2007). Vegetation in *Malha Grande* is of a pioneer scrub dominated by *J. brevifolia* and *Erica azorica*; in *Pico Alto* the vegetation is mainly composed of *Juniperus*-*Laurus* mature forests, dominated by *J. brevifolia*, *Laurus azorica* and *Ilex azorica*; *Santa Bárbara* is located at the top of a volcano of the same name, whose vegetation is dominated by *J. brevifolia* and *Calluna vulgaris* shrubs.

The main parameters of the different population structure in the four localities studied are showed in Table 1. In general, trees of all these populations presented an external healthy appearance. However, only in '*Riscos de la Fortaleza*' several individuals (3%) showed a low vitality (reduced lushness and lighter colour of the crown).

Table 1 Population structure of *Juniperus cedrus* and *J. brevifolia* in the studied localities.

Island	**Locality**	**Sex-ratio male:female**	**Ø max. of the crown (m) mean ± SD**	**Max. height (m) mean ± SD**	**Population size (no. individuals)**	**Demographic structure (adults:immat.)**
Tenerife (Canaries)	Riscos de La Fortaleza	1:1 (n = 94)	4.21 ± 2.53 (n = 96)	2.91 ± 1.33 (n = 96)	≈170	1:0.75 (n = 170)
Terceira (Azores)	Malha Grande	1 : 0.67 (n = 15)	2.16 ± 0.96 (n = 15)	1.50 ± 0.42 (n = 15)	> 500	0.85:1 (n = 41)
Terceira (Azores)	Pico Alto	1: 0.89 (n = 15)	2.83 ± 1.04 (n = 15)	3.00 ± 0.75 (n = 15)	> 500	1:0.43 (n = 40)
Terceira (Azores)	Santa Bárbara	1: 0.89 (n = 15)	0.61 ± 0.28 (n = 15)	0.37 ± 0.16 (n = 15)	> 500	1 : 0.69 (n = 99)

Procedures

To study the female cone phenology, 20 female plants of *J. cedrus* were selected from the population at *Riscos de La Fortaleza*. Ten of them were at the *crag-top* and ten at *crag-base*. Ten branches of each plant were marked with different coloured tags. For two consecutive years (between 2004 and 2006 in the same plants), ripe and unripe female cones were counted seasonally per branch. With regard to *J. brevifolia* on Terceira, where three populations were studied, the methodology used for the female cone phenology was slightly modified. In this case, 15 female plants were selected (between 2003 and 2004) from each of the three populations. However, the main parameter (% of female cones of different types) was calculated for the two species in the four different seasons, which permitted different comparisons to be made. Female cones of the two endemic Macaronesian cedars mature in the second year (Adams 2008). Thus, at the same time, a sole individual could present small unripe receptive or aborted female cones, 1– or 2–year large unripe female cones, and 2–year fleshy mature cones. As this succession is complex, we focused the phenology follow-up on the presence of large female cones and especially the proportion of mature ones, due to the importance of their availability to seed dispersers. Since there is an obvious difference in cone size between the two species (smaller female cones in *J. brevifolia*), in *J. cedrus*, we considered female cones ≥8 mm diameter as large and <8 mm as small. In *J. brevifolia*, cones ≥6 mm were scored as large and <6 mm as small.

To characterise mature cones, 180 female cones were taken from the whole population of *Riscos de la Fortaleza* in Tenerife. These samples were collected from the same plants as in the female cone phenology. In 90 of the female cones, we measured the diameter and water content, weighing them wet and then drying at 60°C in a heater until a constant weight was reached. The remaining 90 female cones were weighed and their seeds removed and counted per cone. From these seeds, 180 were randomly selected, measured for length and width, and weighed independently. In Terceira, 60 female cones were collected from each population, and the same procedure was followed.

To assess seed viability, a total of 40 female plants were selected, 20 at *Riscos de la Fortaleza* (Tenerife) and 20 at *Malha Grande* (Terceira). Twenty female cones from each plant were collected and all their seeds extracted, maintaining the identity of their mother plant to evaluate potential differences in seed viability among the plants. More than 800 seeds of each species were carefully opened using a small bench vice to determine the morphological state of each embryo (healthy: filled seeds, or unhealthy: empty or with a damaged embryo). These seeds were also measured and weighed to compare biometric data and viability. After they were opened, damaged embryos were directly considered as non-viable. Embryos that appeared healthy were immersed for 24 h in water and then for 6 h in gibberellic acid (GA_3, 0.16 mg/ml; gibberellic acid treatment was used to enhance red stain patterns in viable embryos, allowing us to identified non-viable embryos from apparently healthy embryos). This process was followed to avoid false results in the viability test, because juniper embryos exhibit physiological dormancy, as they are unable to develop a radicle due to an inhibition mechanism (Baskin & Baskin 1998). Next, embryos were cut and immersed in 2,3,5–triphenyl-tetrazolium chloride solution (hereafter TTC) diluted to 0.1% for 24 h in the dark, and at room temperature (Scharpf 1970, Tanaka 1984). The TTC used was 'TTC sterile solution 1%, Scharlau Microbiology'. In this method, living cells stain red as the tetrazolium is reduced by dehydrogenase enzymes to form a stable red triphenyl formazan, which is insoluble in water (Tanaka 1984). To avoid any physical dormancy caused by the seminal cover (Cantos *et al.* 1998), the embryos were tested in a completely bare condition. To establish the viability state of the embryos, a total of 15 different patterns of red stains were considered, which were then reduced to three categories: non-viable, potentially viable and viable. This method was very conservative. An embryo was only considered to be viable when nearly all the surface was stained red. Potentially viable embryos were those partially stained and always in areas critical for germination such as shoots and root apices (Kaplan & Cooke 1997, West & Harada 1993).

Statistical analyses

Categorical analyses (Likelihood ratio tests) were performed to compare number of ripe female cones in the two endemic cedar species and among the different seasons. This analysis was also used when comparisons in seed viability were carried out. Student and Mann-Whitney tests, for parametric and non-parametric data, respectively, were applied when the different traits were compared between the two cedars. All analyses were performed using the SPSS statistical package (version 14.0).

Results

Female cones phenology

Juniperus cedrus mature cones were present throughout all seasons (Fig. 1). Among the female cones, unripe ones were more abundant than ripe ones throughout the year ($G_3 = 100.34$, $p < 0.001$). However, ripe female cones were mainly present in summer, autumn and winter; few ripe cones were present in the spring (only 15.7% of the whole ripening process, p

< 0.001 for all comparisons). When the two main sub-populations from El Teide were compared, large mature cones were more abundant at the crag-base than at the crag-top ($G_1 = 205.42$, $p < 0.001$), although the seasonal pattern was rather similar to the whole population.

The female cone phenology of *J. brevifolia* showed a markedly seasonal pattern with respect to *J. cedrus.* Ripe female cones totally disappeared during winter and spring; they were only present during summer and autumn, being significantly more abundant in the summer ($G_1 = 174.39$, $p < 0.001$) (Fig. 1). In contrast, unripe female cones were more abundant in winter (Likelihood ratio tests, $p < 0.001$ for all comparisons). Considering all female cones, unripe ones were present throughout the year and they were highly abundant during winter and spring (Likelihood ratio tests, $p < 0.001$ for all comparisons).

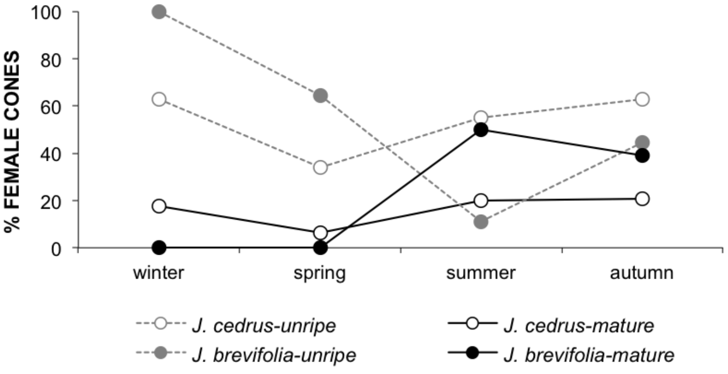

Fig. 1 Female cone phenology of *Juniperus cedrus* in *El Teide* National Park (Tenerife, Canary Islands), and *J. brevifolia* in three localities of Terceira (Azores).

Female cones and seed traits

There were no significant differences in female cone and seed sizes, nor in pulp water content among the three *J. brevifolia* populations studied on Terceira ($p > 0.05$ for all comparisons). Therefore, data analysis of these three populations was merged.

Female cones of *J. cedrus* were 21.4% larger than those of *J.*

brevifolia (Table 2), showing a significantly greater diameter (t_{178} = -12.13, $p < 0.001$) and weight (Z = -3.44, p = 0.001). However, water content was four times larger in *J. brevifolia* female cones than in those of *J. cedrus* (Z = -11.59, $p < 0.001$). Comparing the two areas considered in *Riscos de La Fortaleza* (El Teide), female cones at the *crag-base* were significantly larger than those at the *crag-top* (U = 23.0, p = 0.041). When seeds from the two sub-populations were compared, no differences were found either in number of seeds per cone or in their length or diameter.

Juniperus brevifolia normally has three seeds per female cone while *J. cedrus* produces two or three seeds (G_3 = 9.04, p = 0.029). These are longer (Z = -15.98, $p < 0.001$), wider (Z = -15.56, $p < 0.001$) and heavier (Z = -15.98, $p < 0.001$) than those of *J. brevifolia* (Table 2).

Table 2 Female cones and seed traits of *Juniperus cedrus* (*Riscos de La Fortaleza*, El Teide National Park, Tenerife, Canary Islands) and *J. brevifolia (Malha Grande*, *Pico Alto* and *Santa Bárbara*, Terceira Island, Azores).

	Juniperus cedrus				***Juniperus brevifolia***			
Parameters	**Mean**	**SD**	**Range**	**n**	**Mean**	**SD**	**Range**	**n**
Female cone diameter (mm)	9.94	1.29	7.67-13.79	90	7.81	1.06	4.00-10.00	90
Fresh weight (g)	0.35	0.13	0.12-0.82	90	0.28	0.10	0.11-0.54	90
% water	15.39	9.75	5.86-43.34	90	59.78	4.07	50.00-71.88	90
Number of seeds per cone	2.41	0.69	1.00-4.00	90	2.47	0.82	1.00-4.00	90
Seed length (mm)	6.48	0.79	4.59-8.69	180	4.42	0.62	2.90-5.80	180
Seed width (mm)	4.50	0.77	2.11-6.43	180	2.57	0.63	1.00-4.00	180
Seed weight (mg)	53.16	24.64	12.60-138.40	180	12.81	10.25	2.10-128.00	180

Seed viability

A total of 41.5% of the *J. cedrus* seeds examined contained apparently healthy embryos. A TTC test of these embryos revealed that 26.5% of them were non-viable, 8.9% potentially viable and 64.6% viable. Applying these results to the total number of seeds opened (filled and empty seeds): 69.3% were non-viable, 3.7% potentially viable and 27% viable. No significant differences were found between the size (diameter) of viable and non-viable *J. cedrus* seeds (t_{143} = 0.84, p = 0.40). However, in this species, viable seeds were significantly heavier than non-viable seeds (Z = -4.38, $p < 0.001$). Furthermore, significant differences were also found in the two sub-populations considered in *Riscos de la Fortaleza*, with plants at the crag-base having higher seed viability (25.3%) than those growing at the *crag-top* (5.4%) (G_1= 81.06; $p < 0.001$).

87.1% of the *J. brevifolia* seeds were filled, containing visibly healthy embryos. After TTC testing, 27.8% were found to be non-viable, 27.5% potentially viable and 44.7% viable. Considering the total number of seeds opened (filled and empty seeds), 37.1% were non-viable, 23.9% potentially viable and 39.0% viable. In *J. brevifolia*, no significant differences were found in the size or weight of viable and non-viable seeds.

Comparing both species, seed viability of *J. brevifolia* was significantly higher than *J. cedrus* (G_1 = 185.59, $p < 0.001$) (Fig. 2). On the other hand, the intraspecific comparison between the seed viability of the

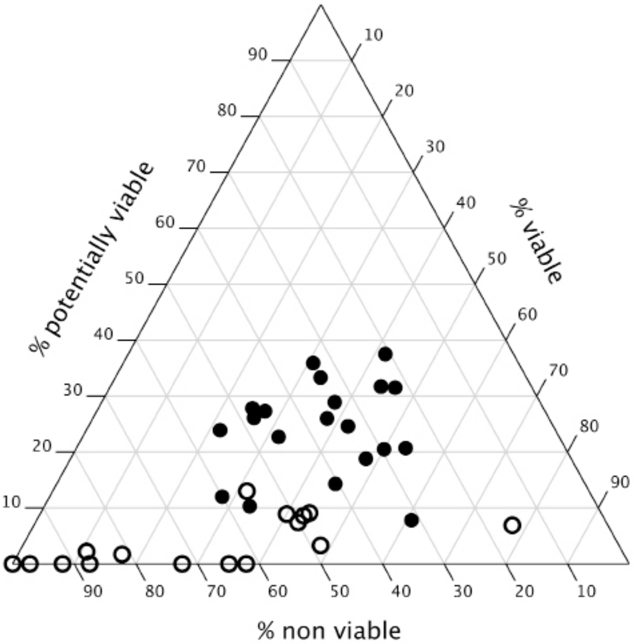

Fig. 2 Ternary plot of seed viability scores (viable, potentially viable and non-viable) of the two Macaronesian endemic cedars (*J. cedrus* and *J. brevifolia*). Each point corresponds with the seed viability score of a plant; filled and empty seeds were considered. Five *J. cedrus* plants share the same values of seed viability (100 % of non-viable seeds) and their correspondent circles are superimposed. Circles: *J. cedrus* and black dots: *J. brevifolia.*

different mother-plants in each species revealed significant differences both in *J. cedrus* and in *J. brevifolia* (G_{19} = 296.93, $p < 0.001$; G_{19} = 49.64, $p < 0.001$, respectively). In all of these categorical analyses, viable and potentially viable seeds were merged in one single group.

Discussion

Female cone phenology

Clear differences were recorded between the female cone phenology of the two endemic cedars in the Macaronesian islands. The *J. cedrus* population had mature female cones throughout the year, whereas *J. brevifolia* had a visibly different pattern in which mature cones were only present during two seasons: summer and autumn. It is remarkable that ripe female cones disappear during winter and spring in *J. brevifolia*, which contrasts with the persistence on the plants, even for years, in other *Juniperus* species (Chambers *et al.* 1999). This process is related to removal rates of frugivores and natural abscission beneath the plants from the Azores, where many rot due to the high environmental humidity and also predated by introduced rats (R.B.E., pers. obs.).

Female cones from the Macaronesian cedars mature in the second year (Adams 2008) and the notable presence of ripe cones in summer and autumn show that these seasons are the most important for female cone maturity. In northern temperate habitats, most species that are dispersed by vertebrates have their ripening peaks in late summer–autumn, whereas Mediterranean forest and shrublands fruits ripen in autumn–winter (see Herrera 2002). This fact is also in concordance with the phenology pattern observed in *J. brevifolia*, which is a temperate species with maturation peaking in summer–autumn. However, *J. cedrus* occurs at lower latitude and higher percentages of mature cones appear in summer–autumn, but also in winter.

In the mutualistic interaction between temperate fleshy fruits and birds, the timing of fruit ripening affects the probability of seed dispersal by birds in continental ecosystems (Thompson & Willson 1979). According to these authors, weekly removal rates of fruits are faster for autumn fruiting species than for summer and winter species but, the two latter strategies should be more profitable at lower temperate latitudes due to the greater year-round presence of frugivores. In the present study, both summer and autumn are the most important seasons for the seed dispersal systems of these insular cedar species, because the highest percentages of mature cones occur then. However, in the case of *J. cedrus* (which grows at subtropical latitudes), the presence of mature female cones is also relatively important in winter, showing a greater availability for all year-round frugivores, as described above.

Female cone sizes and pulp water content

Size is an important factor in fruits, because it limits ingestion to relatively small-sized dispersers, like birds, that swallow them whole (Herrera 2002, Howe & Westley 1990). However, this factor is probably less important in consumption by large vertebrates with wide mouths (Herrera 2002), whereas small birds tend to consume small fruits (Howe & Westley 1990, Noma & Yumoto 1997). In a thorough investigation carried out by Jordano (1995), in which the fleshy fruit characteristics of 910 angiosperm species were analysed, it was observed that there is a strong relationship between fruit diameter and disperser type. Although *J. cedrus* and *J. brevifolia* are both insular species evolving under particular ecological conditions, differences in female cone characteristics indicate two different evolutionary histories in their respective seed dispersal systems. *Juniperus cedrus* has evolved with large vertebrate dispersers such us the raven *Corvus corax* (Nogales *et al.* 1999), now presumably extinct in the study area, and almost certainly by different species of the endemic giant lizards (genus *Gallotia*) present in the Canaries. Mature cones on Tenerife are currently consumed by the medium-size lizard *G. galloti* (Valido 1999) and by some *Turdus* species that had been recorded in the surrounding area of the plants (B.R., pers. obs.). In this regard, it is interesting to note that with the exception of the extinct giant lizards, no frugivores that could have interacted with *J. cedrus* have been recorded in the palaeontological deposits studied in Tenerife. However, ravens and native lizards are absent in the Azores archipelago, where medium size blackbirds *T. merula* (R.B.E., pers. obs.) and possibly small warblers *S. atricapilla* are the dispersers of *J. brevifolia*.

The high water content in the *J. brevifolia* female cones (four times higher than *J. cedrus*), seems to be related to the wet climate of the Azores archipelago, with an average rainfall of 3,000 mm/year (Elias 2007, Marzol *et al.* 2006). Pulp water content may also be a single phenotypic response to the different environmental setting. In plants, this parameter seems to be linked to climatic features noted during fruit development (Debussche *et al.* 1987).

Seed viability

Differences in the number of filled seeds (seeds with apparently healthy embryos) were observed between the two species of *Juniperus* present in the Macaronesian islands, with *J. brevifolia* having more potential for natural regeneration. Junipers bear a high proportion of externally well-developed but empty seeds (Chambers *et al.* 1999, García *et al.* 2000a, Thomas *et al.* 2007). In a study of *J. communis* throughout its range in Europe (García *et al.* 2000b), a strong correlation was found between seed production, population fragmentation, distance between populations and climatic stress. Consequently, the production of filled seeds declined gradually towards the limits of *J. communis* distribution and juniper seed viability strongly diminished in regions with harsher environments. So, the low percentage of filled seeds in *J. cedrus* could be a consequence of the high-mountain climatic stress and pollination constraints probably due to the low proportion of sexually mature individuals (Table 1) and the fragmented population as described for other species of junipers (Chambers *et al.* 1999, Thomas *et al.* 2007, Wesche *et al.* 2005). As it is shown in Table 1, despite the balanced condition of the sex-ratio, the number of individuals in *J. cedrus* population is much lower than that found in populations of *J. brevifolia*, where, in addition, individuals are less dispersed and must suffer a minor pollination restriction. Furthermore, the strong reduction in the *J. cedrus* population as a result of excessive human exploitation for timber purposes could have involved certain genetic impoverishment caused by inbreeding depression.

As occurs with female cone size and in the two sub-populations considered at *Riscos de La Fortaleza* (El Teide), differences in seed viability were also found between these two areas, where female plants at the *crag-base* had higher values of seed viability. Furthermore, these plants also presented higher values of total crown cover (Z = -2.50, $p < 0.05$). This fact seems to confirm the significant effect of the habitat conditions and the climatic stress on seed viability, even within a population and over a short spatial scale.

TTC results indicate values significantly lower in viable seeds of *J. cedrus* (27%) with respect to *J. brevifolia* (44.7%). Although the viability of *J. cedrus* seeds was relatively low, the percentage of filled seeds with viable embryos (64.6%) was much higher than in a previous study with reforested trees in which the highest viability value was 21% (Jordán de Urríes 1997). TTC tests were also used in other studies on different populations of the endangered *J. oxycedrus* ssp. *macrocarpa* (Juan *et al.* 2003, 2006), and always indicated low values of viable seeds (< 12% of totally stained embryos). However, in an unthreatened species like the redberry juniper *J. pinchotii* (Adams 2008), TTC results yielded 100% viability in filled seeds (44% of the seeds analysed) (Warren 2001 in (Petersen *et al.* 2005). These results suggest that the endemic Macaronesian cedars are in an intermediate status with regard to their seed viability, between a critical situation like the endangered cedar *J. oxycedrus* ssp. *macrocarpa* (classified as in danger of extinction (Blanca *et al.* 1999), and other juniper species in a better conservation status.

Significant differences between seed viability of the mother-plants analysed were found in *J. cedrus* (range: 0-77.6%) and also *J. brevifolia* (range: 21.7-60.8%). These intraspecific differences show the important effect of the seed source on their subsequent success. Similar wide variations in the percentage of filled seeds were found in different populations of *J. oxycedrus* (ranging from 0% to 60% in different individuals) (Ortiz *et al.* 1998). Therefore, the mother-plant effect must be considered an important ecological factor for upcoming interpretations in seed dispersal studies, and also for conservation purposes.

Final considerations

This contribution, based on some reproductive biology aspects, such us female cone phenology, size, weight, pulp water content and seed viability, is the first to present basic information about the two endemic insular species, *J. cedrus* and *J. brevifolia*. Therefore, this paper provides support for future research, essential for promoting regeneration of these endangered island species. Lastly, our results reveal that some environmental factors such as harsh conditions, high population fragmentation and the dependence on large dispersers have compromised the fitness of *J. cedrus* in the Canary Islands.

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II

The key role of a ring ouzel *Turdus torquatus* wintering population in seed dispersal of the endangered endemic *Juniperus* cedrus in an insular environment

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Abstract

Although the ring ouzel *Turdus torquatus* has been considered accidental in the Canary Islands, it has been observed for the last five years in the high mountain zone of Tenerife (Canary Islands), showing it to be a regular winter visitor. Its interaction with female *Juniperus cedrus* cones and seeds was studied by analysing its wintering diet during January–April 2008. This juniper is endemic to the Canaries and Madeira and is classified as endangered by the IUCN. Previously, its sole long-distance disperser was known to be the raven *Corvus corax*, which is unfortunately now extinct in the distribution range of *J. cedrus*. The results show that the diet of *T. torquatus* in Tenerife consists mostly of female *J. cedrus* cones (95% of the biomass consumed). Due to the large amount of seeds dispersed by ring ouzels and the effectiveness in seed germination, this winter visitor constitutes an important vector in the dispersal of *J. cedrus* that appears to have gone undetected in the past. This passerine plays a key role in its seed dispersal system, being able to move seeds over long distances, thus connecting fragmented populations of this plant. The present work is one of the few cases described in which a plant endemic to an oceanic island is so dependent on a winter visitor, and highlights the importance of insular environments in the wintering range of the ring ouzel. Owing to the absence of native long-distance seed dispersers, the future fate of this conifer is now probably dependent on the wintering population of this thrush.

Keywords: Ring ouzel • *Turdus torquatus* • Frugivorous diet • Seed dispersal • *Juniperus cedrus* • High mountain shrub • Tenerife • Canary Islands

In fleshy-fruited plants, frugivores play a determinant role in the seed dispersal effectiveness by determining the landscape pattern of seed distribution and density over available microhabitats (*sensu* Schupp 1993). Furthermore, dispersal also affects the rate of gene flow, influencing the genetic structure within and between populations (Ouborg *et al.* 1999). Among vertebrates, birds constitute an important seed dispersal vector for many plant species, allowing seed movements over long distances (Whittaker & Fernández-Palacios 2007). On the other hand, island ecosystems harbour a biota that mainly presents low population sizes and isolated evolution, resulting in a high endemic component, which is highly vulnerable to anthropogenic disturbances (Simberloff 2000, Traveset & Riera 2005). For this reason, the disruption of mutualisms on islands can have drastic consequences, and the future of a plant could be seriously threatened if its main seed disperser disappears (e.g. Clout & Hay 1989, Cox *et al.* 1991, Meehan *et al.* 2002, Temple 1977, Traveset & Riera 2005) .

Only one species of juniper occurs in the high mountain of the Canary Islands: 'cedro canario' *Juniperus cedrus*. This endemic species is included in the IUCN Red List as endangered (IUCN 2009), principally due to its limited distribution and abundance as a consequence of severe deforestation in the past, and the low estimated number of mature individuals. Despite its delicate conservation status and the abundance of seed dispersal studies on other juniper species in mainland ecosystems (e.g. García 2001, Holthuijzen & Sharik 1985, Jordano 1993, Santos & Tellería 1994, Santos *et al.* 1999), there is little information about the interactions with its dispersers. Until now, it has been recorded that *J. cedrus* female cones in Tenerife have been consumed by lizards *Gallotia galloti* (Valido 1999), and ravens *Corvus corax* (Nogales *et al.* 1999). Unfortunately, this latter species is nowadays extinct in the *J. cedrus* distribution area of Tenerife, and hence, the long-distance seed dispersal of this juniper would be limited.

However, several wintering individuals of ring ouzel *Turdus torquatus* were regularly recorded during the last five years (2004–2008) in the high mountain (upper 2,000 m a.s.l.) of Tenerife, in the juniper woodland and areas surrounding it (pers. obs.). So far, this species has been considered accidental in the Canary Islands (Cramp 1998), where Martín & Lorenzo (2001)regard it as an irregular and scarce wintering visitor. Apart from Southern Spain, Malta, Cyprus and Greece, individuals from European races (*T. t. torquatus* and *T. t. alpestris*) winter in north-west Africa, especially in the Saharan Atlas on dry and bare slopes or crests with juniper woodland (Cramp 1998). Southward migration starts in September, reaching NW Africa from mid–October, but main arrival is from mid–November; they leave North-Africa in March–April (Cramp 1998, del Hoyo *et al.* 2005). In general, migratory birds breed in areas that do not allow wintering endurance, so they need to exploit wintering grounds rich in resources for survival (Alerstam & Hedenström 1998). Although the diet of the ring ouzel in spring and early summer is mainly composed of invertebrates, its autumn and wintering diet is principally fruits; indeed, individuals that winter in north-west Africa feed mainly on juniper berries (Cramp 1998, del Hoyo *et al.* 2005, Ryall & Briggs 2006).

The main aim of this work was to evaluate the importance of the winter visitor *T. torquatus* as an effective seed disperser of the endangered endemic juniper *J. cedrus* in an insular environment (Tenerife, Canary Islands), by analysing its wintering diet.

The Canary Islands lie in the Atlantic Ocean between 27–29°N and 13–18°W. The archipelago has a volcanic origin and is 96 km from the Western Sahara. This work was carried out in Tenerife, which is the largest (2,034 km^2) and the highest island (3,718 m a.s.l.) of the archipelago. The two main populations of *J. cedrus* existing in Tenerife Island were selected to collect samples. These populations are located in '*El Teide*' National Park at 9.3 km distant, respectively. One of them is called '*Riscos de La Fortaleza*' (2,170 m a.s.l.), a craggy geological formation located at the NE side of the Park; the other, '*Siete Cañadas*', consists of an arc of mountains aligned to the south. The climate is typical for high mountain habitats, with great temperature variations throughout the year (differences of about 10 °C between maximum and minimum monthly average temperatures) and a mean annual temperature of 10.7 °C (Bustos & Delgado 2004). The mean temperature during the winter is 7.15 °C; the National Park receives an annual precipitation of about 300–500 mm, most of which falls during the winter months (Bustos & Delgado 2004). The vegetation consists principally of endemic plants, among which *Juniperus cedrus* and shrubs like *Descurainia bourgeauana*, *Spartocytisus supranubius*, *Pterocephalus lasiospermus* and A*denocarpus viscosus* stand out (Wildpret de la Torre & Martín Osorio 2004).

The study was carried out during the winter–spring of 2008; birds' droppings were collected in January, February, March and even April (due to the persistence of the wintering ring ouzels in the area). Droppings were collected in different microhabitats: mainly in surface of rocks around junipers (see Zamora 1990), on the ground, and in a watering point and its surroundings. We consider that practically all these samples corresponded to ring ouzel droppings because although blackbird *Turdus merula* was also detected in the area, this latter species was really scarce. Furthermore, we captured a total of 10 blackbirds during the winter of 2007 and none of them contained seeds from J. cedrus in their droppings.

To estimate the number of *T. torquatus* present in the study area, a census of the species was carried out combining linear transects and observation points when the field was very rough (Tellería 1986). Because of the clearly association between ring ouzels and junipers areas (200 ha approximately), it was difficult to assess the number of birds in the whole area of the National Park. However, it was estimated that the wintering population of ring ouzel did not exceed 50 individuals. Considering the great capacity of displacement and the high rate of fruit consumption of these thrushes, all seems to indicate that the samples studied in the present work are rather representative of this wintering population.

To identify the ingested prey items, each dropping was individually analysed with a 16x stereomicroscope, following the method of Ralph *et al.* (1985). Vegetable remains were identified by comparison with fruits collected in the study area, and the invertebrate fraction by comparing remains in the droppings with exoskeleton collections (IPNA–CSIC).

A germination experiment with seeds that were collected during the winter of 2006–2007 was performed in a greenhouse over six months (October 2007–March 2008). A total of 230 seeds extracted from the droppings of *T. torquatus,* and 230 control seeds directly collected from 20 mother plants were sown. Each seed was randomly and independently sown in a 4 cm^2 pot using a standard substrate (50% culture soil, 25% volcanic sand and 25% turf). Seeds were buried approximately 5 mm deep. All pots were watered every two days and germination was monitored every five days. Germination was defined as the emergence of any seedling part from the soil surface.

Percentage of prey, percentage biomass, and frequency of occurrence, were the main parameters used for expressing diet composition. To overcome possible bias from these measurements, an index of relative importance (thereafter IRI) was calculated for invertebrates, because it is suitable for ranking the relative importance of food types in the diet (Hart *et al.* 2002). The IRI was calculated for each invertebrate category, using the formula:

$$\mathrm{IRI} = F\,(N + W)$$

where N is the numerical percentage, W is the biomass percentage, and F is the percentage frequency of occurrence (see Hart *et al.* 2002).

To calculate the percentage of biomass for *J. cedrus* female cones, we subtracted the average weight of seeds (53.16 mg; n = 180 seeds measured) from the fresh weight (350 mg; n = 90 female cones), considering that each cone contains an average of 2.41 seeds (Rumeu *et al.* 2009). Therefore, we assigned 222.0 mg to the pulp of each cone consumed. For each dropping, we estimated intake by considering that three seeds corresponded to a minimum of one female cone (Adams 2008, Rumeu *et al.* 2009). The same procedure was followed in the case of *Rhamnus integrifolia*. In this case, the weight assigned to the pulp of each fruit was 156.5 mg.

Likelihood ratio tests were performed to compare number of seeds and invertebrates per dropping between the two areas selected, and also in the germination analyses. SPSS statistical package (version 15.0) was employed.

A total of 1,020 ring ouzel droppings were collected (498 at Riscos de La Fortaleza and 522 at Siete Cañadas). All of the 1,439 seeds were identified (1,392–96.7% from *J. cedrus*, and 47–3.3% from *R. integrifolia*); only four *J. cedrus* seeds were externally damaged. This strong interaction between ring ouzels and *J. cedrus* shows that this bird species acts as an important seed disperser for this endemic juniper during its wintering stay. A similar situation appears in high-mountain scrubland of Sierra Nevada in SE Spain (Zamora 1990), where ring ouzels feed almost exclusively on *Juniperus communis*, dispersing large quantities of their seeds. Furthermore, in the Middle and High Atlas (Morocco), female cones of *J. oxycedrus* and *J. phoenicea* make up the greater part of the ring ouzel winter diet (see Ryall & Briggs 2006).

The animal portion of the diet was exclusively composed of invertebrates, and a total of 229 prey items were identified (Table 1). The percentage of prey and the IRI showed that Myriapoda (Diplopoda) and Coleoptera (especially Scarabaeidae, Curculionidae and Dasytidae) were the main groups consumed (Table 1). However, invertebrates only contributed 5% to the biomass, while plant material from *J. cedrus* female cones constituted the main part of this parameter (92.5%). This invertebrate portion presumably supplements the diet, since fruit pulp is in general rich in water and carbohydrates, but low in proteins (Herrera 2002). The genus *Juniperus* includes trees with needles and berries quite rich in resin and essential oils (Adams 1998, Shahmir *et al.* 2003). Energy for migration is deposited mainly in the form of fat, which is the major energy store in birds (Schaub & Jenni 2000), so essential oils included in juniper female cones should provide the ring ouzel much of the energy needed for its spring migration.

A striking homogeneity of the diet of *T. torquatus* was appreciated between the two localities studied, *J. cedrus* female cones being heavily consumed (739 seeds in 498 droppings at *Riscos de La Fortaleza*; 653 seeds in 522 droppings at *Siete Cañadas*; $Z = -1.947$, $p = 0.052$). Thus, data from the two areas were analysed as a single group.

Table 1 Composition of the diet of ring ouzels during their winter stay in *El Teide* National Park (Tenerife, Canary Islands). *%Pr* – percentage of prey; *% FO* – frequency of occurrence; *%B* – percentage of biomass (in the case of plant material, referring to pulp content); *IRI* – Index of Relative Importance.

Prey items	% Pr	% FO	% B	IRI
Plant material	–	99.9	95.04	–
Juniperus cedrus	–	98.8	92.54	–
Rhamnus integrifolia	–	2.9	2.50	–
Plant material indet.	–	0.1	–	–
Seeds	–	68.3	–	–
Juniperus cedrus	–	66.9	–	–
Rhamnus integrifolia	–	1.7	–	–
Invertebrates	100.0	16.1	1.92	1638.8
Mollusca	1.3	0.3	0.11	0.4
Gasteropoda	1.3	0.3	0.11	0.4
Arachnida	3.1	0.7	0.05	2.1
Araneae indet.	3.1	0.7	0.05	2.1
Myriapoda	33.6	6.9	0.29	232.8
Diplopoda	33.6	6.9	0.29	232.8
Hexapoda	62.0	10.4	1.46	659.6
Coleoptera	37.6	6.9	1.03	264.8
Cerambycidae	0.4	0.1	0.01	0.0
Chrysomelidae	0.9	0.2	0.00	0.2
Curculionidae	9.6	2.0	0.25	19.3
Dasytidae	6.6	1.1	0.06	7.1
Elateridae	0.4	0.1	0.00	0.0
Scarabaeidae	13.1	2.6	0.45	35.9
Tenebrionidae	1.3	0.3	0.02	0.4
Coleoptera indet.	5.2	1.2	0.23	6.4
Diptera	0.4	0.1	0.00	0.0
Hemiptera	3.5	0.8	0.09	2.8
Hymenoptera	5.2	1.2	0.14	6.3
Lepidoptera (larvae)	8.3	1.7	0.07	13.9
Orthoptera (juv.)	3.1	0.7	0.13	2.2
Insecta indet	3.9	0.9	–	–
Inorganic material	–	5.8	–	–
Soil	–	5.8	–	–

Juniperus cedrus mature cones are available throughout the year, autumn being the season with greatest quantities of berries (Rumeu *et al.* 2009); therefore, the ring ouzels can find them on their arrival, becoming an important seed dissemination vector, unknown until now. Moreover, probably due to the harsh winter conditions of the high mountain and the inaccessibility of the sites where junipers are located (mainly in protected craggy areas with restricted access), the interaction between ring ouzels and *J. cedrus* has gone unnoticed in the past. However, some individuals of *T. torquatus* have been observed for five consecutive years in the area of *J. cedrus* populations (2004–2008, pers. obs.), showing it to be a regular winter visitor. The results yielded by the germination experiments demonstrate that *T. torquatus* is a clear legitimate disperser of *J. cedrus* and the effect on germination was significantly higher respect to the control seeds ($G_1 = 18.52$, $p < 0.001$). Thus, 34.4% of seeds planted from *T. torquatus* droppings germinated, whereas only 17.0% of control seeds did. This is especially important due to the delicate conservation status of *J. cedrus*, since the raven, known to have been its long-distance disperser (Nogales *et al.* 1999) is now almost extinct on the island. Therefore, taking into account the large amount of seeds dispersed, the ring ouzel constitutes a key species in the *J. cedrus* seed dispersal system and population dynamics in Tenerife. After the extinction of the raven in the range of these juniper populations, and due to lizards being short-distance dispersers, *J. cedrus* is nowadays absolutely dependent on the wintering *T. torquatus* for long-distance seed dispersal. This ecological process probably connects fragmented populations, contributing to the maintenance of their genetic biodiversity. On the other hand, migratory birds can decline in three regions: the breeding grounds, the winter quarters, and passage areas (Berthold *et al.* 1998). Due to the high proportion of *Juniperus* berries in the wintering diet of the ring ouzel, it is important that a decline in the high mountain juniper woodland in the Canary Islands could have negative implications on ring ouzel survival in the future. Thus, as a consequence of the degraded and ageing state of juniper woodland in the Atlas Mountains of Morocco, Ryall & Briggs (2006) predict that juniper berry availability could be a limiting factor for ring ouzel if the woodland destruction continues.

Within the genus *Turdus*, the fruit-eating habits of most of the European species and their important role as seed dispersers are well described (e.g. Herrera 1995, Jordano 1993, Jordano & Schupp 2000, Ryall & Briggs 2006, Zamora 1990). However, this work highlights that the winter stay of the ring ouzel is not only important in continental areas; it can also be significant in insular environments. In this case, it is crucial to the survival and maintenance of genetic biodiversity of the threatened *J. cedrus*. Furthermore, this is one of the few cases described in which an endemic plant from an oceanic island is so dependent on a winter visitor. Future studies considering the other islands where *J. cedrus* is distributed will be necessary to better understand the actual role of the ring ouzel in the survival of the threatened endemic juniper *J. cedrus* in the Canary Islands.

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III

Differential seed dispersal systems of endemic junipers in two oceanic Macaronesian archipelagos: the influence of biogeographic and biological characteristics

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Abstract

This article evaluates the seed dispersal systems of two congeneric and endemic fleshy-fruited plants in the context of two relatively close oceanic archipelagos. For this purpose, representative populations of the endangered junipers *Juniperus cedrus* in the Canary Islands and Madeira, and *J. brevifolia* in the Azores were studied. Despite both species sharing the same biogeographic region, we set out to test whether different conditions of the islands and biological characteristics of each juniper species determine the distinctive guilds of seed dispersers involved. We assessed the quantitative and qualitative role of the potential frugivores, showing that the wintering *Turdus torquatus* and the native *T. merula* were the main seed dispersers for *J. cedrus* and *J. brevifolia*, respectively (Frequency of occurrence: 74.9%, 80.2%; germination increase respect to controls: 11.6%, 15.5%; for *J. cedrus* and *J. brevifolia*, respectively). The endemic lizard *Gallotia galloti* was quantitatively outstanding as seed disperser of *J. cedrus*, although its qualitative effect does not appear to be beneficial. The introduced rabbit *Oryctolagus cuniculus* acts as a disruptor in both natural seed dispersal systems, as inferred from the high percentage of damaged seeds found in their droppings. Our results indicate that *J. cedrus* and *J. brevifolia* are primarily adapted to ornithochory processes, *T. torquatus* and *T. merula* being their respective legitimate long-distance dispersers. Although these birds should be playing a key role in the connectivity of fragmented populations, the dependence of *J. cedrus* on a migrant bird involves a notable fragility of the system.

Keywords: Plant-animal interactions • Ornithochory • Saurochory •
Island ecosystems • Conservation

Introduction

Plant-animal interactions have been the focus of numerous studies, demonstrating the importance of frugivores in the seed dispersal system of many plant species (Jordano 2000). They determine the seed shadow patterns, i.e. the spatial distribution of dispersed propagules with respect to their source plant and conspecifics (*sensu* Schupp 1993) and so they can have a significant effect on both the demography and genetic make-up of animal-dispersed plants (Jordano & Godoy 2002). The disperser contribution is determined by its effectiveness, which depends on the quantity of seeds dispersed and the quality of dispersal each seed is submitted to (Schupp 1993). The seed treatment in the disperser digestive tract may have a marked effect on the subsequent germinability of seeds, since both morphological and physiological traits affect the state of the seeds after their ingestion (Nogales *et al.* 2005). Thereby, the role of seed dispersers as modifiers of germination patterns becomes crucial to the effectiveness of dispersal.

As far as we know, this contribution assesses for the first time, in the context of oceanic archipelagos, the influence of different chronology, biogeography and biological characteristics in the seed dispersal systems of two congeneric fleshy-fruited plant species. We present data on the seed dispersal system of two juniper species endemic to the Macaronesian islands: (1) *Juniperus cedrus* (Webb and Berth.) distributed over the Canary Islands and Madeira; and (2) *Juniperus brevifolia* (Seub.) Antoine, restricted to the Azores. These archipelagos differ in latitude, distance from mainland, geological age, area and altitude of islands, habitat diversity and climate. Hence, the species studied have evolved under distinct ecological conditions; in particular, they vary substantially in the degree of fragmentation of their populations, phenology and main characteristics of the female cones, and groups of potential disperser guilds. In the case of *J. cedrus*, previous information points to the raven *Corvus corax* (nowadays in serious decline in the Canaries) as its long-distance seed disperser in the Canary Islands (Nogales *et al.* 1999), together with the endemic lizard *Gallotia galloti*, at least on Tenerife (Valido 1999), as short-distance disperser. Nevertheless, recent findings have demonstrated the interaction with the wintering *Turdus torquatus*, a thrush species that actively consumes *J. cedrus* female cones during its stay in the high mountain of Tenerife (92.5% of the total biomass ingested; Rumeu *et al.* 2009b). However, ravens, wintering thrushes and native lizards are absent in the Azores, where to our knowledge, there is only one study in which authors report the presence of *J. brevifolia* plant material in the digestive tract of one blackcap *Sylvia atricapilla* (Neves *et al.* 2004). Therefore, at least *S. atricapilla* and *T. merula* (R.B.E., pers. obs.) may be the dispersers of *J. brevifolia* in this archipelago. Lastly, rabbits (*Oryctolagus cuniculus*; probably introduced during the 15th century in both archipelagos) may consume female cones of both juniper species, as happens with other junipers (Santos *et al.* 1999, Schupp *et al.* 1997a, Schupp *et al.* 1997b). Many studies in continental ecosystems reveal that birds and mammals are the agents that primarily disperse juniper seeds (Santos *et al.* 1999, Schupp *et al.* 1997a), thrushes (g. *Turdus)* playing a key role (García 2001, Jordano 1993, Livingston 1972, Zamora 1990). However, little information is available about the seed dispersal systems of junipers on oceanic islands, although the effectiveness of these processes is crucial for the persistence of their populations (Schupp 1993).

Two representative populations of the Macaronesian endemic junipers were selected for the present study, in which the main goals were: (1) to identify the main dispersers acting in the seed dispersal systems of both endemic junipers; (2) to assess the quantitative contribution of each frugivore to the seed dispersal system; (3) to compare the qualitative component of the seed dispersal, with special emphasis on morphological damage to seeds; (4) to test, when possible, the seasonal variation in the seed dispersal process; and (5) to evaluate the effect of seed passage through disperser guts by assessing percentages of germination and emergence rates.

In general, we would expect to find the Canary juniper has a more complex seed dispersal system, as due to biogeographical factors (mainly the short distance from the mainland and the great diversity of habitats), the Canary Islands have a more diverse range of potential dispersal agents than the Azores. Beside this, variation in female cone size is significantly grater in the case of *J. cedrus* (Rumeu *et al.* 2009a), presumably attracting larger seed dispersers in the Canary Islands system. We expect to find differences among the guild of dispersers in their qualitative effect on seed dispersal, although major damage due to seed passage through the digestive tract would not be likely, as juniper seeds have a very hard coat (Adams 2008, Chambers *et al.* 1999). However, as lizards present longer gut passage time (hereafter GPT) in comparison to birds (see Traveset 1998 and references therein), they could facilitate germination by producing deeper seed scarification. At least in the case of ectothermic lizards, we expect a greater contribution to the dispersal system during the warmer months, when they are most active.

Material and methods

Study species

Juniperus cedrus natural populations are very rare and fragmented (IUCN 2009). In the Canary Islands, it grows on La Palma, La Gomera, Tenerife and Gran Canaria (Adams 2008), and nowadays it is mainly restricted to craggy and inaccessible areas. *Juniperus brevifolia*, which is endemic to the Azores archipelago, is distributed throughout all the islands except Graciosa (Adams 2008, Elias 2007), where it is currently extinct. Natural populations on most of the Azores are also scarce and/or fragmented, but there are some islands (Terceira and Flores) where it is still possible to find extensive natural areas occupied by communities dominated by *J. brevifolia* (Elias 2007, Elias & Dias 2009). Both species are considered as endangered (IUCN 2009).

These junipers develop globose female cones with contrasting traits (see Rumeu *et al.* 2009a for further details). It is worth noting here the marked differences in cone size (*J. cedrus* average diameter: 9.94 ± 1.29 mm; *J. brevifolia*: 7.81 ± 1.06 mm) and phenology. Female cones of both Macaronesian species mature in the second year (Adams 2008) but, while in the case of *J. cedrus* they are available to dispersers throughout the four seasons, *J. brevifolia* presents mature female cones only during summer and autumn (Rumeu *et al.* 2009a).

Sites

The Canary Archipelago consists of seven main volcanic islands located between 27–29°N and 13–18°W, and only 96 km off the Atlantic coast of northwest Africa. The study of the *J. cedrus* seed dispersal system was carried out on Tenerife, which emerged 12 Ma ago and is the largest of the islands, 2,058 km^2 in size. We selected one of the most important natural populations, at *Riscos de La Fortaleza* (2,170 m a.s.l.), a craggy geological formation included in *El Teide* National Park. This timberline area is subjected to wide thermal fluctuations throughout the year (about 10°C between maximum and minimum monthly average temperatures), and presents an annual mean temperature of 10.7°C. Annual rainfall is about 368 mm and most of it falls during the winter months (Bustos & Delgado 2004).

Located in the North Atlantic between 36–40°N and 24–32°W, the Azores Archipelago consists of nine main islands of volcanic origin. The distance between them (São Miguel) and the most westerly point of the European continent (Cabo da Roca) is about 1,584 km. The population selected to study the seed dispersal system of *J. brevifolia* was located at *Malha Grande* (505 m a.s.l.), on the island of Terceira (402 km^2). The mean annual temperature is 14.0°C and total annual rainfall is 2168 mm, which occurs mainly in autumn and winter (Elias 2007).

Seed collection

Fieldwork was conducted from 2004 to 2009. To identify the main seed dispersers for *J. cedrus*, droppings from the potential frugivores were collected. Faeces and regurgitations from birds were sampled monthly (November 2006–November 2007) by trapping them with five mist nets (117.5 m^2), set up at the only population site where there was a concentration of junipers. Nets were left open for 10.2 hours per month (total number: 133 hours) and birds captured were ringed and kept in individual ringing bags until they defecated (≈ 30–40 min). Later, they were released in the same place where captured. We also placed plastic films under five female plants in that area to collect droppings and regurgitations from undetermined birds and increase the number of samples. Unfortunately, in late July 2007 a fire swept the netted area, and although the procedure was continued there to complete the year, we replaced the nets the following year (2008) in another nearby population (*Siete Cañadas*) from August to October. As *T. torquatus* was confirmed as a regular wintering bird (Rumeu *et al.* 2009b), droppings of this species were also collected from January to April of 2008 from the rocks surrounding *J. cedrus* plants, which are commonly used by thrushes as surveillance and resting perches (Livingston 1972, Zamora 1990). We considered that practically all these samples corresponded to *T. torquatus* because, although *T. merula* was also present in the area, the latter was scarce and when captured (n = 7), no *J. cedrus* seeds were found in its droppings.

As described above, natural populations of *C. corax* are in drastic decline in the archipelago and extinct in the study area. However, to test the role that they could play as seed dispersers of *J. cedrus*, we carried out a captivity experiment. Mature cones from six female plants of *J. cedrus* were collected separately. Two *C. corax* kept in captivity at two recovery centres for wild animals on different islands (Tenerife and Gran Canaria) were fed with female cones from each individual plant. Cages were inspected every day to gather pellets and recover a minimum of 30 seeds per plant. To control mother plant effects and avoid mixing seeds of different plants, after the last female cones were consumed from a given plant, we waited two days in case any other seeds were recovered from droppings or pellets.

In the field, we also collected 758 droppings from the native lizard *G. galloti* during the summer (when lizards have a higher metabolic activity) of 2004, at 17 different sites in the areas with *J. cedrus* mother-plants. Moreover, 3001 droppings from *O. cuniculus* were collected in 15 different sites of the population during the same period. In addition, to establish the role of lizards throughout the year, we collected droppings seasonally (spring 2008–winter 2009) by setting a radius of 2 m around each of 13 randomly selected female plants. To ensure that only fresh droppings were sampled, all faecal material was removed before the beginning of the sample collection process. When possible, the diameter of all droppings collected was measured to relate it with lizard body size (Valido & Nogales 2003).

In contrast to *J. cedrus*, which maintains mature cones throughout the year, *J. brevifolia* mature cones are only present during the summer and autumn (Rumeu *et al.* 2009a). For this reason, fieldwork in the Azores was carried out from August to October of 2008. Faeces and regurgitations from birds were collected by trapping them with 3–5 mist nets (67.5–117.5 m^2) located in four zones (≈ 630 m apart), which were evenly distributed within the study site. Due to the sampling being seasonally restricted, we tried to trap birds whenever weather conditions permitted fieldwork. Nets were left open for 6.2 hours per day (total number: 136.5 hours). To increase the number of samples, droppings from *T. merula* were also collected from the surface of rocks around *J. brevifolia* plants. Droppings from *O. cuniculus* (n = 2500) were collected at 27 different sites of the juniper population.

For both juniper species, control seeds were extracted directly from female cones on the plants. Whenever an individual plant could be associated with seeds recovered (as in pellets from ravens in captivity), we used seeds from those plants as controls, and the identity of the source mother plant was followed up throughout the seed germination assessment.

Seed procedures

To assess the quantitative role of each seed disperser, droppings and pellets were stored independently and seeds were manually extracted and counted. Then, to compare the qualitative effect of the different dispersers or treatments, we use a stereomicroscope (10x) and noted the external state of the seeds, classifying them into two categories (damaged and undamaged). To evaluate the effect of seed passage through the disperser guts, different germination experiments were performed in a greenhouse. For further germination details, see recommendations by Traveset & Verdú (2002), and Nogales *et al.* (2005, 2006).

Juniperus cedrus seeds from lizard and rabbit droppings were sown in October 2004 and monitored for three consecutive years (until March 2007). Since the highest percentage of germination (14.5%) was recorded in the first year (compared to 2.8% in the second year and 0% in the third), we decided to carry out the remaining experiments for only one year. As reported by Rodríguez-Pérez *et al.* (2005), seed emergence patterns in germination experiments can be contradictory in some cases when comparing field vs. greenhouse-garden conditions. For this reason, to examine the quality of dispersal, two in-situ germination experiments were also performed at both sites during the rainy period (November 2008–March 2009 in *Riscos de La Fortaleza*; October 2008–June 2009 in *Malha Grande*). Seeds were planted independently using meshes of grades: 5.5 cm^2 or 3.5 cm^2 for *J. cedrus* and *J. brevifolia*, respectively. These experiments were protected by covering them with another finer grade mesh (1.2 cm^2) to avoid predation of seeds and seedlings. In the case of *J. cedrus*, the tested disperser effects were those of *G. galloti*, *T. torquatus*, and control seeds. For *J. brevifolia* in *Malha Grande*, disperser effects tested were *T. merula* and control seeds (n = 200 seeds in all treatments).

Statistical analyses

Categorical analyses (Likelihood ratio tests) were performed to compare the number of seeds per dropping from *G. galloti* over the four different seasons and also the number of germinated seeds after each treatment. In those cases where the use of the same data set was required, we applied the more conservative sequential *Bonferroni* technique to assess statistical significance. An ANOVA test was used to compare the sizes of *G. galloti* droppings between seasons, and Kolmogorov-Smirnov tests were employed to evaluate differences in germination rates between treatments. To avoid possible bias in these latter analyses, caused by the differential percentages of seed germination from one treatment to another, the only data considered were those from the period when the first seedling emerged until the germination curve was established (Nogales *et al.* 2006). All analyses were performed using the SPSS statistical package (version 15.0).

Results

Seed-disperser interactions

The relative contribution of each potential frugivore to seed dispersal differed among species (Table 1). In the case of *J. cedrus*, *T. torquatus* showed the highest values of seeds per dropping, frequency of occurrence: 74.9% and percentage of undamaged seeds: 99.7%. The number of seeds was significantly lower for the other dispersers (likelihood ratio test; $p < 0.001$ for all comparisons); interaction with *E. rubecula* was rare, with only one undamaged seed having been recovered from its droppings. On the other hand, in the few samples analysed from *T. merula*, no seeds were found. The endemic lizard *G. galloti* was next in the number of seeds dispersed, the frequency of occurrence of seeds in its droppings being significantly higher than those found for *O. cuniculus* and *E. rubecula* (G_1 = 1,728.11, $p < 0.001$; G_1 = 21.91, $p < 0.001$; respectively). Furthermore, this lizard also presented a lower number of damaged seeds in comparison to *O. cuniculus* (G_1 = 147.88, $p < 0.001$). Thus, the lowest values of seeds per dropping, frequency of occurrence and percentage of undamaged seeds corresponded to the alien rabbit *O. cuniculus*.

Turdus merula was the main disperser for *J. brevifolia*, showing the highest numbers of seeds per dropping and frequency of occurrence (81.1%). This latter measure was significantly higher than that of O. cuniculus and *S. atricapilla* (G_1 = 896.16, $p < 0.001$; G_1 = 138.58, $p < 0.001$; respectively). Seeds defecated by *S. atricapilla* were scarce but it was the second disperser in importance found for the Azorean juniper system. Lastly, as was the case in the Canaries, the introduced rabbit *O. cuniculus* presented fewer seeds per dropping, lower frequency of occurrence and percentage of undamaged seeds.

Table 1 Relative contribution of each potential frugivore to *Juniperus cedrus* and *J. brevifolia* seed dispersal systems in the respective study areas (*Riscos de La Fortaleza*, Tenerife, Canary Islands; and *Malha Grande*, Terceira, Azores).

Juniper species	Potential frugivores	No. of seeds per dropping (mean ± SD)	F.O. (%)	Undamaged seeds (%)	No. of seeds found analysed	No. of droppings analysed
J. cedrus	*Turdus torquatus*	1.48 ± 1.41	74.9	99.7	739	498
J. cedrus	*Turdus merula*	0.00 ± 0.00	0	0	0	6
J. cedrus	*Erithacus rubecula*	0.06 ± 0.24	5.9	100	1	17
J. cedrus	*Fringilla teydea*	0.00 ± 0.00	0	0	0	11
J. cedrus	*Gallotia galloti*	1.15 ± 1.36	59.2	95.3	1669	1444
J. cedrus	*Oryctolagus cuniculus*	0.04 ± 0.24	3.9	56.3	118	3001
J. brevifolia	*Turdus merula*	2.32 ± 2.08	81.1	98.9	761	328
J. brevifolia	*Sylvia atricapilla*	0.11 ± 0.47	6.1	100	7	66
J. brevifolia	*Erithacus rubecula*	0.00 ± 0.00	0	0	0	17
J. brevifolia	*Fringilla coelebs*	0.00 ± 0.00	0	0	0	30
J. brevifolia	*Oryctolagus cuniculus*	0.07 ± 0.31	5.9	41.2	177	2500

F.O., frequency of occurrence of juniper seeds in the droppings analysed

Seasonal patterns of seed dispersal

Seasonal variation in seed dispersal agents and their quantitative contribution show that dispersal of *J. cedrus* seeds does not occur equally through the year. No frugivorous birds were captured during the summer (Fig. 1), and lizard droppings at *Riscos de La Fortaleza* (n = 1444) were more abundant during the summer season (41.9% of total droppings found) (Table 2). The frequency of occurrence of *J. cedrus* seeds in *G. galloti* droppings, and the number of seeds per dropping were clearly different between seasons ($G_3 = 65.88$, $p < 0.001$; $\chi^2_3 = 18.52$, $p < 0.001$, respectively), droppings containing seeds and number of seeds per dropping being more frequent during the summer than in the other seasons ($p < 0.05$ for all comparisons). Diameter of *G. galloti* faeces varied significantly through the year (ANOVA I test; $F_3 = 12.85$, $p < 0.001$); those found in summer were larger than those collected during the other seasons ($p < 0.001$ for all comparisons), indicating that seed dispersal mainly relies on bigger lizards during the warmer period. While *T. torquatus* dispersed 60.5% of seeds during the winter months, *G. galloti* spread the seeds mainly in the warmest season (56.4%). Therefore, a seasonal replacement pattern of the main seed disperser agents allows *J. cedrus* seeds to be dispersed throughout the year (Fig. 2).

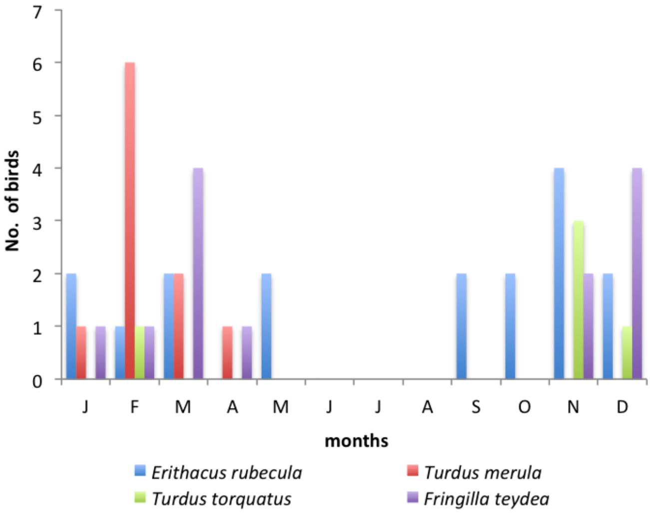

Fig. 1 Frugivorous birds trapped in *Riscos de La Fortaleza* over 1 year (November 2006–October 2007; total mist-netting effort: 133h). Data from August to October were obtained in a nearby population (*Siete Cañadas*) during 2008 (after the fire that affected the original population).

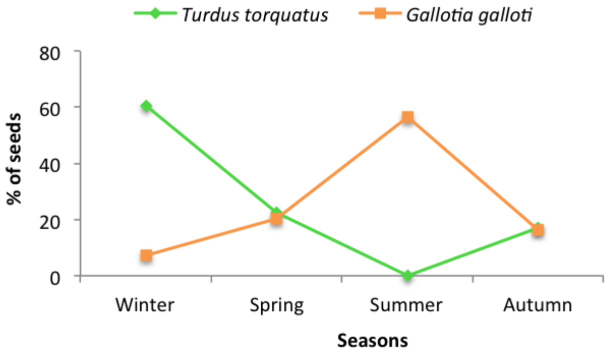

Fig. 2 Percentage of *Juniperus cedrus* seeds dispersed by the main frugivores present in *Riscos de La Fortaleza* over 1 year.

Seed germination

Frugivores affected the percentages and germination rates of *J. cedrus* seeds tested in different ways (Fig. 3). Both *C. corax* and *T. torquatus* significantly increased the percentage of seeds germinated (G_1 = 7.70, p = 0.006; G_1 = 8.33, p = 0.004, respectively). Whereas 22.5% of control seeds emerged from the soil surface, the figures were 34.1 and 34.5% after gut passage through *C. corax* and *T. torquatus* respectively. The effect of lizards was different; the endemic *G. galloti* significantly reduced the number of germinated seeds with respect to control ($G_1 = 13.29$, $p = 0.001$), and slowed down their germination (Kolmogorov-Smirnov test; $Z = 1.79$, $p = 0.003$) (Fig. 3). Unfortunately, due to the low number of juniper seeds found in *O. cuniculus* faeces, and a high proportion of externally well developed but empty seeds (see Chambers *et al.* 1999), we could not present any data about germination success after the *O. cuniculus* gut treatment. We did not obtain conclusive results in the in-situ germination experiment with *J. cedrus* seeds in any of the treatments. In all three treatments, only two control seeds (1% of the control seeds sown) were able to germinate at *Riscos de La Fortaleza.*

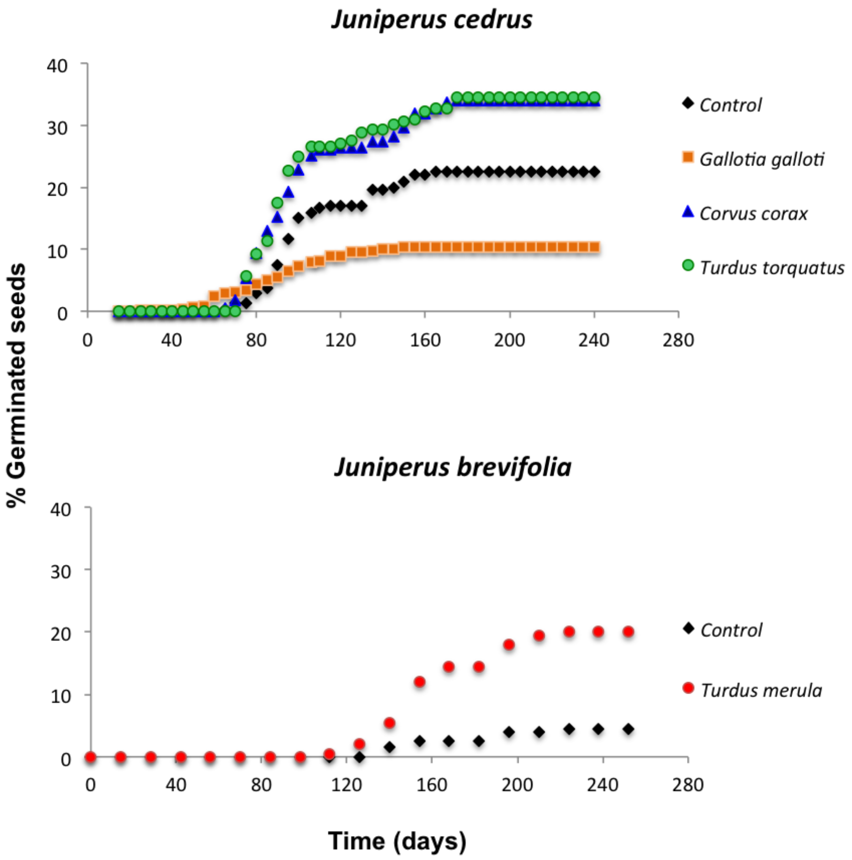

Fig. 3 Germination percentages and rates of *Juniperus cedrus* and *J. brevifolia* seeds obtained from droppings and regurgitations of the main seed dispersers. Data from *J. cedrus* derive from the greenhouse experiments, while those for *J. brevifolia* are from the in situ experiment.

In the case of *J. brevifolia*, germination results presented are those derived from the field experiment, since no seeds germinated in the greenhouse. In the in-situ test, a higher proportion of those seeds ingested by the thrush species *T. merula* germinated, compared to controls (20.0% and 4.5%, respectively; $G_1 = 23.93$, $p < 0.001$), but they did not emerge faster ($Z = 1.34$, $p = 0.056$) (Fig. 3).

Discussion

Seed dispersal by frugivores

The largest number of *J. cedrus* seeds was found in droppings of the wintering *T. torquatus* and the native lizard *G. galloti*. Frugivore interactions in continental ecosystems between thrushes and different juniper species are well described (García 2001, Jordano 1993, Livingston 1972, Santos *et al*. 1999, Zamora 1990). However, after the extinction of *C. corax* in the range of this juniper population, the wintering *T. torquatus* is now the sole long-distance disperser for *J. cedrus* seeds. Hence, its regular annual arrival in the high mountain zone of Tenerife is crucial in the population dynamics of this juniper, enabling distant population nuclei to be linked and contributing to the maintenance of its genetic diversity. An interesting finding revealed during this work, is that although the endemic finch *F. teydea* does not act as *J. cedrus* seed disperser, during the winter months they leave their optimal habitat (pine forest) and take advantage of the timberline resources, actively consuming *J. cedrus* seeds (pers. obs.). In addition, saurochory is mainly an insular phenomenon (Olesen & Valido 2003), and the large number of seeds moved by *G. galloti* confirms that the seed-dispersing role of lizards on islands is more important than previously thought (Cooper & Vitt 2002, Olesen & Valido 2003, Rodríguez *et al.* 2008). In fact, this high consumption of *J. cedrus* female cones by *G. galloti* becomes relatively important to the dispersal system, given the high density of these lizards in all habitats of the Canary Islands (Olesen & Valido 2003). However, unlike *T. torquatus*, lizards are considered as short-distance dispersers (home range areas reported for *G. galloti* < 100 m^2; Molina-Borja 1985).

With respect to *J. brevifolia* on Terceira, the native *T. merula* was the main seed disperser, showing the highest values of seeds per dropping and frequency of occurrence. This case, as described above, is in concordance with the predominant pattern described in continental ecosystems. Nevertheless, to our knowledge and despite its opportunistic frugivore behaviour, the interaction between *S. atricapilla* and junipers is not common in continental environments (see Jordano 1987, Jordano & Herrera 1981). Average fruit size consumed is strongly correlated with gape-width of warblers (Jordano 1987), since fruit size impedes ingestion by those relatively small-sized dispersers that swallow them whole (Herrera 2002, Howe & Westley 1990). Hence, the small female cone size of *J. brevifolia* (7.81± 1.06 mm; Rumeu *et al.* 2009a) facilitates this interaction.

The introduced rabbit *O. cuniculus* had a negative effect on the seed dispersal systems of the two Macaronesian junipers, due to the high percentage of damaged seeds (43.7 and 58.8% for *J. cedrus* and *J. brevifolia*, respectively). Lagomorphs can disperse seeds of fleshy-fruited plants, including juniper species (Santos *et al.* 1999, Schupp *et al.* 1997a, Schupp *et al.* 1997b). However, some studies have shown that rabbits break seeds and therefore reduce seed survival and germination when feeding on fleshy fruits, in continental systems (Muñoz-Reinoso 1993) and also in insular environments like the Canary Islands (Nogales *et al.* 2005). Taking into account the recent introduction date of rabbits in both archipelagos (in that the endemic junipers have not been able to evolve in contact with them) and the low quantitative and qualitative effectiveness of seed dispersal, *O. cuniculus* represents another example of the impact that alien species can have on native mutualisms (e.g. Nogales *et al.* 2005).

Replacement of seed dispersers

In *J. cedrus*, a seasonal variation in frugivorous interactions was detected. While no frugivorous bird species were captured during the summer months, their role in seed dispersal becomes very significant during the winter, when the migrant *T. torquatus* disperses a high quantity of *J. cedrus* seeds. However, its return to breeding sites in March–April coincides with the time when the temperature starts to rise in the timberline zone and lizards become more active. Thus, these short-distance dispersers reach their peak of seed dispersal activity during the summer months, when larger droppings were found. According to Valido & Nogales (2003), there is a positive relationship between diameter of faecal pellets and *G. galloti* body size, so our results show that during the summer, bigger lizards actively consume *J. cedrus* female cones. In general, recorded schedules of fruit production and depletion suggest adaptation to dispersal opportunities (Howe & Smallwood 1982). In this case study, the endemic Macaronesian junipers show contrasting phenology. Whereas *J. brevifolia* seed dispersal is restricted to summer and autumn, *J. cedrus* presents mature female cones throughout the year (Rumeu *et al.* 2009a), allowing year-round dispersal of their seeds by different frugivores.

Effect of disperser gut treatments on seed germination

External damage on *J. cedrus* and *J. brevifolia* seeds was low in the case of birds and reptiles, contrasting with the higher values reported for *O. cuniculus.* For the two juniper species studied, seeds ingested by birds germinated in greater proportions than controls. These results agree with those found in *J. virginiana* (Holthuijzen & Sharik 1985). Nevertheless, they contrast with other studies on junipers, in which passage through bird digestive tracts did not increase germination percentages with respect to controls (Livingston 1972, Salomonson 1978). After recording the positive effect of *C. corax* in the seed dispersal system of *J. cedrus*, it is striking that without the presence of *T. torquatus* during the winter months, the extinction of this native corvid could have negative consequences on the fitness of *J. cedrus*. However it is probable that the interaction between *J. cedrus* and the wintering *T. torquatus* has been occurring for a long time, but has passed unnoticed (Rumeu *et al.* 2009b).

When saurochory has an effect on percentage germination, it is often positive (Traveset 1998). However, although the interaction between *G. galloti* and *J. cedrus* has been going on for much longer on an evolutionary time scale, the effect of this endemic lizard on germination percentage and rate was negative. Consequences of asynchronous germination are complex, but the effect of lizards on *J. cedrus* germination rate could guarantee that seedling mortality is spread over a longer period, increasing the likelihood of success of undamaged seeds (Harper 1977). Similar results were previously reported when assessing the effect of *G. galloti* on the seed germination of *Rubia fruticosa* (Padilla & Nogales 2009). As this latter study reflects, the reduction in seed viability could be due to the enzymatic effects in the long gut (mean snout-vent length of *G. galloti*: 106.4 ± 12.1 mm) and because herbivorous lizards have long GPT (*G. galloti* mean GPT: 6.9 ± 3.8 days; Valido & Nogales 2003) compared to passerine birds (5–20 min for regurgitated seeds; 0.3–1.5 h for defecated seeds) (see Traveset 1998 and references therein). Nevertheless, taking into account the important effect of the *J. cedrus* mother plant on seed viability (Rumeu *et al.* 2009a), further germination experiments with lizards kept in captivity (which permit the control of this mother effect) are essential to clarify the relationship between native lizards and endemic junipers in the Canary Islands.

Concluding remarks

Our results show that the seed dispersal systems of the two endemic Macaronesian junipers differ in some important quantitative and qualitative aspects, like the dispersal agents involved and the seasons in which seed dispersal occurs. As expected, *J. cedrus* presented a more complex seed dispersal system than *J. brevifolia* (in which only *T. merula* is quantitatively and qualitatively important), and the ensemble of legitimate known seed dispersers is in concordance with the size of female cones. So, while *J. cedrus* has evolved with large seed dispersers (like *C. corax* and probably giant lizards of the endemic genus *Gallotia* that were abundant in the past), the female cones of *J.*

brevifolia are mostly consumed by small and medium size birds such as *S. atricapilla* and *T. merula.* Despite the biogeographic peculiarities of the island systems these junipers inhabit, they are in both cases well adapted to ornithochory, which coincides with the patterns described in continental species. *Juniperus brevifolia* presents a solid seed dispersal system, where the main frugivore involved is a native, ubiquitous and abundant thrush, capable of the maintenance of seed gene flow among populations. However, even though the *J. cedrus* seed dispersal system is more complex than that of *J. brevifolia*, the dependence of *J. cedrus* on the wintering of *T. torquatus* for the long-distance dispersal of its seeds implies great fragility in the system. Moreover, although *T. torquatus* is not globally threatened (IUCN 2009), there has been a decrease in numbers and a reduction in its range in Britain (Burfield & Brook 2005), where it has been listed as a species deserving high conservation concern (see Sim *et al.* 2007). Thus, the conservation of this migrant bird species could be the key in the persistence of *J. cedrus* in Tenerife.

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IV

The colonization history of *Juniperus brevifolia* (Cupressaceae) in the Azores islands

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Abstract

Background: A central aim of island biogeography is to understand the colonization history of insular species using current distributions, fossil records and genetic diversity. Here, we analyze five plastid DNA regions of the endangered *Juniperus brevifolia*, which is endemic to the Azores archipelago.

Methodology/Principal Findings: The phylogeny of the section *Juniperus* and the phylogeographic analyses of *J. brevifolia* based on the coalescence theory of allele (plastid) diversity suggest that: (1) a single introduction event likely occurred from Europe; (2) genetic diversification and inter-island dispersal postdated the emergence of the oldest island (Santa Maria, 8.12 Ma); (3) the genetic differentiation found in populations on the islands with higher age and smaller distance to the continent is significantly higher than that on the younger, more remote ones; (4) the high number of haplotypes observed (16), and the widespread distribution of the most frequent and ancestral ones across the archipelago, are indicating early diversification, demographic expansion, and recurrent dispersal. In contrast, restriction of six of the seven derived haplotypes to single islands is construed as reflecting significant isolation time prior to colonization.

Conclusions/Significance: Our phylogeographic reconstruction points to the sequence of island emergence as the key factor to explain the distribution of plastid DNA variation. The reproductive traits of this juniper species (anemophily, ornithochory, multi-seeded cones), together with its broad ecological range, appear to be largely responsible for recurrent inter-island colonization of ancestral haplotypes. In contrast, certain delay in colonization of new haplotypes may reflect intraspecific habitat competition on islands where this juniper was already present.

Keywords: *Juniperus brevifolia* • Phylogeny • Phylogeography • Plastid DNA • Haplotype diversity • Island colonization • Azores

Introduction

Volcanic islands are geographic units that emerged from the ocean floor. After colonization by plants, oceanic barriers isolate island populations from the mainland and from each other, thus reducing gene flow (Emerson 2002, Whittaker & Fernández-Palacios 2007). It is expected that plants displaying traits favorable for dispersal, establishment and distribution, such as anemophily, zoochory and self-compatibility, have been more successful in the colonization of remote archipelagos and their islands (García-Verdugo *et al.* 2010, Vargas 2007).

Located in the Atlantic Ocean, the Macaronesian oceanic insular hotspot harbors three endemic juniper species (Cupressaceae), each distributed over a different archipelago: (1) *Juniperus brevifolia* (Seub.) Antoine in the Azores, (2) *J. cedrus* Webb & Berth. in the Canary Islands, and (3) *J. maderensis* (Menezes) R. P. Adams in Madeira (Adams *et al.* 2010). These three taxa belong to the section *Juniperus*, which contains 11 of the ca. 67 species included in the genus (Adams 2011). Besides the Macaronesian junipers, the section comprises seven species currently distributed in the Mediterranean region and eastern Asia, plus *J. communis*, which has a widely circumboreal distribution. Their presence in the Atlantic archipelagos per se entails at least one long-distance dispersal event from the continent, though preliminary molecular phylogenies suggest at least two events (see Vargas 2007). In particular, the presence of *J. brevifolia* in the Azores implies one of the most remote juniper colonizations known to date (distance of about 1,300 km from western Europe, 1,600 km from eastern North America, and 800 km from north-west Madeira, which is the nearest Macaronesian archipelago). The Azores archipelago is located between 36°–40° N and 24°–32°W, and it comprises nine main islands of different geological ages, divided into Western (Corvo and Flores), Central (Faial, Pico, São Jorge, Graciosa and Terceira), and Eastern (São Miguel and Santa Maria) groups, with respective ages of 0.71–2.16 Ma, 0.25–3.52 Ma, and 4.01–8.12 Ma (Fig. 1). These islands are the result of the active volcanism associated with the divergence of the African, Eurasian and American tectonic plates. Lying over a 615 km long axis, the minimum distance between western and central groups is currently of 218 km, and of 139 km between central and eastern groups.

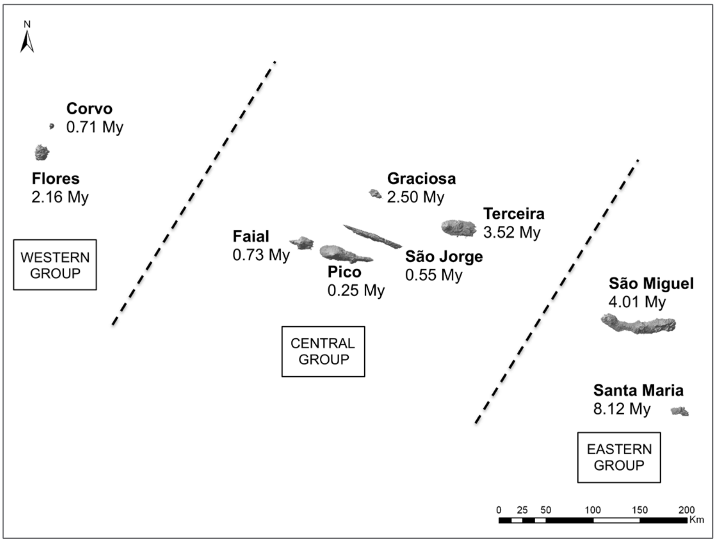

Fig. 1 The Azores archipelago. Island groups and maximum geological ages according to França *et al.* 2003.

Fossil-calibrated phylogenetic studies suggest a high number of relict Tertiary lineages in Macaronesia (Andrus *et al.* 2004, Carine *et al.* 2004, Vargas 2007). However, molecular studies have also shown that several presumed Macaronesian relict lineages have a recent origin (Emerson 2002, Vargas 2007). As a general pattern, the presence of plants in oceanic islands probably is the result of many different colonization processes since the islands emerged, as revealed by the contrasting evolutionary histories of the lineages assessed. In particular, the colonization history of the remote archipelago of Azores is poorly understood. Even though some of the 149 native angiosperms (Schaefer *et al.* 2011a) have been analyzed under phylogenetic perspectives (e.g. *Bellis*, Fiz *et al.* 2002; *Euphorbia,* Molero *et al.* 2002; *Vaccinium,* Powell & Kron 2002; *Pericallis,* Swenson & Manns 2003; *Lotus*, Allan *et al.* 2004; *Azorina,* Roquet 2008, *Euphrasia*, Gussarova *et al.* 2008; *Laurus,* Rodríguez-Sánchez *et al.* 2009), only a few of them have been the focus of deeper phylogeographic studies in the Azores archipelago (e.g.

Festuca, Díaz-Pérez *et al.* 2008; *Picconia*, Ferreira *et al.* 2011; *Ammi*, *Angelica*, *Azorina*, *Euphorbia*, *Pericallis*, Schaefer *et al.* 2011b), far fewer phylogeographic results related to interisland colonization are known.

The study of representatives from different Azorean plant groups allows us to single out the most successful combinations of traits to thrive in these remote islands, and provides a general framework to understand the different processes that foster colonization. Regardless of being dioecious and, hence, self-incompatible, *J. brevifolia* represents one of the best examples of endemic species displaying traits favorable for long-distance dispersal and colonization, i. e. anemophily, zoochory, multi-seeded cones (Adams 2011) and mesic habitat requirements (Elias 2007), among others. If *J. brevifolia* derives from a recent colonization, low levels of genetic diversity would be expected overall, whereas if colonization by *J. brevifolia's* ancestor occurred far back in time we would expect that extant populations have high genetic diversity between island populations due to genetic drift favoring/fixing different alleles and haplotypes (Hedrick 1999, Zhang *et al.* 2005). Consequently, DNA data may provide us with important variables to reconstruct the chronological sequence of diversification in the Azorean juniper.

In angiosperms, both plastid DNA (cpDNA) and mitochondrial DNA (mtDNA) are maternally inherited in most cases (Hipkins *et al.* 1994), and the degree of genetic structuring shaped by organelle DNA can only be interpreted in terms of seed dispersal. However, previous conifer studies show predominant paternal inheritance of cpDNA in this group and in members of the Cupressaceae family (Hipkins *et al.* 1994, Mogensen 1996, Neale *et al.* 1991, 1989, Petit *et al.* 2005). Furthermore, because the spatial pattern of the adult plants is a consequence of the seed dispersal (Jordano 2000), molecular variables make it possible to relate the haplotype distribution of the Azores juniper to the contribution of both seed and pollen gene flow.

In this investigation, we used plastid DNA sequences of *J. brevifolia* to: (1) infer the temporal and spatial origin, (2) estimate genetic diversity levels on each island, and (3) reconstruct the phylogeographic history in the Azores archipelago.

Methods

Study species

As the remaining juniper species, *J. brevifolia* is dioecious and wind-pollinated, shedding pollen from the male cones principally during spring (Adams 2011). It develops fleshy female cones that ripen in summer and autumn (Rumeu *et al.* 2009), and are consumed mainly by birds (Rumeu *et al.* 2011), as recorded for continental congeners (García 2001, Jordano 1993, Livingston 1972, Zamora 1990).

As a consequence of deforestation since the onset of human settlement in the 15th century, natural populations of the two Azorean native conifers (*Taxus baccata* and *J. brevifolia*) have been drastically reduced. Thus, Schirone *et al.* (2010) predict imminent extinction for *T. baccata* and report only 5 living individuals on Pico island, whereas *J. brevifolia* populations are nowadays scarce and/or fragmented and the species is considered as 'endangered' on a global scale (IUCN 2011). Notably, although *J. brevifolia*'s range is much more restricted than in the past, and it is nowadays extinct on Graciosa and critically endangered on Santa Maria, its current distribution entails a wide ecological range, and there are still extensive natural areas on some islands where plant communities are dominated by this juniper (Elias 2007, Elias & Dias 2009).

Ethics statement

The 'Secretaria Regional do Ambiente e do Mar–Direcção Regional do Ambiente' from the Azorean Autonomous Region provided us with the required permit for the collection of wild plant leaves (LICENÇA N° 59 / 2008 / DRA).

Plant material and DNA plastid sequencing

For the inference of intraspecific patterns of *J. brevifolia* cpDNA sequence variation, needles were collected from trees on all the islands of occurrence.

Except for Santa Maria, where only two samples could be collected, about 50 trees were sampled from each island. The geographic coordinates of each sample were recorded using a hand-held GPS navigator. In total, needles of 367 trees were sampled and stored in zippered plastic bags containing silica gel. Total DNAs were extracted from silica-gel dried needles using the CTAB 2x method (Doyle & Doyle 1987, Palmer *et al.* 1988). The concentration of the total DNA obtained was measured in an Eppendorf biophotometer, and its quality assessed in 1% agarose gels.

To perform phylogenetic analyses of section *Juniperus*, we took *trn*L intron and *trn*L-*trn*F intergenic spacer (Taberlet *et al.* 1991) sequences from previous studies (Mao *et al.* 2010, Martínez & Vargas, in prep.). A matrix using these cpDNA regions was constructed with nine *trn*L and *trn*L-*trn*F sequences from Mao *et al.* (2010), and 36 *trn*L-*trn*F sequences from Martínez & Vargas (unpublished data); the latter were completed with their corresponding 36 *trn*L sequences, which were newly generated by us for this study. In addition, the matrix was extended with two samples of the Macaronesian junipers *J. cedrus* and *J. maderensis* and eight samples of our focal species (*J. brevifolia*). Based on previous phylogenetic analyses of *Juniperus* (Mao *et al.* 2010), we also added sequences of three outgroup samples of *J. drupacea* (section *Caryocedrus*) (Tables 1/S1). PCR conditions were based on Taberlet *et al.* (1991) with some variations: 95° C for 1 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 2 min, and 72 °C for 2 min, with a final extension step of 72 °C for 10 min.

To perform phylogeographic analyses of *J. brevifolia*, we tested two to eight individuals from different islands for 19 plastid DNA regions in an exploratory study based on previous phylogenetic and phylogeographic analyses (Adams *et al.* 2009, Fazekas *et al.* 2008, Hamilton 1999, Hwang *et al.* 2000, Kress & Erickson 2007, Provan *et al.* 2008, Sang *et al.* 1997, Shaw *et al.* 2005, 2007, Taberlet *et al.* 1991). The three most variable plastid DNA regions (*pet*N-*psb*M, Adams *et al.* 2009; *trn*S-*trn*G, Hamilton 1999; *trn*T-*trn*L, Taberlet *et al.* 1991) were used to sequence 9–10 individuals per island (except for Santa Maria, where only two trees are known) in order to assemble the phylogeographic matrix (Tables 2/S2).

Table 1 Taxon names and origin of the *Juniperus* samples included in the phylogenetic analysis. Coding numbers as in Fig. 2. Taxonomy follows that of Adams *et al.* 2011. See Table S1 for details.

Coding no	Taxon	Origin
1	*J. brevifolia*	Portugal, Azores, Santa Maria, Almagreira
2	*J. brevifolia*	Portugal, Azores, São Miguel, Serra da Tronqueira
3	*J. brevifolia*	Portugal, Azores, Terceira, Malha Grande
4	*J. brevifolia*	Portugal, Azores, São Jorge, R.F. Pico da Esperança
5	*J. brevifolia*	Portugal, Azores, Pico, Cachorro
6	*J. brevifolia*	Portugal, Azores, Pico, Lagoa do Capitão
7	*J. brevifolia*	Portugal, Azores, Faial, Caldeira
8	*J. brevifolia*	Portugal, Azores, Faial, Caldeira
9	*J. brevifolia*	Portugal, Azores, Flores, Alto da Cova
10	*J. brevifolia*	Portugal, Azores, Corvo, Lomba Redonda
11	*J. cedrus*	Spain, Canary Islands, Tenerife, La Orotava
12	*J. cedrus*	Spain, Canary Islands, La Palma, Pared de Roberto
13	*J. communis* var. *communis*	Spain, Burgos, Covarrubias
14	*J. communis* var. *communis*	Spain, Granada, Pico Trevenque
15	*J. communis* var. *communis*	France
16	*J. communis* var. *depressa*	USA, New Mexico
17	*J. communis* var. *saxatilis*	Spain, Madrid, La Pedriza
18	*J. communis* var. *saxatilis*	Georgia, Caucasus
19	*J. communis* var. *saxatilis*	Pakistan
20	*J. deltoides*	Turkey
21	*J. formosana var. mairei*	China, Gansu
22	*J. macrocarpa*	Spain, Cádiz, Barbate, Trafalgar Cape
23	*J. macrocarpa*	Spain, Cádiz, Tarifa
24	*J. macrocarpa*	Spain, Valencia, El Saler
25	*J. macrocarpa*	Spain, Cádiz, Chiclana
26	*J. macrocarpa*	Italy, Sardinia, Santa Teresa Gallura
27	*J. macrocarpa*	Italy, Sicily, Ragusa
28	*J. maderensis*	Madeira, Fajã da Nogueira
29	*J. navicularis*	Portugal, Apostiça
30	*J. navicularis*	Portugal, Apostiça
31	*J. navicularis*	Portugal, Estremadura
32	*J. oxycedrus* var. *badia*	Spain, Huesca, Sierra de Guara
33	*J. oxycedrus* var. *badia*	Spain, Madrid, Villalba
34	*J. oxycedrus* var. *badia*	Spain, Ciudad Real, Puebla de Don Rodrigo
35	*J. oxycedrus* var. *badia*	Spain, Jaén
36	*J. oxycedrus* var. *badia*	Spain, Jaén
37	*J. oxycedrus* var. *badia*	Morocco, Kjbel Kelti
38	*J. oxycedrus* var. *badia*	Morocco, Kjbel Kelti
39	*J. oxycedrus* var. *badia*	Turkey, Gümüshane, Torul
40	*J. oxycedrus* var. *oxycedrus*	Spain, Granada, El Peñón
41	*J. oxycedrus* var. *oxycedrus*	Spain, Granada, El Peñón
42	*J. oxycedrus* var. *oxycedrus*	Spain, Balearic Islands, Menorca
43	*J. oxycedrus* var. *oxycedrus*	Greece, Lemo
44	*J. oxycedrus* var. *oxycedrus*	Greece, Lemo
45	*J. oxycedrus*	Turkey, Istanbul, Kartal

Table 1 (Continued)

Coding no	Taxon	Origin
46	*J. oxycedrus*	Greece, Kalavryta, Diakoftó
47	*J. oxycedrus*	Tunisia, Cap Bon, Sidi Daoud
48	*J. oxycedrus*	France
49	*J. rigida* var. *conferta*	Cultivated (Spain, Pontevedra, Lourizán)
50	*J. rigida* var. *conferta*	Japan
51	*J. rigida* var. *rigida*	Japan
52	*J. rigida* var. *rigida*	Japan
53	*J. taxifolia*	Japan
54	*J. taxifolia*	Japan, Bonin Island
55	*J. taxifolia* var. *lutchuensis*	Japan
	OUTGROUP	
56	*J. drupacea*	Greece
57	*J. drupacea*	Greece
58	*J. drupacea*	Greece

PCR protocols varied slightly for the three DNA regions, and consisted of: 30–35 cycles of 94 °C for 30 s, 50–56 °C for 2 min and 72 °C for 2 min, preceded by an initial denaturation at 95 °C for 1 min and followed by a final extension at 72 °C for 5–10 min. A volume of 1μl of bovine serum albumin (BSA) at 1mg ml^{-1} was included in each 25μl reaction to improve the efficiency of the amplification. PCR products were sequenced using an ABI Prism ® 3730xi DNA sequencer at the Macrogen Institute (Macrogen Co., Korea). Sequences were aligned and manually adjusted using MAFFT v6.814b implemented in the Geneious 5.1.7 software. All new sequences have been deposited in GenBank (see Tables S1 and S2 for accession numbers).

To extend the phylogeographic analysis, we also amplified the *psbA*-*trn*H region (Sang *et al.* 1997) (following the above-mentioned conditions, but without using BSA). In this case, the presence of a 58 bp insertion/deletion detected in the preliminary screening was checked in 1.5% agarose gels for the 367 samples collected in the field (Table 3).

Data analysis

To obtain the taxonomic relationships within *Juniperus* section *Juniperus* and to infer the temporal and spatial origin of *J. brevifolia*, Maximum

Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) analyses were conducted for the *trn*L intron and *trn*L-*trn*F dataset. Large indels were found in the *trn*L-*trn*F spacer; as gap characters represent in this case a considerable portion of the potential phylogenetic information (Simmons & Ochoterena 2000), all indels detected were manually coded following the method of Simmons & Ochoterena (2000) and included in the analyses. To determine the model of sequence evolution that best fits the sequence data (F81+G), the Akaike Information Criterion (AIC; Akaike 1979) was implemented in this dataset using jModeltest 0.1.1 (Posada 2008). ML analyses were performed using PhyML (Guindon & Gascuel 2003) including the model parameters previously obtained with jModeltest. ML bootstrap analysis was carried out with the same software and settings, using 500 non-parametric bootstrap replicates. Parsimony analyses were run in tnt 1.1 (Goloboff *et al.* 2008) under traditional heuristic search. We first used the Tree Bisection-Reconnection (TBR) branch-swapping algorithm with 10,000 replicates (saving two most-parsimonious trees per replicate); subsequently, the trees obtained in the first search were used to start a second heuristic search that retained all best trees. Branch support was evaluated using 1,000 bootstrap replicates, collapsing groups with branch support values below 50. The BI analysis was used to estimate divergence times within section *Juniperus*, and of *J. brevifolia*. We implemented a relaxed molecular-clock approach in beast v.1.6.0 (Drummond *et al.* 2006, Drummond & Rambaut 2007), which simultaneously estimates phylogenetic relationships and node ages. The molecular clock analysis was carried out with two data partitions: (1) the *trn*L intron and *trn*L-*trn*F intergenic spacer dataset (as only two nucleotide substitution models were available in beast v.1.6.0, we used the HKY+G model as the closest to our dataset following the AIC criterion), and (2) indels from partition 1 coded as binary data (binary simple substitution model, Simmons & Ochoterena 2000) using the software SeqState 1.4.1 (Müller 2006). For the temporal calibration we used several divergence times formerly obtained by Mao *et al.* (2010): (1) the split between sections *Juniperus*-*Caryocedrus* (49.1–29.9 Ma), (2) the crown of section *Juniperus* (29.9–11.1 Ma), and (3) the crown of the 'blue seed cone group' (BSG) in section *Juniperus* (17.5–4.7 Ma). The substitution rate variation was modeled using an uncorrelated lognormal distribution, and a Birth-Death process (Gernhard 2008) was employed as tree prior. Two MCMC analyses were run for 10 million generations with a sample frequency of 1,000, and discarding the first 10% generations as burn-in. Analysis with TRACER 1.4 (Rambaut & Drummond 2007) confirmed adequate sample size, with ESS values above 200. Both analyses were combined using LOGCOMBINER 1.4.8, and trees were summarized in a maximum clade credibility tree obtained in TREEANOTATOR 1.4.8.

To infer connectivity between island populations of *J. brevifolia*, the *pet*N-*psb*M, *trn*S-*trn*G and *trn*T-*trn*L sequences obtained were concatenated, and a single analysis was performed based on the common inheritance without recombination that can be assumed for cpDNA markers (Birky 2001). One of the species (*J. oxycedrus*) most closely related to *J. brevifolia* according to the phylogenetic analysis was also included. Here, indels were also manually coded following Simmons & Ochoterena (Simmons & Ochoterena 2000). We performed a phylogeographic analysis based on the coalescence theory (Avise 2000). A statistical parsimony method (Templeton *et al.* 1992) implemented in the TCS 1.21 software (Clement *et al.* 2000) was used to infer genealogical relationships among haplotypes. The maximum number of differences resulting from single substitutions among haplotypes was calculated with 95% confidence limits, treating gaps as the fifth state.

The nearest-neighbor statistic (*Snn*) was calculated to assess genetic differentiation in *J. brevifolia*, as we expected isolation by distance among island groups, and also due to temporal differences in island emergence. This statistic is a measure of how often the ‘nearest neighbors’ (similar sequences) belong to the same pre-defined cluster (Hudson 2000). The closer *Snn* is to 1, the more differentiated are the populations within the partitions of a dataset; if *Snn* is close to 0.5, the partitions are construed as components of a single panmictic population. To detect the genetic differentiation attributable to geography, the combined *pet*N-*psb*M, *trn*S-*trn*G and *trn*T-*trn*L dataset of *J. brevifolia* was split into three according to the geographic island groups (Fig. 1). To assess the chronological component of genetic differentiation, the dataset was partitioned into two age groups: islands emerging before the Pleistocene (Santa Maria, São Miguel and Terceira; >2.5 Ma), and during the Pleistocene (Pico, Faial, São Jorge, Flores and Corvo; <2.5 Ma) (França *et al.* 2003). In both cases, *Snn* was calculated using DnaSP v5 (Librado & Rozas 2009), with indels previously coded. Permutation tests with 1,000 replicates were performed to evaluate significance, and the *Bonferroni* correction for multiple comparisons to control for the occurrence of Type I-error.

In order to determine the statistical relationship between genetic (*Fst*) and geographic distances (in km) between all possible population pairs, we ran a Mantel test (Mantel 1967) as implemented in genalex 6.3 (Peakall & Smouse 2006). We considered islands as single populations, and measured the distances between them using straight-line distances in ArcGis 9.3 (Environmental Systems Research Institute, Redlands, CA). *Fsts* were calculated using DnaSP v5 (Librado & Rozas 2009).

To assess the geospatial distribution and diffusion process of *J. brevifolia* through time, an additional beast analysis was performed with the *pet*N-*psb*M, *trn*S-*trn*G, *trn*T-*trn*L dataset. The molecular clock analysis was also carried in two data partitions: (1) the concatenated *pet*N-*psb*M, *trn*S-*trn*G, *trn*T-*trn*L dataset (HKY+G nucleotide substitution model), and (2) indels from the previous partition (1) coded as binary data (binary simple substitution model, Simmons & Ochoterena 2000) using the the software SeqState 1.4.1 (Müller 2006). For the temporal calibration, we set the diversification time of *J. brevifolia* as 8.27–0.69 Ma, obtained in the previous molecular dating analysis. Hence, we constrained the crown node of *J. brevifolia* to this age (mean 4.48, SD 2.305 with normal distribution). The substitution rate variation was modeled with an uncorrelated lognormal distribution, and a coalescent (constant size) tree prior was employed. Four MCMC analyses were run for 10 million generations, with a sample frequency of 1,000, discarding the first 10% generations as burn-in. We also confirmed adequate sample size after combining the four analyses with LogCombiner 1.4.8. Following the instructions described in http://beast.bio.ed.ac.uk, the beauti file (.xml) was modified assigning a fixed spatial location to each sample (here, eight locations for the ingroup, corresponding to each of the Azorean islands sampled). Finally, a discrete phylogeographic analysis was performed using a standard continuous-time Markov chain as described in Lemey *et al.* (2009). This analysis determined the probability distribution of the eight locations in the nodes of the Maximum clade credibility tree. A Bayesian stochastic variable selection model (BSSVS, which is an extension of the discrete phylogeographic model) using the Bayes Factor (BF) test, allowed us to achieve statistical significance for the rates of the dispersal events. Using a tool added to the BEAST code (RateIndicatorBF), we visualized the well-supported rates of dispersal in GOOGLE EARTH.

Results

Phylogenetic analyses and lineage divergence times

Phylogenetic relationships using *Juniperus* sequences of the *trn*L intron and the *trn*L-*trn*F region and Bayesian (BEAST) and ML analyses suggested the monophyly of *J. brevifolia*, albeit this result was not supported by the MP analysis (Fig. 2). The diversification time of *J. brevifolia* was estimated to occur at between 8.27–0.69 Ma. In addition, all three analyses pointed to a sister group of closely related species from Portugal (*J. navicularis*) and the eastern Mediterranean basin (*J. deltoides* and populations of *J. oxycedrus*). The divergence time between the clade of *J. brevifolia* + *J. navicularis* and its sister group was estimated at between 17.03 – 5.07 Ma (95% highest posterior density interval), in the upper Miocene – lower Pliocene.

Haplotype networking

Within *J. brevifolia*, we detected 16 haplotypes as a result of variation of *pet*N-*psb*M, *trn*S-*trn*G and *trn*T-*trn*L sequences in four nucleotide substitutions and seven gaps. The resulting indels (treated as a fifth character) were distributed as follows: in *pet*N-*psb*M, 14 bp between 216–229, 1 bp at position 563, 24 bp between positions 566–589; in

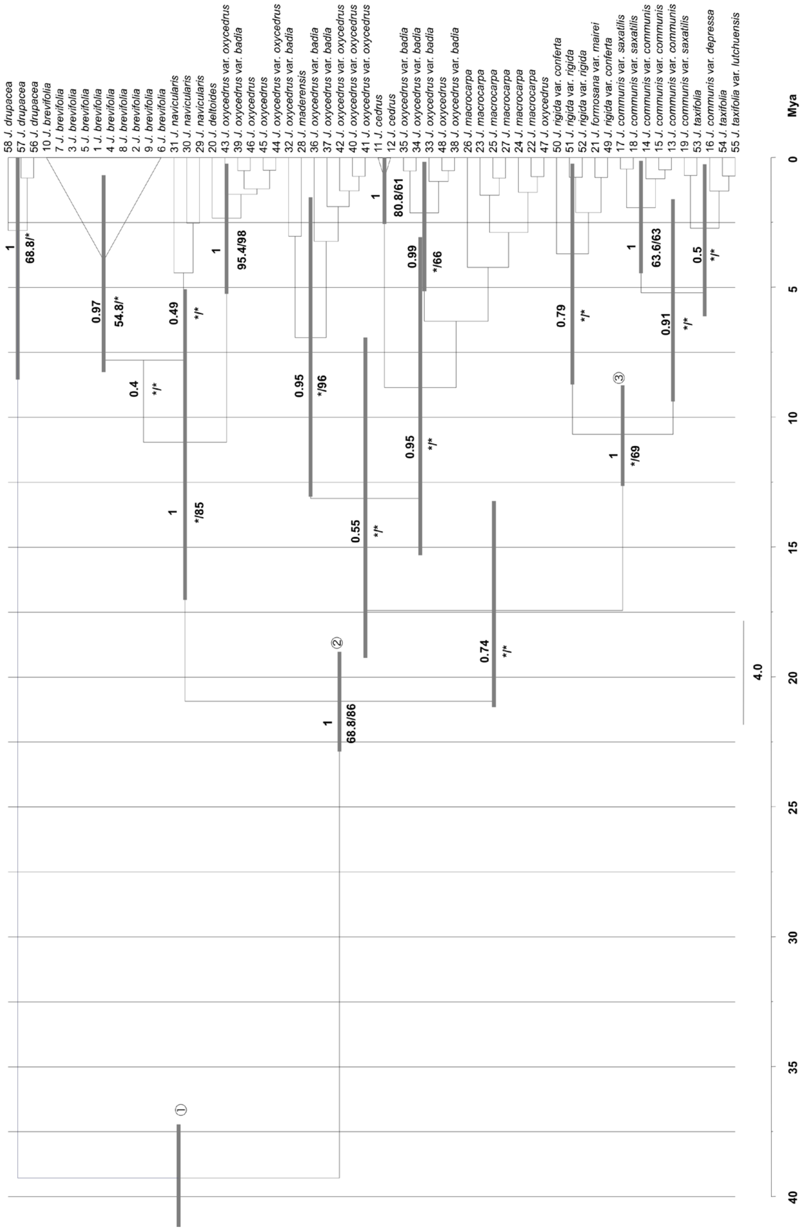

Fig. 2 Phylogenetic relationships within *Juniperus* section inferred from *trn*L and *trn*L-*trn*F, and divergence time-scale derived from BEAST. Numbers before taxon names refer to the coding no. given in Tables 1/S1. Numbers above branches are BEAST posterior probabilities; numbers below branches are Maximum Likelihood bootstrap support values (before slashes); and Maximum Parsimony bootstrap support values (after slashes). Asterisks indicate absence of support. Gray bars represent divergence times (95% highest posterior density intervals) for each node, while numbers in white circles represent calibration points obtained from Mao *et al.* (2010): (1) split between sects. *Juniperus-Caryocedrus* (49.1-29.9 Ma), (2) crown of sect. *Juniperus* (29.9-11.1 Ma) and (3) crown of BSG in sect. *Juniperus* (17.5-4.7 Ma). BEAST posterior probability values for calibration nodes were inferred from Mao et al. (2010).

*trn*S-*trn*G, 120 bp between 331–450; in *trn*T-*trn*L, 2 bp between 266–267, 1 bp at position 267 and 1 bp at 303. Table 2 shows the haplotype found in each locality; for the haplotype distribution of the 72 samples analyzed, see Table S2. The statistic parsimony analysis connected the outgroup accession of *J. oxycedrus* var. *oxycedrus* (haplotype 1) with the network of *J. brevifolia*, needing 14 mutation steps. Only two loops were retrieved, while two distinct clades (A and B) were obtained (Fig 3). The internal haplotypes 8 and 14 are widely distributed over the three island groups, whereas the other internal haplotypes are shared between the central and the western groups (5, 6, 16), and between the central and eastern groups (9). Six tip haplotypes are exclusive of a single island: Terceira (7, 13, 17), São Miguel (10, 11) and São Jorge (15). A lineage formed by haplotypes 9, 10, 11, 12 and 13 was clearly associated with the easternmost islands.

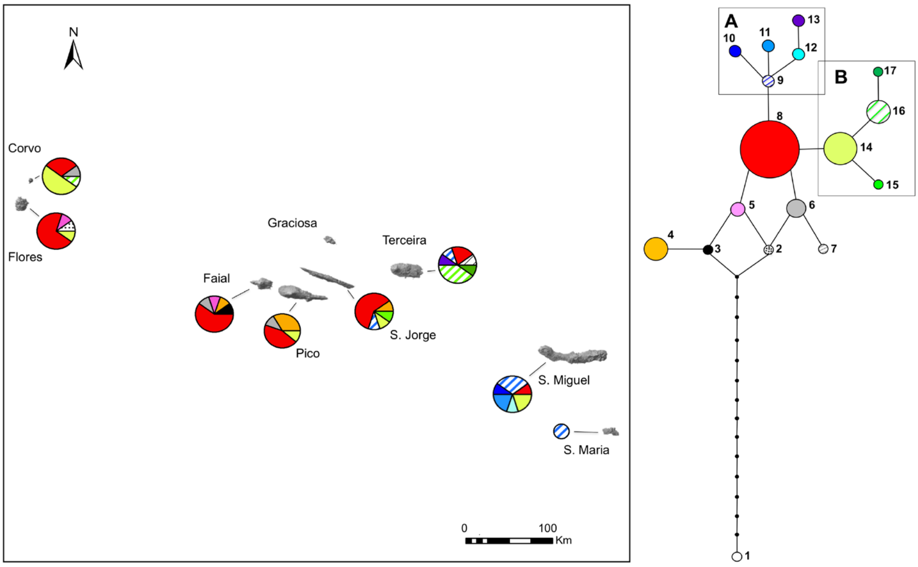

Fig. 3 cpDNA (*pet*N-*psb*M/*trn*S-*trn*G/*trn*T-*trn*L) haplotype network and its spatial distribution in the Azores archipelago. Each haplotype is represented by both a number and a color. Haplotype sizes are proportional to the number of individuals displaying them. Distinct clades (A and B) are shown within boxes.

Table 2 Plant material of *Juniperus brevifolia* used for sequencing *pet*N-*psb*M, *trn*S-*trn*G and *trn*T-*trn*L from 71 samples. *N* is the number of samples collected in each locality and *H* corresponds to the haplotypes found according to Fig. 3. Outgroup taxon (last row): *Juniperus oxycedrus* var. *oxycedrus*. See Table S2 for voucher source, haplotype number of each tree sampled, and the GenBank accession numbers of each haplotype found.

Geographical area/locality	N	H
Portugal, Azores, Santa Maria, Almagreira	2	9
Portugal, Azores, São Miguel, Monte Escuro	4	8, 11, 9
Portugal, Azores, São Miguel, Serra da Tronqueira	6	12, 9, 14, 10
Portugal, Azores, Terceira, Malha Grande	3	8, 16, 13
Portugal, Azores, Terceira, Pico Alto	3	8, 16, 7
Portugal, Azores, Terceira, Santa Bárbara	2	17, 9
Portugal, Azores, Terceira, Fajãzinha	2	16
Portugal, Azores, São Jorge, Bocas do Fogo	2	8, 9
Portugal, Azores, São Jorge, R.F. Pico da Esperança	4	14, 8, 4
Portugal, Azores, São Jorge, Serra do Topo	4	8, 15
Portugal, Azores, Pico, Cachorro	1	8
Portugal, Azores, Pico, Cerrado de Sonicas	1	14
Portugal, Azores, Pico, Montanha	1	4
Portugal, Azores, Pico, Baldios	1	4
Portugal, Azores, Pico, Lagoa do Capitão	1	8
Portugal, Azores, Pico, Curral Queimado-R.F. Prainha W	1	8
Portugal, Azores, Pico, Curral Queimado-R.F. Prainha	1	6
Portugal, Azores, Pico, Piquete do Caveiro W	1	4
Portugal, Azores, Pico, Manhenha	1	8
Portugal, Azores, Faial, Quebrada	2	8
Portugal, Azores, Faial, Grotão	1	8
Portugal, Azores, Faial, Caldeira	4	8, 6, 3
Portugal, Azores, Faial, Cabeço dos Trinta	3	4, 8, 5
Portugal, Azores, Flores, Lagoa	1	8
Portugal, Azores, Flores, Alto da Cova	2	8
Portugal, Azores, Flores, Pico da Casinha	1	8
Portugal, Azores, Flores, Caldeirães	1	8
Portugal, Azores, Flores, Morro Alto e Pico da Sé	2	5, 14
Portugal, Azores, Flores, Caldeira Funda e Rasa	2	2, 8
Portugal, Azores, Flores, Fajãzinha	1	8
Portugal, Azores, Corvo, Lomba Redonda	6	14, 8, 6
Portugal, Azores, Corvo, Cabeçeira	2	14, 16
Portugal, Azores, Corvo, Alqueve	2	14
OUTGROUP		
Greece, Lemo	1	1

psbA-trnH length polymorphism in J. brevifolia

Two different fragments of the *psb*A-*trn*H region were detected when analyzing the 367 *J. brevifolia* trees (Table 3), with lengths of 408 bp and

466 bp. None of the 99 trees from the western group, and only one of the 52 trees from the eastern group, showed the 408 bp amplicon. However, high levels of sequence length variation were detected in samples from the central group, where 28.6% of trees displayed the shorter fragment, and 71.4% the longer one. Across islands, the presence of the shorter amplicon in the samples from the central group was distributed as follows: 22.7% on Terceira, 14.0% São Jorge, 36% Pico and 16% Faial.

Table 3 *Juniperus brevifolia* individuals (n = 367) used to infer the amplicon length of the *psb*A-*trn*H region. *N*: number of individuals sampled in each locality. Voucher abbreviations: *BR*: B. Rumeu collection numbers as coded in the DNA Bank at the Jardín Botánico Canario 'Viera y Clavijo'- Unidad Asociada-CSIC. Length of the *psb*A-*trn*H amplicon is shown in the last column: number of individuals without brackets, amplicon length (bp) in brackets. Representative sequences of the two amplicon types of different lengths were deposited in the GenBank (JF951047, 408 bp; JF951048, 466 bp)

Geographical area/Locality	N	Vouchers	*psb*A-*trn*H amplicon length (bp)
Azores, Santa Maria, Almagreira	2	BR4710, BR4711	2 (466)
Azores, São Miguel, Monte Escuro	15	BR4494-BR4508	15 (466)
Azores, São Miguel, Serra da Tronqueira	35	BR4509-BR4543	1 (408); 34 (466)
Azores, Terceira, Malha Grande	20	BR4544-BR4563	5 (408); 15 (466)
Azores, Terceira, Pico Alto	20	BR4564-BR4583	3 (408); 17 (466)
Azores, Terceira, Santa Barbara	20	BR4584-BR4603	6 (408); 14 (466)
Azores, Terceira, Fajãzinha	6	BR4604-BR4608	1 (408); 5 (466)
Azores, São Jorge, Bocas do Fogo	2	BR4444, BR4445	2 (466)
Azores, São Jorge, R. F. Pico da Esperança	21	BR4446-BR4466	3 (408); 18 (466)
Azores, São Jorge, Serra do Topo	27	BR4467-BR4493	4 (408); 23 (466)
Azores, Pico, Cachorro	4	BR4344-BR4347	2 (408); 2 (466)
Azores, Pico, Cerrado dos Sonicas	8	BR4348-BR4353; BR4359-BR4360	4 (408); 4 (466)
Azores, Pico, Montanha	5	BR4354-BR4358	4 (408); 1 (466)
Azores, Pico, Baldios	2	BR4361-BR4362	1 (408); 1 (466)
Azores, Pico, Lagoa do Capitão	9	BR4363-BR4371	2 (408); 7 (466)
Azores, Pico, Curral Queimado-R. F. Prainha W	2	BR4372-BR4373	2 (408)
Azores, Pico, Curral Queimado-R. F. Prainha	5	BR4374-BR4378	3 (408); 2 (466)
Azores, Pico, Curral Queimado-R. F. Prainha E	2	BR4379-BR4380	2 (466)
Azores, Pico, Piquete do Caveiro W	6	BR4381-BR4386	1 (408); 5 (466)
Azores, Pico, Piquete do Caveiro E	2	BR4387-BR4388	2 (466)
Azores, Pico, Piquete do Caveiro S	1	BR4389	1 (466)
Azores, Pico, Manhenha	4	BR4390-BR4393	4 (466)
Azores, Faial, Quebrada	10	BR4395-BR4402	1 (408); 9 (466)
Azores, Faial, Grotão	4	BR4404-BR4406; BR4424	4 (466)
Azores, Faial, Caldeira	17	BR4497-BR4423	3 (408); 14 (466)
Azores, Faial, Cabeço dos Trinta	19	BR4425-BR4443	4 (408); 15 (466)
Azores, Flores, Lagoa	1	BR4660	1 (466)

Table 3 (Continued)

Geographical area/Locality	N	Vouchers	*psb*A-*trn*H amplicon length (bp)
Azores, Flores, Alto da Cova	18	BR4661-BR4678	1 (408); 17 (466)
Azores, Flores, Pico da Casinha	5	BR4679-BR4683	5 (466)
Azores, Flores, Caldeirães	2	BR4684-BR4685	2 (466)
Azores, Flores, Morro Alto e Pico da Sé	7	BR4686-BR4692	7 (466)
Azores, Flores, Caldeira Funda e Rasa	8	BR4693-BR4700	8 (466)
Azores, Flores, Fajãzinha	9	BR4701-BR4709	9 (466)
Azores, Corvo, Lomba Redonda	27	BR4610-BR4637	27 (466)
Azores, Corvo, Cabeceira	17	BR4638-4654	17 (466)
Azores, Corvo, Alqueve	5	BR4655-4659	5 (466)

Genetic differentiation analysis and Mantel Test

Values of *Snn* (Table 4) rendered significant results when comparing the genetic differentiation of the three island groups (eastern, central and western). We detected a highly significant genetic differentiation of eastern *J. brevifolia* populations with respect to the rest of the archipelago. However, no significant differences were found when comparing the two other island groups with the rest of the archipelago. There was also a highly significant genetic differentiation associated with the ages of the islands predating the Pleistocene *vs*. those that emerged during this period.

Table 4 Genetic differentiation associated with isolation according to island group distances and island ages. Nearest-neighbor statistic (*Snn*) values calculated from the combined *pet*N-*psb*M, *trn*S-*trn*G and *trn*T-*trn*L dataset of *J. brevifolia*. Significance levels in brackets were those obtained after *Bonferroni* correction.

	Snn
Isolation by island group distances	
Eastern group – Central group – Western group	0.52, $p = 0.002$**
Eastern group – Rest	0.85, $p < 0.001$(***)
Central group – Rest	0.57, $p = 0.032$ (ns)
Western group – Rest	0.62, $p = 0.109$ (ns)
Isolation by island ages	
Predating Pleistocene – Postdating Pleistocene	0.79, $p < 0.001$***

ns, not significant; *, $0.01 < p < 0.05$; **, $0.001 < p < 0.01$; ***, $p < 0.001$
(Significant level after *Bonferroni* correction = 0.017)

The Mantel test between geographic distance and *FST* values revealed a weak but significant isolation-by-island distance effect, indicated by a low relationship between geographic distance and global *FST* values (1,000 permutations, $R^2 = 0.38$, $p = 0.001$) (Table 5).

Table 5 Population pairwise *FST* estimates based on haplotype sequences (above the diagonal) and geographic distances (in km, below the diagonal). Islands have been considered as single *Juniperus brevifolia* populations and are abbreviated as follows: MA, Santa Maria; SM, São Miguel; TE, Terceira; JO, São Jorge; PI, Pico; FA, Faial; FL, Flores; CO, Corvo.

	MA	SM	TE	JO	PI	FA	FL	CO
MA	-	0.123	0.473	0.630	0.696	0.696	0.730	0.741
SM	92	-	0.200	0.188	0.304	0.309	0.297	0.325
TE	270	198	-	0.129	0.198	0.228	0.189	0.047
JO	306	245	60	-	-0.006	0.028	0.005	0.110
PI	325	271	95	36	-	-0.031	0.067	0.218
FA	362	308	127	68	38	-	-0.042	0.280
FL	599	543	350	298	274	236	-	0.203
CO	603	543	348	299	277	240	30	-

Negative values should be interpreted as no genetic differentiation between populations from the two islands and likely reflects the imprecision of the algorithm used by the software to estimate this value.

Discrete Phylogeographic Analysis

The Bayesian maximum clade credibility tree of the *pet*N-*psb*M, *trn*S-*trn*G and *trn*T-*trn*L dataset showed a considerably uncertainty in the geographic origin of *J. brevifolia* (Fig. 4). For the most ancestral supported node, a wide diversification time window of 4.99–0.65 Ma was estimated (data not shown). The only highly supported lineages indicated a most probable ancestral range in São Miguel for haplotypes 9, 10, 11, 12 and 13, and in Terceira for haplotypes 16 and 17. Six main migration routes were supported by the BF test (Fig 4): Terceira-Corvo (*BF* = 11.5), São Miguel-Terceira (*BF* = 11.34), Pico-Faial (*BF* = 9.75), Faial-Flores (*BF* = 9.62), Santa Maria-São Miguel (BF = 6.29), Pico-São Jorge (BF = 5.92) and São Jorge-Corvo (*BF* = 4.14). Both analyses support (1) the importance of Terceira as a stepping-stone island within the Azores, and (2) an east-west colonization of the haplotypes 9 and 16.

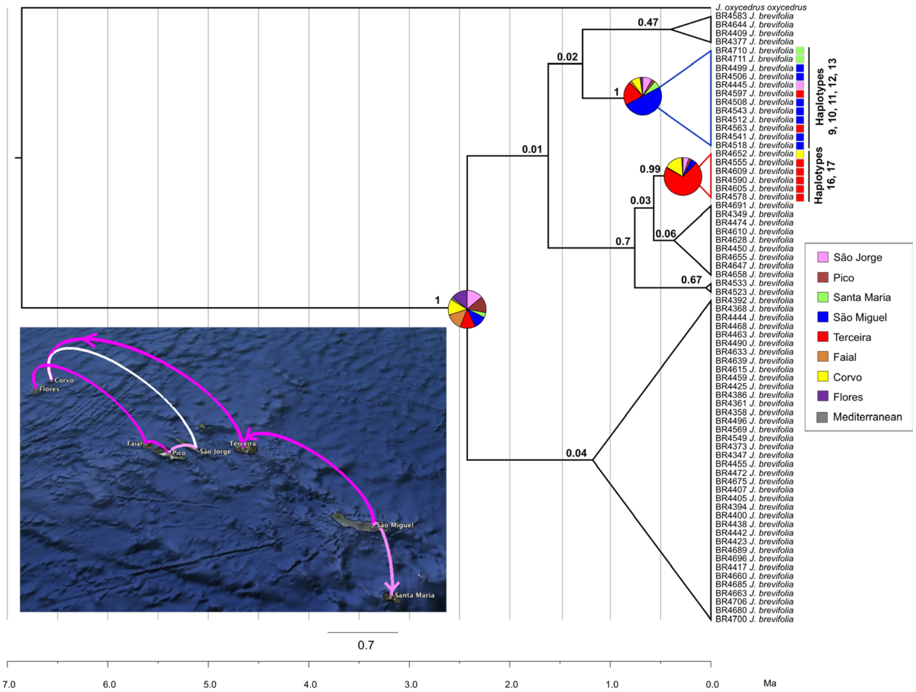

Fig. 4 Relaxed molecular-clock chronogram and phylogeographic reconstruction of *Juniperus brevifolia*. Maximum clade credibility tree summarized from the geospatial Bayesian analysis of cpDNA (*pet*N-*psb*M, *trn*S-*trn*G and *trn*T-*trn*L sequences) of 71 individuals of *J. brevifolia*. Pie charts represent posterior probability distributions of ancestral range at well-supported nodes of interest. Colored rectangles represent the sample's island of origin. The haplotype relatedness is also shown in the well-supported clades. Colonization routes supported by a *BF*>3 are shown on the map. The color of each route represents its relative support, with more intense colors indicating stronger support. Arrows specify directionality in the colonization route, inferred from well-supported nodes of interest in the geospatial Bayesian analysis. The map is based on satellite images available in Google Earth (http://earth.google.com).

Discussion

The results support a hypothesis in which a single introduction event likely from Europe, followed by inter-island dispersal, explains best the current distribution of *J. brevifolia*. However, DNA sequence data failed to support a clear sister-group relationship hypothesis, which prevented from estimating a particular time of colonization from the continent. This colonization may have been favored by the occurrence of several ancient islands (60–0.018 Ma), that could have acted as stepping-stones between continental Europe and the Azorean islands (Fernández-Palacios *et al.*

2011). Nevertheless, divergence time estimates revealed that genetic differentiation of *J. brevifolia* postdated the emergence of the oldest island of Santa Maria (8.12 Ma, França *et al.* 2003).

High diversity and dynamic colonization on the oldest islands

Apart from Santa Maria, which has been dramatically deforested for centuries, and where only two trees could be found and sampled, a high diversity of haplotypes has been detected in the remaining seven islands. The easternmost Azorean islands harbor the highest diversity levels with six haplotypes (São Miguel, Terceira), followed by São Jorge and Faial with five, and the remaining three islands with four. These findings agree with Carine & Schaefer's (2010) hypothesis of the 'Azorean diversity enigma', whereby most of the endemics are widespread across the archipelago. Moreover, they also agree with Schaefer *et al.* (2011b), who show that a range of Azorean endemic plant lineages contain high levels of intra-specific genetic variation comparable to (or even higher than) those found among the abundant congeneric single island endemics from the Canary Islands (e.g. Rumeu *et al.* for the Canarian juniper, unpublished).

The key role of São Miguel and Terceira in the diversification of *J. brevifolia* is also evidenced by the Discrete Phylogeographic Analysis, which points to these two islands as the source of seven different haplotypes (Fig. 4). Notably, São Miguel and Terceira are the oldest islands (both predating the Pleistocene) with still large populations and also the closest to the continent, which suggests the hypothesis that either age or distance from the continent have been of paramount importance for early dispersal and establishment of *J. brevifolia* in the Azores. A similar positive correlation between genetic diversity and island age or proximity to the continent has been detected for the Canaries based on allozyme diversity, thus far the largest population genetics database for these islands' flora (Caujapé-Castells 2011). Results derived from our *Snn* analysis revealed significant genetic differentiation due to isolation-by-distance of the eastern group of the Azores with respect to the other two island groups, and also on a time-scale (i. e., considering whether islands emerged before or during the Pleistocene). However, temporal isolation rather than isolation-by-distance from the continent appears to have played a more determinant role for the first colonization, given similar geographic distances between island groups (Santa Maria-São Miguel, Terceira-São Jorge-Pico-Faial) and the mainland in a large scale.

Combining these results with those obtained by the BF test, it is possible to underscore the importance of Terceira in fostering genetic connectivity within the archipelago. Despite the migration route from Terceira to Corvo being well supported by the Discrete Phylogeographic Analysis and the BF test, it is also important to note that Graciosa is located on this route. Although *J. brevifolia* is extinct in Graciosa, this island may have constituted a stepping stone connecting the western group. The additional analysis of the *psb*A-*trn*H length polymorphism in 367 juniper trees also reflects a high dispersibility of *J. brevifolia* among the central islands and, to a lesser extent, some connectivity between the central and the eastern groups, which implies hopping a minimum distance of 139 km. In particular, the great connectivity among the central group may have had its peak during the colder phase of the last glaciation (~18,000 years BP), when the sea level dropped more than 100 m (Kawamura *et al.* 2007, Sima *et al.* 2004) and Pico and Faial formed a single landmass (Borges & Brown 1999, Martins 1993).

The ease of colonization by the Azorean juniper is evidenced by its widespread range (all the islands except for Graciosa, where it is extinct), and has been reflected by the widespread distribution of ancestral haplotypes. This scenario correlates with recurrent inter-island colonization over long periods of time despite considerable geographic distances between island groups, and supports the hypothesis that Azorean endemic lineages have maintained substantial gene flow between islands (Cardoso *et al.* 2010). However, this hypothesis has been recently challenged by results reported by Schaefer *et al.* (2011b), who analyzed the molecular variation of five endemic lineages (*Ammi*, *Euphorbia stygiana*, *Angelica lignescens*, *Azorina vidalii* and *Pericallis malviflora*) within the Azores range, and suggested that dispersal-mediated allopatry has been an extensive process in the archipelago, and considerable distances between some of the islands or island groups are effective barriers to gene flow.

Overall, the total proportion of single-island haplotypes displayed by *J. brevifolia* (50%) was lower than that observed by Schaefer *et al.* (2011b) for the five endemic lineages studied (71%), which indicates higher connectivity of the Azorean junipers. In contrast, significant distribution of ancestral haplotypes of *J. brevifolia* parallels the widespread haplotype distribution of *Picconia azorica* across the islands (Ferreira *et al.* 2011), another wind pollinated and endozoochorous tree species endemic to the Azores (Dias *et al.* 2007, Ferreira *et al.* 2011). Using ISSRs and RAPDs, Silva *et al.* (2011) also found that the largest portion of the *J. brevifolia* genetic variability resides within populations and among populations within islands, whereas the between island component is irrelevant. These results, together with the weak isolation-by-distance detected for *J. brevifolia* with the Mantel test, adds up to the idea of recurrent gene flow between island groups, supporting that the significant genetic differentiation observed is best interpreted by the temporal sequence of island emergence.

Limited colonization of recent lineages

The widespread distribution of the ancestral haplotypes in the network may entail dispersal soon after *J. brevifolia* colonized the archipelago (Fig. 3), and furnishes molecular evidence for colonization related to island ages in the Azores. In contrast, six of the seven recent-most haplotypes are restricted to a single island, indicating that they may not have had enough time for inter-island dispersal. This pattern needs to be further investigated in the flora of the Azores given that a widespread distribution of ancient haplotypes, as opposed to a geographic restriction of the most recent (derived) ones, is also featured by *Picconia azorica* (Ferreira *et al.* 2011). Alternatively, new colonization may have been prevented by the presence of already established junipers containing ancestral haplotypes. This finding is related to the 'Darwin's naturalization hypothesis', as recently tested by Schaefer *et al.* (2011a) for the Azorean flora. This hypothesis proposes that naturalization is more likely for aliens with no close relatives in the new land, due to lack of competitive exclusion (Darwin 1859) i. e. closely related species are more likely to have similar ecological niches due to common ancestry, and therefore would be competing for the same resources. Extending this hypothesis not only for closely related species but also for intraspecific lineages, our results suggest that colonization of *J. brevifolia* could have been hindered by the presence of early juniper lineages already occupying a similar ecological niche. Thus, restriction of six recent haplotypes to single islands may be due to the occurrence of habitat competition with early lineages or plant traits unfavorable for long-distance dispersal in relatively short periods of time.

Traits promoting long-distance dispersal

The reproductive traits of *J. brevifolia* appear to have been favorable for long-distance dispersal. Feasibly, pollen flow among the present islands may have been relatively dynamic because of the prevailing winds. The Azores are usually under the influence of either tropical or polar maritime air masses, as a consequence of the seasonal drifting of the high-pressure Azores Anticyclone (Andrade *et al.* 2008). Furthermore, whenever the high-pressure center is dissipated or displaced, a polar atmospheric front shifts southwards, and several low-pressure fronts may sweep the whole archipelago. During the extended winter (October to March), the Azores region is frequently crossed by the North Atlantic storm-track (Andrade *et al.* 2008). As pollen shed occurs mainly during spring, the strong winds still frequent at this season could move pollen over long distances (Burrows 1975). On the other hand, as *J. brevifolia* presents fleshy female cones edible for passerine birds, the gene flow estimated in this paper could be also due to the long-distance dispersal of seeds. A recent study on this juniper's seed dispersal system (Rumeu *et al.* 2011) revealed that birds, mainly blackbirds (*Turdus merula*) and blackcap warblers (*Sylvia atricapilla*), are active dispersal agents (frequency of occurrence of seeds in droppings: 81.1% and 6.1%, respectively). The question remains as to whether these birds are responsible for distribution of junipers in the Azores islands. Gut passage times are clearly different between them, *T. merula*'s being longer because of its larger size; consequently, this species defecates the seeds instead of regurgitating them, as it often happens in the smaller *S. atricapilla*. Since the emergence of the islands, successive and occasionally very explosive eruptions (e.g. in São Miguel, Terceira and Faial, Walker 1973) also may have promoted the movement of birds among islands in the attempt to escape from these disturbances. Furthermore, strong winds caused by the North Atlantic storm-track (Andrade *et al.* 2008), may have promoted bird dispersal within the Azores archipelago. Therefore, blackbirds could have been largely responsible for the seed movements of at least the recent haplotypes.

Concluding remarks

In summary, successful gene flow through pollen (anemophily) and seeds (ornithochory), may have promoted a relatively dynamic colonization by early junipers followed by a more parsimonious establishment of lineages. In addition, colonization success also depends on environmental suitability (Carlquist 1965, Whittaker & Fernández-Palacios 2007), and the ability of the species to thrive in the habitat reached. In this respect, the Azores archipelago presents a remarkable habitat homogeneity and climatic stability –at least over the past 6,000 years (Carine & Schaefer 2010)–, and *J. brevifolia* has been described as a pioneer species with a broad ecological range (0–1500 m a.s.l.), capable of colonizing recent substrates (Elias 2007, Elias & Dias 2004). In the long term, both ecological factors may have facilitated the establishment of new propagules, and thereby contributed to the successful colonization of *J. brevifolia* since island formation.

Supporting information

Table S1. Taxon names, geographical area, locality, voucher information and GeneBank accession numbers for the *Juniperus* samples included in the phylogenetic analysis. Coding numbers as in Fig. 2. Voucher abbreviations: *BR*: B. Rumeu collection numbers, as coded in the DNA Bank at the Jardín Botánico Canario 'Viera y Clavijo'-Unidad Asociada CSIC; *JM*: J. Martínez voucher numbers; *MA*: herbarium of the Royal Botanic Garden of Madrid; *E*: Royal Botanic Garden, Edinburgh, Scotland, U.K. Taxonomy follows that of Adams *et al.* 2011.

Table S2. Plant material used for sequencing *pet*N-*psb*M, *trn*S-*trn*G and *trn*T-*trn*L from 72 samples. *N* is the number of samples collected in each locality, voucher source indicates each tree sampled and *H* is the haplotype found. Voucher abbreviations: *BR*: B. Rumeu collection numbers, as coded in the

DNA Bank at the Jardín Botánico Canario 'Viera y Clavijo'- Unidad Asociada CSIC. Sequences of each haplotype were deposited in the GenBank.

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Table S1. Taxon names, geographical area, locality, voucher information and GeneBank accession numbers for the *Juniperus* samples included in the phylogenetic analysis. Coding numbers as in Fig. 2. Voucher abbreviations: *BR*: B. Rumeu collection numbers, as coded in the DNA Bank at the Jardín Botánico Canario 'Viera y Clavijo'-Unidad Asociada CSIC; *JM*: J. Martínez voucher numbers; *MA*: herbarium of the Royal Botanic Garden of Madrid; *E*: Royal Botanic Garden, Edinburgh, Scotland, U.K. Taxonomy follows that of Adams *et al.* 2011.

Coding no.	Taxon	Origin/Voucher	GenBank
1	*J. brevifolia*	Portugal, Azores, Santa Maria, Almagreira, BR4710	JF950948
2	*J. brevifolia*	Portugal, Azores, São Miguel, Serra da Tronqueira, BR4512	JF950949
3	*J. brevifolia*	Portugal, Azores, Terceira, Malha Grande, BR4546	JF950950
4	*J. brevifolia*	Portugal, Azores, São Jorge, R.F. Pico da Esperança, BR4450	JF950951
5	*J. brevifolia*	Portugal, Azores, Pico, Cachorro, BR4347	JF950952
6	*J. brevifolia*	Portugal, Azores, Pico, Lagoa do Capitão, Fiz, O. (Martínez & Vargas in prep.)	JF950953
7	*J. brevifolia*	Portugal, Azores, Faial, Caldeira, BR4415	JF950954
8	*J. brevifolia*	Portugal, Azores, Faial, Caldeira, Fiz, O. (Martínez & Vargas in prep.)	JF950955
9	*J. brevifolia*	Portugal, Azores, Flores, Alto da Cova, BR4663	JF950956
10	*J. brevifolia*	Portugal, Azores, Corvo, Lomba Redonda, BR4615	JF950957
11	*J. cedrus*	Spain, Canary Islands, Tenerife, La Orotava, Martínez, J. (Martínez & Vargas in prep.)	JF950958
12	*J. cedrus*	Spain, Canary Islands, La Palma, Pared de Roberto, BR5000	JF950959
13	*J. communis* var. *communis*	Spain, Burgos, Covarrubias, Vargas, P. (Martínez & Vargas in prep.)	JF950960
14	*J. communis* var. *communis*	Spain, Granada, Pico Trevenque, MA612184 (Martínez & Vargas in prep.)	JF950961
15	*J. communis* var. *communis*	France, WO-2111 (Mao *et al.* 2010)	HM024557
16	*J. communis* var. *depressa*	USA, New Mexico, BU-10936 (Mao *et al.* 2010)	HM024558
17	*J. communis* var. *saxatilis*	Spain, Madrid, La Pedriza, Martínez, J (Martínez & Vargas in prep.)	JF950962
18	*J. communis* var. *saxatilis*	Georgia, Caucasus, MA576696 (Martínez & Vargas in prep.)	JF950963
19	*J. communis* var. *saxatilis*	Pakistan, G.S.Miehe-3011 (Mao *et al.* 2010)	HM024559
20	*J. deltoides*	Turkey, WO-2002 (Mao *et al.* 2010)	HM024561
21	*J. formosana* var. *mairei*	China, Gansu, J.Q.Liu-1469 (Mao *et al.* 2010)	HM024568
22	*J. macrocarpa*	Spain, Cádiz, Barbate, Trafalgar Cape, Nieto, G.& Fuertes, J. (Martínez & Vargas in prep.)	JF950964
23	*J. macrocarpa*	Spain, Cádiz, Tarifa, JM5E8	JF950965

Table S1 (Continued)

Coding no.	Taxon	Origin/Voucher	GenBank
24	*J. macrocarpa*	Spain, Valencia, El Saler, Martínez, J. (Martínez & Vargas in prep.)	JF950966
25	*J. macrocarpa*	Spain, Cádiz, Chiclana, Eliçabe, L.M. (Martínez & Vargas in prep.)	JF950967
26	*J. macrocarpa*	Italy, Sardinia, Santa Teresa Gallura, Nieto, G.& Fuertes, J. (Martínez & Vargas in prep.)	JF950968
27	*J. macrocarpa*	Italy, Sicily, Ragusa, MA645785 (Martínez & Vargas in prep.)	JF950969
28	*J. maderensis*	Madeira, Fajã da Nogueira, BR6174	JF950970
29	*J. navicularis*	Portugal, Apostiça (Martínez & Vargas in prep.)	JF950971
30	*J. navicularis*	Portugal, Apostiça (Martínez & Vargas in prep.)	JF950972
31	*J. navicularis*	Portugal, Estremadura, MA565189 (Martínez & Vargas in prep.)	JF950973
32	*J. oxycedrus* var. *badia*	Spain, Huesca, Sierra de Guara, Vargas, P (Martínez & Vargas in prep.)	JF950974
33	*J. oxycedrus* var. *badia*	Spain, Madrid, Villalba, Martínez, J. (Martínez & Vargas in prep.)	JF950975
34	*J. oxycedrus* var. *badia*	Spain, Ciudad Real, Puebla de Don Rodrigo, MA615745 (Martínez & Vargas in prep.)	JF950976
35	*J. oxycedrus* var. *badia*	Spain, Jaén (Martínez & Vargas in prep.)	JF950977
36	*J. oxycedrus* var. *badia*	Spain, Jaén (Martínez & Vargas in prep.)	JF950978
37	*J. oxycedrus* var. *badia*	Morocco, Kjbel Kelti, Pérez, R. (Martínez & Vargas in prep.)	JF950979
38	*J. oxycedrus* var. *badia*	Morocco, Kjbel Kelti, Pérez, R. (Martínez & Vargas in prep.)	JF950980
39	*J. oxycedrus* var. *badia*	Turkey, Gümüshane, Torul, Herrero, A. *et al.* (Martínez & Vargas in prep.)	JF950981
40	*J. oxycedrus* var. *oxycedrus*	Spain, Granada, El Peñón, JM1E7	JF950982
41	*J. oxycedrus* var. *oxycedrus*	Spain, Granada, El Peñón, JM4E8	JF950983
42	*J. oxycedrus* var. *oxycedrus*	Spain, Balearic Islands, Menorca, Martínez, J. (Martínez & Vargas in prep.)	JF950984
43	*J. oxycedrus* var. *oxycedrus*	Greece, Lemo (Martínez & Vargas in prep.)	JF950985
44	*J. oxycedrus* var. *oxycedrus*	Greece, Lemo (Martínez & Vargas in prep.)	JF950986
45	*J. oxycedrus*	Turkey, Istanbul, Kartal, EOO138135 (Martínez & Vargas in prep.)	JF950987
46	*J. oxycedrus*	Greece, Kalavryta, Diakoftó, Vargas, P. (Martínez & Vargas in prep.)	JF950988
47	*J. oxycedrus*	Tunisia, Cap Bon, Sidi Daoud, Aldasoro *et al.* (Martínez & Vargas in prep.)	JF950989
48	*J. oxycedrus*	France, WO-2113 (Mao *et al.* 2010)	HM024581
49	*J. rigida* var. *conferta*	Cultivated (Spain, Pontevedra, Lourizán), MA547007 (Martínez & Vargas in prep.)	JF950990
50	*J. rigida* var. *conferta*	Japan, J.Q.Liu-TBG-JRIC (Mao *et al.* 2010)	HM024591

Table S1 (Continued)

Coding no.	**Taxon**	**Origin/Voucher**	**GenBank**
51	*J. rigida* var. *rigida*	Japan (Martínez & Vargas in prep.)	JF950991
52	*J. rigida* var. *rigida*	Japan, J.Q.Liu-TBG-JRIR (Mao *et al.* 2010)	HM024592
53	*J. taxifolia*	Japan, J.Q.Liu-TBG-JTA (Mao *et al.* 2010)	HM024602
54	*J. taxifolia*	Japan, Bonin Island (Martínez & Vargas in prep.)	JF950992
55	*J. taxifolia* var. *lutchuensis*	Japan (Martínez & Vargas in prep.)	JF950993
	OUTGROUP		
56	*J. drupacea*	Greece (Martínez & Vargas in prep.)	JF950994
57	*J. drupacea*	Greece (Martínez & Vargas in prep.)	JF950995
58	*J. drupacea*	Greece, BU-5651 (Mao *et al.* 2010)	HM024563

Table S2. Plant material used for sequencing *pet*N-*psb*M, *trn*S-*trn*G and *trn*T-*trn*L from 72 samples. *N* is the number of samples collected in each locality, voucher source indicates each tree sampled and *H* is the haplotype found. Voucher abbreviations: *BR*: B. Rumeu collection numbers, as coded in the DNA Bank at the Jardín Botánico Canario 'Viera y Clavijo'- Unidad Asociada CSIC. Sequences of each haplotype were deposited in the GenBank.

Geographical area/locality	**N**	**Voucher-source**	**H**	**GenBank accession no. (*pet*N-*psb*M / *trn*S-*trn*G / *trn*T-*trn*L)**		
Portugal, Azores, Santa Maria, Almagreira	2	BR4710, BR4711	9	JF951004	JF951025	JF951039
Portugal, Azores, São Miguel, Monte Escuro	4	BR4496	8	JF951003	JF951020	JF951038
		BR4499, BR4506	11	JF951006	JF951026	JF951042
		BR4508	9	–	–	–
Portugal, Azores, São Miguel, Serra da Tronqueira	6	BR4512	12	JF951007	JF951027	JF951041
		BR4518, BR4541	9	–	–	–
		BR4523, BR4533	14	JF951009	JF951021	JF951043
		BR4543	10	JF951005	JF951029	JF951040
Portugal, Azores, Terceira, Malha Grande	3	BR4549	8	–	–	–
		BR4555	16	JF951011	JF951023	JF951045
		BR4563	13	JF951008	JF951028	JF951034
Portugal, Azores, Terceira, Pico Alto	3	BR4569	8	–	–	–
		BR4578	16	–	–	–
		BR4583	7	JF951002	JF951019	JF951033
Portugal, Azores, Terceira, Santa Bárbara	2	BR4590	17	JF951012	JF951024	JF951046
		BR4597	9	–	–	–
Portugal, Azores, Terceira, Fajãzinha	2	BR4605, BR4609	16	–	–	–
Portugal, Azores, São Jorge, Bocas do Fogo	2	BR4444	8	–	–	–
		BR4445	9	–	–	–
Portugal, Azores, São Jorge, R.F. Pico da Esperança	4	BR4450	14	–	–	–
		BR4455, BR4463	8	–	–	–
		BR4459	4	JF950999	JF951016	JF951036
Portugal, Azores, São Jorge, Serra do Topo	4	BR4468, BR4472, BR4490	8	–	–	–
		BR4474	15	JF951010	JF951022	JF951044
Portugal, Azores, Pico, Cachorro	1	BR4347	8	–	–	–

Table S2 (Continued)

Geographical area/locality	N	Voucher-source	H	GenBank accession no. (*pet*N-*psb*M / *trn*S-*trn*G / *trn*T-*trn*L)		
Portugal, Azores, Pico, Cerrado de Sonicas	1	BR4349	14	–	–	–
Portugal, Azores, Pico, Montanha	1	BR4358	4	–	–	–
Portugal, Azores, Pico, Baldios	1	BR4361	4	–	–	–
Portugal, Azores, Pico, Lagoa do Capitão	1	BR4368	8	–	–	–
Portugal, Azores, Pico, Curral Queimado-R.F. Prainha W	1	BR4373	8	–	–	–
Portugal, Azores, Pico, Curral Queimado-R.F. Prainha	1	BR4377	6	JF951001	JF951018	JF951032
Portugal, Azores, Pico, Piquete do Caveiro W	1	BR4386	4	–	–	–
Portugal, Azores, Pico, Manhenha	1	BR4392	8	–	–	–
Portugal, Azores, Faial, Quebrada	2	BR4394, BR4400	8	–	–	–
Portugal, Azores, Faial, Grotão	1	BR4405	8	–	–	–
Portugal, Azores, Faial, Caldeira	4	BR4407, BR4417	8	–	–	–
		BR4409	6	–	–	–
		BR4423	3	JF950998	JF951015	JF951035
Portugal, Azores, Faial, Cabeço dos Trinta	3	BR4425	4	–	–	–
		BR4438	8	–	–	–
		BR4442	5	–	–	–
Portugal, Azores, Flores, Lagoa	1	BR4660	8	–	–	–
Portugal, Azores, Flores, Alto da Cova	2	BR4663, BR4675	8	–	–	–
Portugal, Azores, Flores, Pico da Casinha	1	BR4680	8	–	–	–
Portugal, Azores, Flores, Caldeirães	1	BR4685	8	–	–	–
Portugal, Azores, Flores, Morro Alto e Pico da Sé	2	BR4689	5	JF951000	JF951017	JF951037
		BR4691	14	–	–	–
Portugal, Azores, Flores, Caldeira Funda e Rasa	2	BR4696	2	JF950997	JF951014	JF951031
		BR4700	8	–	–	–

Table S2 (Continued)

Geographical area/locality	N	Voucher-source	H	GenBank accession no. (*pet*N-*psb*M / *trn*S-*trn*G / *trn*T-*trn*L)		
Portugal, Azores, Flores, Fajãzinha	1	BR4706	8	–	–	–
Portugal, Azores, Corvo, Lomba Redonda	6	BR4610, BR4628	14	–	–	–
		BR4615, BR4633, BR4639	8	–	–	–
		BR4644	6	–	–	–
Portugal, Azores, Corvo, Cabeçeira	2	BR4647	14	–	–	–
		BR4652	16	–	–	–
Portugal, Azores, Corvo, Alqueve	2	BR4655, BR4658	14	–	–	–
OUTGROUP						
Greece, Lemo	1	Martínez & Vargas in prep.	1	JF950996	JF951013	JF951030

V

Origin and genetic differentiation of the Canarian endangered *Juniperus cedrus* (Cupressaceae)

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Abstract

Aim: The strong human-mediated fragmentation has driven the natural populations of the Canarian endemic juniper (*Juniperus cedrus*) to a very restricted distribution mainly in craggy inaccessible areas. Here we use data from four plastid DNA regions and amplified fragment length polymorphism (AFLP) to infer the origin and diversification of *J. cedrus* and to contribute to better-informed management.

Location: The Canary Islands oceanic archipelago.

Methods: Phylogenetic relationships within section *Juniperus* were inferred based on Maximum Likelihood, Maximum Parsimony and Bayesian inference with *trn*L and *trn*L-*trn*F sequences to test the monophyly and the spatiotemporal origin of *J. cedrus*. A phylogeographic analysis, based on the coalescence theory of allele diversity of the plastid sequences *pet*N-*psb*M and *trn*T-*trn*L, was conducted to investigate inter-island connections. AFLP fingerprint data for eight populations of the Canarian juniper were analysed to assess patterns of genetic diversity and differentiation in each island.

Results: The cpDNA phylogeny suggested the monophyly of *J. cedrus*, although neither the phylogenetic approach nor the haplotype network gave us enough resolution to point out a particular temporal and geographical origin of this Canarian juniper. Plastid markers also revealed a low genetic diversity, as shown by only four haplotypes detected within the Canaries. The highest AFLP genetic diversity was found in La Gomera and the moderate population structure reflected some insular isolation.

Main conclusions: Our results suggest that the genetic composition of *J. cedrus* has been impoverished by the dramatic fragmentation of its populations. The island of La Gomera is a reservoir of genetic diversity either because its old geological age (12 Ma) and stability have allowed long periods of differentiation, or because it may have suffered less intense felling than the larger islands, thereby preserving a much wider allelic diversity. Management efforts prioritizing this island are necessary to safeguard the persistence of *J. cedrus*.

Keywords: AFLPs • Canary Islands • Cupressaceae • Gene-flow • Genetic diversity • *Juniperus cedrus* • Phylogeography • Plastid DNA

Introduction

Isolation is a crucial process in plant evolution, and oceanic archipelagos offer an ideal spatiotemporal framework to study the patterns of genetic variation associated with speciation. Thus, oceanic islands have long been attractive environments for biogeographical studies related to the origin and evolution of their biotas (Carlquist 1965, Emerson 2002, Whittaker & Fernández-Palacios 2007). Specifically, the Macaronesian archipelagos are characterized by their harbouring a high number of plant endemics (Hansen & Sunding 1993, Hobohm 2000, Humphries 1979), and the Canary Islands are particularly well-studied in terms of historical plant evolution and high levels of diversification (Caujapé-Castells 2011, Francisco-Ortega *et al.* 2000, Vargas 2007).

Most endemic Macaronesian plant lineages are positioned in derived clades sister to western Mediterranean clades (Carine *et al.* 2004, Kim *et al.* 2008, Vargas 2007). Consequently, the origins of most Macaronesian endemic plants are due to single colonization events from that region followed by in-situ speciation, although evidence is accumulating for multiple colonization events from other mainland enclaves (Carine *et al.* 2004, Caujapé-Castells 2011, Díaz-Pérez *et al.* 2008, Ojeda *et al.* in press., Vargas 2007). The latter process would be favoured by the relatively short distance from the African and European continents, including several palaeo-archipelagos between the Canary Islands and the mainland (now submerged), that could have acted as stepping stones facilitating the colonization process (e.g. Fernández-Palacios *et al.* 2011).

Mainly due to the characteristics associated with the island syndrome (Bramwell 2011, Carlquist 1965), the biota of oceanic islands are also very fragile toward sudden external changes, and there are many examples worldwide of plant extinctions primarily driven by the impact of human activities (Caujapé-Castells *et al.* 2010). Genetic variation in island endemics is determined by the net effects of allelic loss at foundation, subsequent losses caused by finite population size since then, and gains arising from secondary immigration and new mutations (Jaenike 1973).

As a general rule, continental populations show higher levels of genetic diversity than their island congeners (but see Fernández-Mazuecos & Vargas 2011), which are more sensitive to bottlenecks related to founder effects (Frankham 1997), and frequently show little differentiation within islands (Maki 1999, 2001, Nielsen 2004). Insular populations also tend to be small; thus, they are more prone to the adverse effects of genetic drift, and presumably to inbreeding depression (Ellstrand & Elam 1993). Overall, these circumstances determine a higher genetic risk in the island populations than in their continental ones (Frankham, 1998). Furthermore, differences between the effective and census sizes induce a greater stochastic fluctuation of gene frequencies in small than in large populations (Nunney & Elam 1994).

Juniperus cedrus Webb & Berth (Cupressaceae) occurs in the Canarian archipelago and belongs to sect. *Juniperus*, which comprises 11 of the ca. 67 species included in the genus (Adams 2011). Although its distribution range was also thought to encompass the neighbouring archipelago of Madeira, the taxon in the latter area has been recently proposed as a narrow endemic species named *J. maderensis* (Menezes) R. P. Adams (Adams *et al.* 2010), so that *J. cedrus* would be strictly endemic to the Canary Islands.

Located between 27°–29° N and 13°–18° W, the Canaries lie only 96 km off the African coast, and comprise seven main volcanic islands of different geological ages: Fuerteventura (22 Ma) is the oldest and easternmost one, and El Hierro (1.2 Ma) the most recent and westernmost one (Carracedo & Day 2002). *Juniperus cedrus* occurs in La Palma, La Gomera, Tenerife and Gran Canaria (Fig. 1), where the current populations are fragmented and mainly restricted to craggy and inaccessible areas. It is a dioecious, anemophilous and endozoochorous taxon that sheds pollen from the male cones primarily during autumn (Adams 2011). The fleshy female cones are available to seed dispersers throughout the year (Rumeu *et al.* 2009a), and are consumed mainly by birds (Rumeu *et al.* 2011b, Rumeu *et al.* 2009b), as also reported for its continental congeners (García 2001, Jordano 1993, Livingston 1972, Zamora 1990). Its establishment on the islands must have been aided by some of its reproductive traits such as wind pollination, ornithochory and multi-seeded cones, which favour long-distance dispersal and colonization (Vargas 2007).

Although *J. cedrus* occurs nowadays in protected areas and most of its populations have attained a moderately large number of individuals, its extremely narrow distribution reflects to a large extent the strong pressure exerted by humans in the past: its valuable wood has had many uses, such as in cabinetmaking, the wine and tobacco industry (barrels and boxes), instrument manufacturing, or just for firewood. Accordingly, the species is globally catalogued as endangered, mainly due to its severe range fragmentation and the low number of mature individuals within populations (IUCN 2011). This demographic history leads us to expect an ailing genetic situation for *J. cedrus*. The dramatic reductions in population sizes may have created severe genetic bottlenecks, whereby the remaining individuals contain only a small fraction of the original gene pool due to drift, which is accentuated by increasing isolation and decreasing population sizes (Ellstrand & Elam 1993, Young *et al.* 1996).

After several studies about basic reproductive and ecological traits of *J. cedrus* (Rumeu *et al.* 2011b, Rumeu *et al.* 2009a, Rumeu *et al.* 2009b), data to substantiate hypotheses about its origin and genetic variability are essential for any conservation and management strategies. In this study, we used plastid DNA sequences and amplified fragment length polymorphism (AFLP) to: (1) infer the temporal and spatial origin of *J. cedrus*, (2) establish the patterns of genetic diversity and differentiation on each island, and (3) contribute to the design of informed conservation measures based on a better understanding of the genetic diversity structure of the endemic Canarian juniper.

Material and methods

Plant sampling and DNA extraction

Juniperus cedrus needles were collected from trees on all the islands of occurrence, and geographic coordinates of each tree were recorded using a hand-held GPS navigator. Due to both the severe fragmentation of natural populations and the inaccessibility of most trees, the numbers of populations and individuals sampled varied widely between the islands: 13 for La Palma, 28 for La Gomera, 27 for Tenerife and 5 for Gran Canaria (Fig 1). Total DNAs were extracted from silica-gel dried needles using the CTAB 2x method (Doyle & Doyle 1987, Palmer *et al.* 1988). The concentration of total DNA was measured in an Eppendorf biophotometer, and its quality was verified in 1% agarose gels. Aliquots of the DNA extracts have been deposited at the DNA Bank of the Canarian Flora at the Jardín Botánico Canario 'Viera y Clavijo' - Unidad Asociada CSIC (JBCVCSIC).

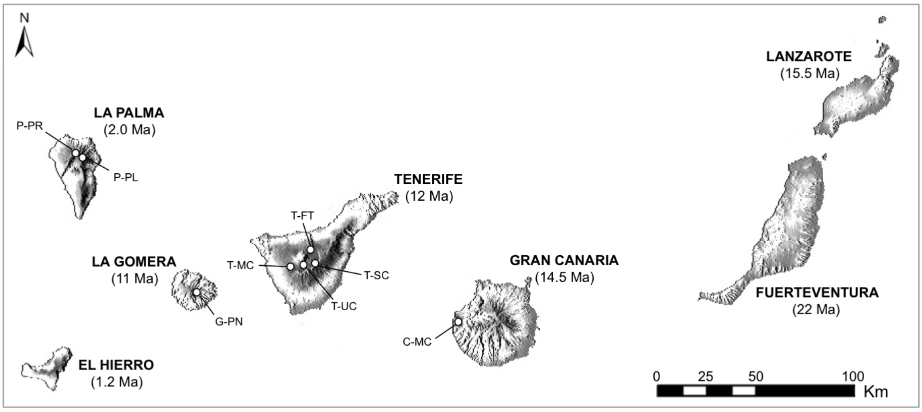

Fig.1 The Canary Islands and their maximum geological ages according to Carracedo & Day (2002). *Juniperus cedrus* populations sampled are indicated with circles. P-PL: La Palma, Piedra Llana; P-PR: La Palma, Pared de Roberto; G-PN: La Gomera, P. N. de Garajonay; T-FT: Tenerife, La Fortaleza; T-SC: Tenerife, Siete Cañadas; T-UC: Tenerife, Ucanca, T-MC: Tenerife, Montaña del Cedro; C-MC: Gran Canaria, Montaña del Cedro.

Plastid DNA markers

To infer the temporal and spatial origin of *J. cedrus*, we performed a phylogenetic analysis of section *Juniperus* using the *trn*L intron and *trn*L-*trn*F intergenic spacer (Taberlet *et al.* 1991) sequences from previous studies (Mao *et al.* 2010, Martínez & Vargas, in prep., Rumeu *et al.* 2011a). A matrix using these cpDNA regions was constructed using nine and 31 *trn*L and *trn*L-*trn*F sequences from Mao *et al.* (2010) and Rumeu *et al.* (2011a), respectively, and three *trn*L-*trn*F from Martínez & Vargas (in prep.). We sequenced the *trn*L regions of these latter samples for this study. In addition, our matrix was extended with newly sequenced samples: six *J. oxycedrus* from Morocco, two *J. maderensis* from Madeira and four *J. cedrus* from the Canary Islands (our study species). Based on previous phylogenetic analyses of *Juniperus* (Mao *et al.* 2010), two outgroup samples of *J. drupacea* (section *Caryocedrus*) were included (Tables 1/S1). Procedures used for amplification and sequencing of plastid DNA regions followed Rumeu *et al.* (2011a).

To obtain more accurate information about *J. cedrus*' putative origin and differentiation in the Canary Islands through a phylogeographic analysis, we first tested between two and four individuals from different islands for 19 plastid DNA regions in an exploratory study based on previous phylogenetic and phylogeographic analyses (Adams *et al.* 2009, Fazekas *et al.* 2008, Hamilton 1999, Hwang *et al.* 2000, Kress & Erickson 2007, Provan *et al.* 2008, Sang *et al.* 1997, Shaw *et al.* 2005, 2007, Taberlet *et al.* 1991). We selected the *trn*T-*trn*L (Taberlet *et al.* 1991), and *pet*N-*psb*M (Adams *et al.* 2009) previously employed by Rumeu *et al.* (2011a) in a study of the Azorean endemic *J. brevifolia*. In order to detect the ancestral haplotype of *J. cedrus*, we assembled a matrix with 5–17 individuals per island based on our previous phylogenetic analysis plus two samples of *J. macrocarpa* from Spain and Italy, and 18 samples of *J. oxycedrus* from Morocco and the Western Mediterranean basin. In order to add information about the genetic relationship between *J. cedrus* and *J. maderensis*, recently proposed as a different species (Adams *et al.* 2010), we also included 14 samples from the Madeiran juniper (Table 2). PCR protocols and sequencing followed Rumeu *et al.* (2011a).

Table 1 Taxon names and origin of the *Juniperus samples* included in the phylogenetic analysis. 'No' is the code for sample identification in Fig. 2. Taxonomy follows that of Adams *et al.* 2011. See Table S1 for details.

No	Taxon	**Geographical area/locality**
1	*J. brevifolia*	Portugal, Azores, Santa Maria, Almagreira
2	*J. brevifolia*	Portugal, Azores, Terceira, Malha Grande
3	*J. cedrus*	Spain, Canary Islands, La Palma
4	*J. cedrus*	Spain, Canary Islands, La Palma, Pared de Roberto
5	*J. cedrus*	Spain, Canary Islands, La Gomera, P. N. Garajonay
6	*J. cedrus*	Spain, Canary Islands, Tenerife, P. N. del Teide
7	*J. cedrus*	Spain, Canary Islands, Gran Canaria, Mña. del Cedro
8	*J. communis* var. *communis*	France
9	*J. communis* var. *communis*	Spain, Granada, Pico Trevenque
10	*J. communis* var. *depressa*	USA, New Mexico
11	*J. communis* var. *saxatilis*	Pakistan
12	*J. communis* var. *saxatilis*	Georgia, Caucasus
13	*J. deltoides*	Turkey
14	*J. formosana* var. *mairei*	China, Gansu
15	*J. macrocarpa*	Spain, Cádiz, Barbate, Trafalgar Cape
16	*J. macrocarpa*	Spain, Cádiz, Tarifa
17	*J. macrocarpa*	Spain, Valencia, El Saler
18	*J. macrocarpa*	Spain, Cádiz, Chiclana, Eliçabe
19	*J. macrocarpa*	Italy, Sardinia, Santa Teresa Gallura
20	*J. macrocarpa*	Italy, Sicily, Ragusa
21	*J. maderensis*	Portugal, Madeira, Fajã da Nogueira
22	*J. maderensis*	Portugal, Madeira, road Ribeira Brava-Paul da Serra (cultivated)
23	*J. maderensis*	Portugal, Madeira, Jardim Botânico (cultivated)
24	*J. navicularis*	Portugal, Apostiça
25	*J. navicularis*	Portugal, Apostiça
26	*J. navicularis*	Portugal, Estremadura
27	*J. oxycedrus* var. *badia*	Spain, Huesca, Sierra de Guara
28	*J. oxycedrus* var. *badia*	Spain, Jaén
29	*J. oxycedrus* var. *badia*	Spain, Jaén
30	*J. oxycedrus* var. *badia*	Morocco, El Ksiba
31	*J. oxycedrus* var. *badia*	Morocco, Kjbel Kelti
32	*J. oxycedrus* var. *badia*	Morocco, Kjbel Kelti
33	*J. oxycedrus* var. *badia*	Morocco, Kjbel Kelti
34	*J. oxycedrus* var. *badia*	Morocco, Marrakech
35	*J. oxycedrus* var. *badia*	Turkey, Gümüshane, Torul
36	*J. oxycedrus* var. *oxycedrus*	Spain, Granada, El Peñón
37	*J. oxycedrus* var. *oxycedrus*	Spain, Granada, El Peñón
38	*J. oxycedrus* var. *oxycedrus*	Spain, Balearic Islands, Menorca
39	*J. oxycedrus* var. *oxycedrus*	Greece, Lemo
40	*J. oxycedrus* var. *oxycedrus*	Greece, Lemo
41	*J. oxycedrus*	Turkey, Istanbul, Kartal
42	*J. oxycedrus*	Greece, Kalavryta, Diakoftó
43	*J. oxycedrus*	Tunisia, Cap Bon, Sidi Daoud
44	*J. oxycedrus*	France

Table 1 (Continued)

No	Taxon	Geographical area/locality
45	*J. oxycedrus*	Morocco, Jebel Lekst
46	*J. oxycedrus*	Morocco, Jebel Imzi
47	*J. oxycedrus*	Morocco, Beni-Mellal
48	*J. oxycedrus*	Morocco, High Atlas mountains, Aït el Qâq – Oukaïmedene
49	*J. oxycedrus*	Morocco, Asni, Tizi n'Test
50	*J. oxycedrus*	Morocco, Agadir, Imouzzer Ida Ou Tane
51	*J. rigida* var. *conferta*	Cultivated (Spain, Pontevedra, Lourizán)
52	*J. rigida* var. *conferta*	Japan
53	*J. rigida* var. *rigida*	Japan
54	*J. rigida* var. *rigida*	Japan
55	*J. taxifolia*	Japan
56	*J. taxifolia*	Japan, Bonin Island
57	*J. taxifolia* var. *lutchuensis*	Japan
	OUTGROUP	
58	*J. drupacea*	Greece
59	*J. drupacea*	Greece

AFLP procedure

Although cpDNA sequences allowed us to infer the genealogical relationships between *J. cedrus* and its relatives, they failed to reveal enough intraspecific genetic diversity to substantiate a population genetic study. Therefore, we opted to use AFLP markers on the grounds of their broad application in plant genetics (Meudt & Clarke 2007) and specifically for their proven usefulness to assess intraspecific genetic diversity in a wide number of plants lineages (Nybom 2004).

Standard AFLP protocols following Vos *et al.* (1995) with modifications by Schönswetter *et al.* (2003) were used. No explicit data on the genome size of *J. cedrus* are available, but IC values of other *Juniper* species were in the range of 10.82 to 12.15 pg (Hizume *et al.* 2001). Following the recommendations on the number of selective bases in Fay *et al.* (2005), and taking into account the three primer combinations chosen by Terrab *et al.* (2008) in a phylogeographic study of *J. thurifera*, we performed an initial screening of selective primers using 18 primer pairs with three and four selective nucleotides to test for their potential to produce scorable fragments. Of these, four primer pairs

(*Eco*RI/*Mse*I plus ACA/CTCG, ACT/CTCG, AGG/CTGA, AGG/CTCG) were chosen according to their reproducibility, levels of polymorphism and previous analyses (Terrab *et al.* 2008). Two to four replicates from the restriction-ligation phase were included in all the subsequent reactions to test reproducibility (Chung *et al.* 2004). Fragments were scored from 100–170 bp to 450–500 bp to minimize the occurrence of fragment size homoplasy, which is more likely with short fragments (Vekemans *et al.* 2002). The reactions were separated on a 7% polyacrylamide gel using an ABI 3730 automated sequencer. GENMAPPER V4.0 (Applied Biosystems) was used for fragment scoring. Following Terrab *et al.* (2008), the criteria for choosing AFLP bands were (1) straightforward interpretability, (2) similar fluorescence intensity, and (3) reproducibility between independent replicates. Bands that did not fulfil these criteria were regarded as potentially unreliable, and therefore discarded from the final presence/absence matrix for the sake of data quality.

Data analyses

Plastid DNA phylogeny

The phylogenetic analysis within *Juniperus* section *Juniperus* used Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) approaches with the *trn*L intron and *trn*L-*trn*F dataset. As large indels were detected in the *trn*L-*trn*F spacer, and these characters represent a considerable portion of the potential phylogenetic information (Simmons & Ochoterena 2000), they were manually coded following these authors and included in the analyses. To determine the model of sequence evolution that best fits the sequence data (HKY+I), the Akaike Information Criterion (AIC) (Akaike 1979) was implemented in this dataset using JMODELTEST 0.1.1 (Posada 2008). ML analyses were performed using RAxML v7.0.3 (Stamatakis 2006) with 100 bootstrap replicates. As this software only implements the GTR evolutionary model, we used the GTRGAMMAI model as the closest to our dataset following the AIC criterion. Parsimony analyses were run in TNT (Goloboff *et al.* 2008) under traditional heuristic search and considering gaps as a 5th character. We first used the Tree Bisection-Reconnection (TBR) branch-swapping algorithm with 10,000 replicates (saving two most-parsimonious trees per replicate). Subsequently, the trees obtained in the first search were used to start a second heuristic search that retained all the best trees. Branch support was evaluated using 1,000 bootstrap replicates, and collapsing groups with branch support values below 50.

The BI analysis was used to estimate divergence times within section *Juniperus*, including the date of origin of *J. cedrus*. We implemented a relaxed molecular-clock approach in BEAST v.1.6.0 to estimate phylogenetic relationships and node ages (Drummond & Rambaut 2007, Drummond *et al.* 2006). The molecular clock analysis was carried out with two data partitions: (1) the *trn*L intron and *trn*L-*trn*F intergenic spacer dataset using the HKY+I model following the AIC criterion, and (2) indels from partition 1 coded as binary data (binary simple substitution model) using the software SeqState 1.4.1 (Müller 2006). For the temporal calibration we used the average of three divergence times formerly obtained by Mao *et al.* (2010): (1) the split between sections *Juniperus*-*Caryocedrus* (49.1–29.9 Ma), (2) the crown of section *Juniperus* (29.9–11.1 Ma) and (3) the crown of the 'blue seed cone group' (BSG) in section *Juniperus* (17.5–4.7 Ma). The substitution rate variation was modelled using an uncorrelated lognormal distribution, and a Birth-Death process (Gernhard 2008) was employed as tree prior. Two MCMC analyses were run for 10 million generations, with a sample frequency of 1,000, and discarding the first 10% generations as burn-in. Analysis with Tracer 1.4 (Rambaut 2007) confirmed adequate sample size, with ESS values above 200. Both analyses were combined using LogCombiner 1.4.8, then trees were summarized in a maximum clade credibility tree obtained in TreeAnotator 1.4.8.

Phylogeographical analysis of plastid DNA variation

To further elucidate the origin of *J. cedrus* and detect the ancestral haplotype, the *pet*N-*psb*M and *trn*T-*trn*L sequences obtained were concatenated into a single matrix, based on the common inheritance without recombination that can be assumed for cpDNA markers (Birky

2001). We then performed a phylogeographic analysis based on the coalescence theory (Avise 2000) to infer the degree of connectivity between the Canarian juniper and its closest relatives, plus *J. maderensis* from the nearest archipelago (Madeira). The genealogical relationships among haplotypes were inferred through a statistical parsimony method (Templeton *et al.* 1992) implemented in the TCS 1.21 software (Clement *et al.* 2000). The maximum number of differences resulting from single substitutions among haplotypes was calculated with 95% confidence limits, treating gaps as the fifth state.

AFLP data analyses

The intraspecific genetic variability of *J. cedrus* was inferred from the AFLP matrix, which was imported from a .xlsx file to the software TRANSFORMER–4 (Caujapé-Castells *et al.* 2011). This software automatically generated the input files needed to run all the population genetic softwares used, and produced reports with the exclusive alleles per population, and the alleles exclusively shared per population pair. The matrix will be freely available in the 'genetic diversity digest' coded D–AFLPS–27 within the Demiurge information system (http://www.demiurge-project.org).

For each population, the total number of AFLP fragments (Frag_{tot}), usable loci (with less than 5% of missing data) ($\text{Frag}_{\text{usable}}$), polymorphic fragments ($\text{Frag}_{\text{poly}}$) and the average gene diversity (HD) were estimated using ARLEQUIN 3.5.1.2 (Excoffier & Lischer 2010), which was also used to carry out an analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992), and to compute exact tests of pair-wise population differentiation. In order to assess the relationship between geographic and genetic distance matrices between population pairs, a Mantel test (Mantel 1967) with 9,999 permutations was performed using GENALEX 6.41 (Peakall & Smouse 2006).

To better inveatigate the spatial genetic structure of populations, we performed a spatial autocorrelation analysis (Sokal & Oden 1978) that allowed us to assess the relationship between AFLP genetic distances as a function of the geographical distances among individuals. We excluded 128 alleles showing frequencies <0.1 or >0.9, as well as 7 alleles where data for >20% individuals were missing. We calculated Moran's I statistics (Moran 1948) for the remaining 84 alleles using the software PASSAGE v2.0 (Rosenberg & Anderson 2011), which built a preliminary correlogram based on 999 nonparametric permutations for 10 distance classes, each containing approximately equal numbers of pairwise comparisons. From these results, we selected the alleles displaying significant values of spatial autocorrelation (n= 47) and constructed a new matrix that was analysed with the software SPAGEDI 1.3 (Hardy & Vekemans 2002) to calculate the average values of Moran's I across distance classes, and their SE (Fig. 4).

A population-assignment test was conducted using the software STRUCTURE 2.3.2 (Pritchard *et al.* 2000) to cluster individuals into subpopulations. This software assumes a model with K populations (K unknown), each characterized by a set of allele frequencies at each locus. Using the Bayesian approach, individuals are probabilistically assigned to either a single population, or jointly to two or more populations if their genotypes indicate admixture. Following Evanno *et al.* (2005), the presence of an AFLP band was coded as 1/(−9), where 1 was the presence of the dominant allele and −9 a missing value. The absence of the AFLP band was coded as 2/2, indicating the recessive allele (2) was homozygous. We set the admixture model and the option of correlated allele frequencies between populations, as this configuration is considered the most appropriate in cases of subtle population structure (Falush *et al.* 2003). Similarly, the program inferred the degree of admixture alpha from the data. After several independent runs for each value of K in a pilot study, we chose a burn-in period of 30,000 iterations and 500,000 steps for the length of the MCMC (Markov chain Monte Carlo). We ran 10 independent simulations of this length for each K, in order to ensure consistent estimates across runs. The possible values of K ranged from 1 to the actual number of sampled populations plus 3. We then followed the method described in Evanno *et al.* (2005) to calculate the true number of populations (K).

Results

Phylogeny of section Juniperus and lineage divergence times

All three analyses using sequences of the *trn*L intron and the *trn*L-*trn*F region support the monophyly of *J. cedrus* (Fig. 2). The diversification window of the Canarian juniper was estimated between 5.3 and 0.18 Ma. Although the sample selection in the phylogeny is biased towards the Western Mediterranean basin and Morocco, *J. cedrus*' probable geographic origin is not consistently supported, and only the Bayesian (BEAST) approach weakly pointed to a closer relationship with a broad sister group formed by *J. macrocarpa* and *J. oxycedrus*. This low resolution and the complex pattern found in *J. oxycedrus* deserve further phylogenetic analyses.

Notably, the monophyly of the Madeiran juniper was supported by the three analyses, and the results suggest that the *J. maderensis* lineage originated and evolved independently from *J. cedrus.* However, our analysis failed to retrieve a consistent sister group relationship for *J. maderensis.*

Haplotype network

We detected a total of 12 haplotypes within *J. cedrus* and its relatives *J. maderensis* (Madeira), *J. macrocarpa* and *J. oxycedrus* (Western Mediterranean basin and Morocco) (Fig 3). Variation in the *pet*N-*psb*M and *trn*T-*trn*L intergenic spacers was the result of 7 nucleotide substitutions and 10 gaps, which were distributed as follows in the alignment: in *pet*N-*psb*M, 35 bp between 145–179, 6 bp between 265–270, 19 bp between 280–298, 5 bp between 431–435, 1 bp at 598, 7 bp between 620–626, 1 bp at position 660 and 2 bp between 659–660; in *trn*T-*trn*L, 1 bp at position 242 and 1 bp at 360. Table 2 shows the haplotype found in each locality; for the haplotype distribution of the 86 samples analysed, see Table S2. Within the Canarian juniper, we detected four exclusive haplotypes (H9, H10, H11, H12); the most ancestral one (H9) needed four steps to connect with the genealogically nearest clade (H3, H4 and H5)

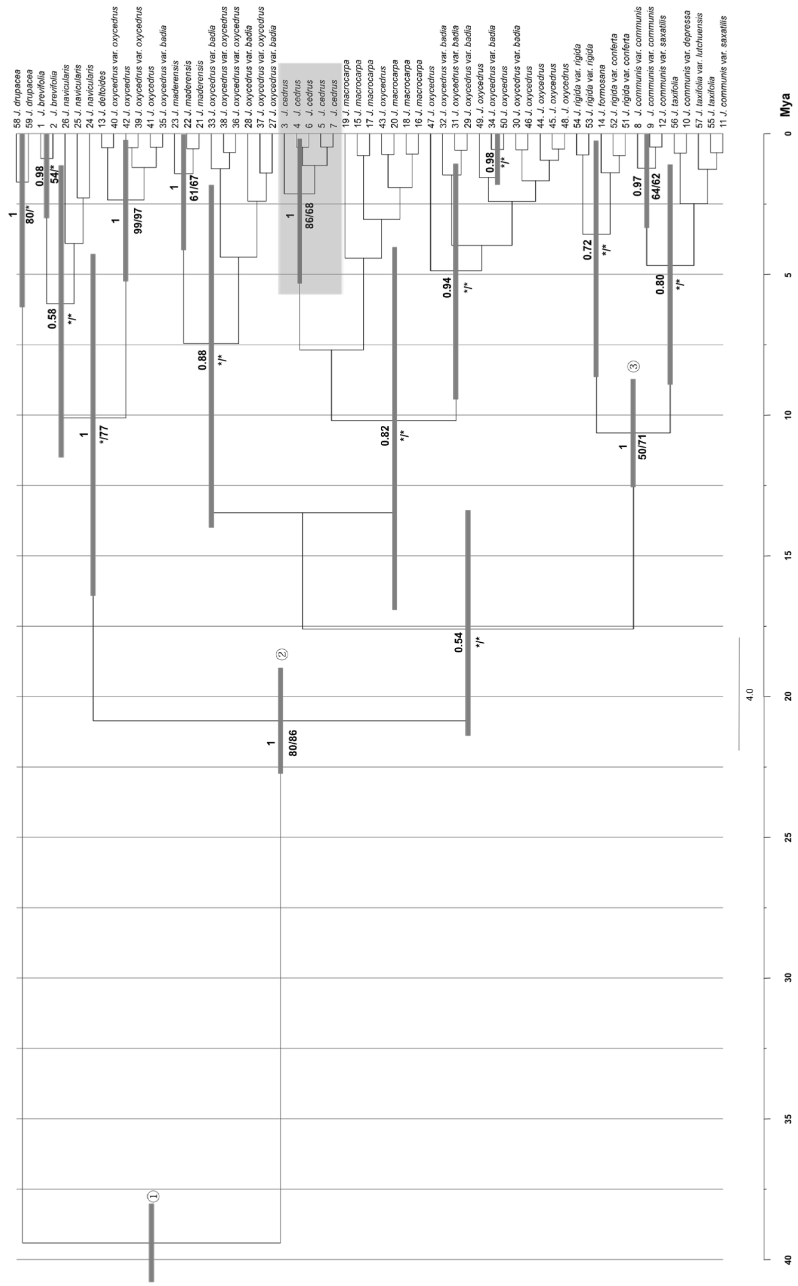

Fig. 2 Molecular phylogenetic relationships and divergence times within section *Juniperus*, inferred from *trn*L and *trn*L-*trn*F intergenic spacers. Numbers above branches are BEAST posterior probabilities, numbers below branches are Maximum Likelihood (before slashes) and Maximum Parsimony (after slashes) bootstrap support values. Asterisks indicate absence of support. Grey bars represent divergence times (95% highest posterior density intervals), while numbers in circles represent calibration points obtained in Mao *et al.* (2010): (1) split between sections *Juniperus*-*Caryocedrus* (49.1-29.9 Ma), (2) crown of section *Juniperus* (29.9-11.1 Ma) and (3) crown of the 'blue seed cone group' in section *Juniperus* (17.5-4.7 Ma).

formed by *J. macrocarpa* and *J. oxycedrus*, and mainly distributed in the Western Mediterranean basin. Five and six steps respectively separated the Moroccan clade (H1 and H2) and the Madeiran clade (H6, H7 and H8) from the Canarian ancestral haplotype H9. The haplotype H10 was the most frequent and widely distributed among the Canary Islands, whereas the derived haplotypes H11 and H12 were shared only by La Palma and Tenerife. These two islands showed the highest diversity with four haplotypes, followed by La Gomera with two and Gran Canaria with only one.

Table 2. Plant material of *Juniperus* used for *pet*N-*psb*M and *trn*T-*trn*L sequencing. *N* is the number of individuals sampled in each locality. *H* is the corresponding haplotype according to Fig. 3. See Table S2 for voucher source, haplotype number of each tree analysed, and the GenBank accession numbers of each haplotype found.

Taxon	**Geographical area/locality**	**N**	**H**
J. cedrus	Spain, Canary Islands, La Palma, Piedra Llana	4	9, 10
J. cedrus	Spain, Canary Islands, La Palma, Pared de Roberto	9	9, 10, 11, 12
J. cedrus	Spain, Canary Islands, La Gomera, P. N. Garajonay	17	9, 10
J. cedrus	Spain, Canary Islands, Tenerife, Riscos de La Fortaleza	6	11, 12
J. cedrus	Spain, Canary Islands, Tenerife, Siete Cañadas	6	9, 10, 11
J. cedrus	Spain, Canary Islands, Tenerife, Mña. del Cedro	3	10
J. cedrus	Spain, Canary Islands, Tenerife, Ucanca	2	10
J. cedrus	Spain, Canary Islands, Gran Canaria, Mña. del Cedro	5	10
J. macrocarpa	Spain, Cádiz, Tarifa	1	5
J. macrocarpa	Italy, Sardinia, Santa Teresa Gallura	1	5
J. maderensis	Portugal, Madeira, road Ribeira Brava-Paul da Serra	3	6, 8
J. maderensis	Portugal, Madeira, road to Pico Arieiro (cultivated)	1	6
J. maderensis	Portugal, Madeira, Fajã da Nogueira	1	6
J. maderensis	Portugal, Madeira, Jardim Botânico (cultivated)	5	6, 7, 8
J. maderensis	Portugal, Madeira, road Santa-Achadas da Cruz (cultivated)	4	6, 7, 8
J. oxycedrus	Tunisia, Cape Bon, Sidi Daoud	1	5
J. oxycedrus	Morocco, Anti-Atlas	2	2
J. oxycedrus	Morocco, Beni-Mellal, Taghzour	1	2
J. oxycedrus	Morocco, High Atlas mountains, Aït el Qâq – Oukaïmedene	1	2
J. oxycedrus	Morocco, Asni, Tizi n'Test	1	2
J. oxycedrus	Morocco, Agadir, Imouzzer Ida Ou Tane	1	1
J. oxycedrus var. *badia*	Morocco, Marrakech	1	1
J. oxycedrus var. *badia*	Morocco, Jebel Kelti	3	2, 3
J. oxycedrus var. *badia*	Morocco, El Ksiba	1	2
J. oxycedrus var. *badia*	Spain, Jaén	2	2, 3
J. oxycedrus var. *badia*	Spain, Huesca, Sierra de Guara	1	3
J. oxycedrus var. *oxycedrus*	Spain, Granada, El Peñón	2	3, 4
J. oxycedrus var. *oxycedrus*	Spain, Balearic Islands, Menorca	1	3

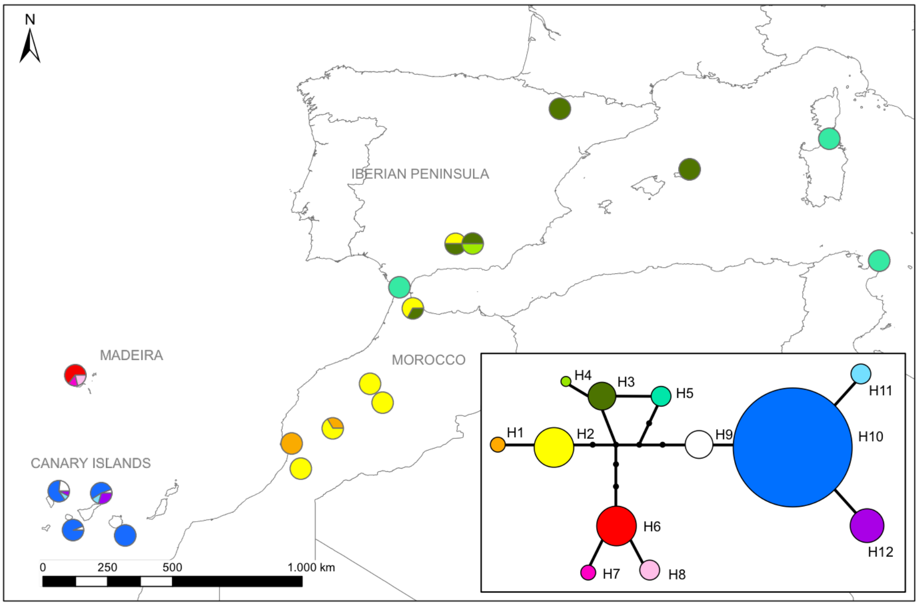

Fig. 3 Haplotype network based on *pet*N-*psb*M and *trn*T-*trn*L cpDNA regions, and its spatial distribution. Each haplotype is represented by both a number and a colour.

AFLP polymorphism of Juniperus cedrus

The four AFLP primer combinations used yielded a total of 219 scorable fragments, and all 73 individuals investigated had unique AFLP profiles. Genetic diversity descriptors are given in Table 3. The populations from La Palma (P-PR) and La Gomera (G-PN) showed the highest values of polymorphic loci. The population G-PN presented a remarkable proportion of exclusive alleles (1.41), followed distantly by C-MC (0.45), P-PR (0.16), T-SC (0.11) and T-FT (0.07). Notably, one individual from La Gomera gathered almost 50% of the exclusive polymorphism detected in the island. We can ensure that there was no contamination in the sampling and DNA extraction of this individual, since the same sample was used in the phylogeographic analysis with no evidence of contamination. In addition, visual examination of the individual leaves did not reveal evidence of fungal contamination, and exclusion of that individual from the data analysis still resulted in La Gomera yielding the highest proportion of exclusive alleles ($^{*}\text{Frag}_{\text{excl}}$ = 0.79, data not shown).

G-PN and T-SC shared more alleles exclusively (five), followed by G-PN and C-MC, and P-PR and G-PN, which exclusively shared three alleles each (data not shown). Two alleles were shared exclusively between T-FT and T-SC, and one between P-PL and T-SC, P-PR and T-SC, P-PL and G-PN, P-PR and CAN, G-PN and T-FT.

Table 3 *Juniperus cedrus* populations sampled for the AFLP analysis, and basic descriptors of polymorphism. *Island/Pop*: name of the island of occurrence and name of the populations. *Abbr:* abbreviation used, *N:* number of individuals analysed. $Frag_{usable}$: AFLP fragments with less than 5% of missing data across the data set, $Frag_{poly}$: no. of polymorphic AFLP fragments, $Frag_{excl}$: no. of exclusive fragments in the population, *$Frag_{excl}$: proportion of exclusive alleles corrected according to the sample sizes and 'usable' fragments of each population. HD ± SD: average gene diversity ± standard deviation

Island / Pop.	Abbr.	Geographical coordinates	N	$Frag_{usable}$	$Frag_{poly}$	$Frag_{excl}$	*$Frag_{excl}$ (%)	HD ± SD
La Palma								
Piedra Llana	P-PL	28º44'N 17º50'W	4	167	49	0	0	0.16 ± 0.11
Pared de Roberto	P-PR	28º45'N 17º52'W	9	211	90	3	0.16	0.16 ± 0.09
La Gomera								
P.N. Garajonay	G-PN	28º06'N 17º12'W	28	132	98	52	1.41	0.16 ± 0.08
Tenerife								
La Fortaleza	T-FT	28º18'N 16º35'W	11	133	54	1	0.07	0.15 ± 0.08
Siete Cañadas	T-SC	28º15'N 16º33'W	12	146	53	2	0.11	0.12 ± 0.07
Mña. del Cedro	T-MC	28º13'N 16º42'W	2	212	15	0	0	0.07 ± 0.07
Ucanca	T-UC	28º13'N 16º38'W	2	189	25	0	0	0.13 ± 0.13
Gran Canaria								
Mña. del Cedro	C-MC	27º58'N 15º48'W	5	179	39	4	0.45	0.11 ± 0.07

The AMOVA was significant for the three sources of variation (among islands, among populations within islands and within populations) (Table 4). However, the highest proportion of genetic variance was maintained within populations (79.2%). The Mantel test revealed a low relationship between geographic and genetic distances ($R^2 = 0.34$; $p = 0.004$), indicating a weak isolation-by-distance (IBD) effect (Table 5).

Moran's *I* correlogram for all samples of *J. cedrus* (Fig. 4) showed a pattern of positive spatial autocorrelation (i.e., a more or less steady decline of Moran's *I* values from the first to the last distance class), with the X-axis intercept at distance-class 4, corresponding to ca. 30 km. The correlogram of the population from La Gomera, which presented

Table 4 Analysis of molecular variance (AMOVA) conducted with the AFLP data of eight populations of *Juniperus cedrus* sampled. Tests of significance were based on 1023 permutations.

Source of variation	**d. f.**	**Sum of squares**	**Variance components**	**Percentage of variation (%)**	***P***
Among groups (islands)	3	150.73	2.08	16.66	< 0.001***
Among populations within groups	4	51.21	0.52	4.18	0.007**
Within populations	65	641.99	9.88	79.16	< 0.001***

Table 5 Population pairwise F_{ST} estimates based on 219 AFLP fragments and eight populations of *Juniperus cedrus* from the Canary Islands. F_{ST} values in bold indicate significance after *Bonferroni* correction (critical *p* = 0.00625).

	P-PL	**P-PR**	**G-PN**	**T-FT**	**T-SC**	**T-MC**	**T-UC**	**C-MC**
P-PL	0.000	–	–	–	–	–	–	–
P-PR	-0.007(ns)	0.000	–	–	–	–	–	–
G-PN	**0.123*** **	**0.112*** **	0.000	–	–	–	–	–
T-FT	**0.282*** **	**0.211*** **	**0.182*** **	0.000	–	–	–	–
T-SC	**0.265*** **	**0.208*** **	**0.178*** **	0.068***	0.000	–	–	–
T-MC	0.364(ns)	0.272*	0.203(ns)	0.101(ns)	0.098(ns)	0.000	–	–
T-UC	0.251(ns)	0.135(ns)	0.065(ns)	0.033(ns)	0.076(ns)	0.209(ns)	0.000	–
C-MC	0.358**	**0.267*** **	**0.292*** **	**0.346*** **	**0.365*** **	0.506*	0.406(ns)	0.000

the highest number of polymorphic loci (98) and exclusive alleles (52), reflected a weak pattern of positive spatial autocorrelation. The X-axis intercept was at distance-class 3, corresponding to ca. 500m.

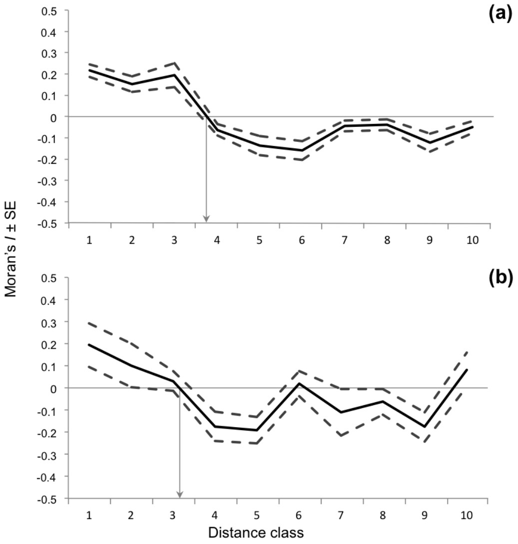

Fig. 4. Summary spatial correlogram (Moran's *I*) for *Juniperus cedrus* in the Canary Islands (a), and in La Gomera (b). The solid line represents the average of the 44 and 20 I values respectively for separate alleles displaying significant values of spatial autocorrelation. Dashed lines represent the standard deviation (SE). Distance classes contain approximately equal numbers of individual pairs. The arrow indicates the distance at which the correlogram intercepts with the x-axis.

The STRUCTURE analyses indicated that $K = 2$ appropriately represented the number of optimal Bayesian groups for our sample of the Canarian junipers (Fig. 5a). This result clearly separated the populations of Tenerife from those of the remaining islands. An AMOVA test performed to assess genetic differentiation between these two population groups confirmed their significant differgence ($p < 0.001$). Further sub-structuring analyses within the two previously described STRUCTURE clusters supported (1) the existence of six groups ($K = 6$) when excluding the populations from Tenerife, and (2) two sub-clusters within Tenerife (Fig. 5b). In the $K = 6$ group, the individuals from Gran Canaria (G-MC) form a clearly distinct group (cluster 6). Individuals from La Palma (populations P-PL and P-PR), and some from La Gomera were mainly included in cluster 1, whereas the remaining clusters (2, 3, 4 and 5) mainly comprised individuals from La Gomera, confirming the substantial genetic heterogeneity detected within this island. Notably, cluster 4 was formed by a single individual from La Gomera, which presented a particular AFLP profile with 24 exclusive alleles, as indicated above. Within Tenerife, individuals belonging to cluster 2 were all from population T-FT.

Discussion

Origin and inter-island colonization of J. cedrus

Our phylogenetic analyses support the monophyly and a distinct evolutionary history of *J. cedrus* from the junipers endemic to Azores (*J. brevifolia*) and Madeira (*J. maderensis*). This result agrees with Adams *et al.* (2010), who recently proposed the Madeiran juniper as a distinct species from *J. cedrus* based on data from nrDNA and cpDNA sequences, and leaf terpenes. Therefore, all phylogenetic reconstructions of the Macaronesian junipers (Rumeu *et al.* 2011a, and in this study) consistently suggest three independent colonization events in each of the archipelagos where these junipers grow (Azores, Madeira and the Canary Islands). Thus, the phylogeny of the Macaronesian junipers supports the prediction of multiple colonization events for plants displaying an endozoochorous syndrome (e.g. *Olea*, *Hedera*; Vargas 2007).

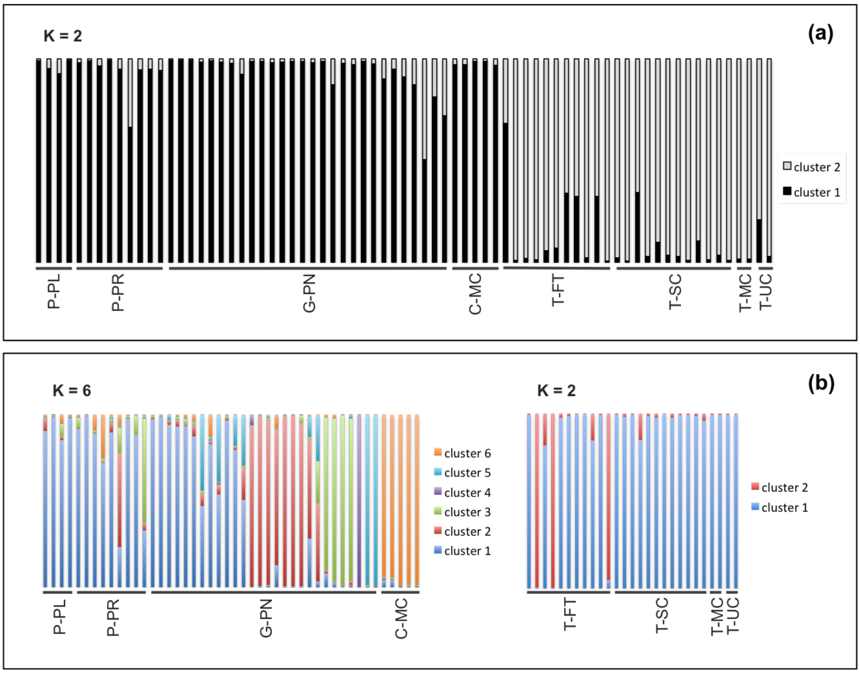

Fig. 5 Bayesian estimation of the genetic population structure of *Juniperus cedrus* based on the AFLP dataset. Each individual is represented by a thin vertical bar, which is portioned into *K* coloured segments that represent the individual's estimated membership fractions in *K* clusters. Horizontal lines on the bottom separate individuals according to populations (labelled below the figure). (a) *Juniperus cedrus* individuals from all populations sampled. (b) Estimated population sub-structure based on clusters obtained in 'a'. Individuals from La Palma, La Gomera and Gran Canaria in the left-plot. Individuals from Tenerife in the right-plot. The figure shown for a given *K* is based on the highest probability run at that *K*.

Nonetheless, the DNA sequence data used in the phylogenetic analyses failed to support a clear sister-group relationship hypothesis for *J. cedrus*. Despite our including a considerable number of juniper samples from the western Mediterranean in the analyses, neither the phylogenetic approach nor the haplotype network gave us enough resolution to infer a western European or North West African origin for this Canarian juniper. The plastid phylogenetic topology (Fig. 2) indicates a closer relationship with populations of *J. macrocarpa* and *J. oxycedrus*, mainly distributed in the western Mediterranean. A Mediterranean origin of the Canarian juniper would be consistent with many colonization histories of Macaronesian islands endemic plants (Carine *et al.* 2010, Caujapé-Castells 2011, Vargas 2007). These authors report a putative Mediterranean origin for ca.

35% plant colonizations examined with molecular data. Nevertheless, our results are inconclusive and indicate that this complex history of colonization needs further research to better understand the temporal and geographical origins of the endemic *J. cedrus*.

With only four cpDNA haplotypes, the genetic makeup of *J. cedrus* is narrow in comparison to the Azorean *J. brevifolia* (16 haplotypes; Rumeu *et al.* 2011a) or the maritime juniper *J. macrocarpa* (14 haplotypes; (Juan *et al.* 2011), despite the fact that these two species also have suffered substantial reductions in population sizes. However, fragmentation of natural populations has been especially severe in the Canary Islands, and the consequent demographic bottlenecks could have caused a dramatic loss of variation, resulting in genetically more depauperated populations (Ellstrand & Elam 1993).

Consistent with the results obtained by Rumeu *et al.* (2011a) for *J. brevifolia* in the Azores, *J. cedrus*' ancestral haplotypes (H9 and H10) showed a wider distribution than more recent ones (H11 and H12), suggesting recurrent inter-island colonization over long periods of time. Anemophily and ornithochory may have favoured gene flow, as these traits are the most suitable features for long-distance dispersal to Macaronesia (Vargas 2007). In contrast, a more restricted distribution of the derived haplotypes may reflect a substantial time needed to succeed in colonization. As proposed by Rumeu *et al*. (2011a), this scenario may be due to intraspecific habitat competition on the islands where junipers are already present. However, both its narrow distribution across the Canarian archipelago (only four islands), and the small number of extant individuals on each island, prevents us from unequivocally testing this hypothesis.

High genetic differentiation on La Gomera

Our results show that the populations with the highest values of polymorphic loci are located on the islands with the smallest areas (La Palma, La Gomera). Although only two plastid DNA haplotypes were detected on La Gomera, it is remarkable that this island showed the highest values of exclusive AFLP alleles (Table 3). The result of the STRUCTURE analyses also highlights La Gomera as the island showing a greater genetic sub-structuring, with individuals clustered into five different groups (Fig. 5b), one of which (cluster 4) consists of a single individual. Even excluding that individual from the analyses, La Gomera continues to have the highest numbers of unique alleles and different structure clusters. Although Gran Canaria is the oldest island where *J. cedrus* occurs, and several parts of Tenerife emerged ca. 12 Ma, La Gomera also has an ancient geological origin (11 Ma), and probably suffered less intensive felling than the larger islands of Tenerife and Gran Canaria, where the past distribution of *J. cedrus* may have covered much larger areas. The combination of these factors probably allowed La Gomera to act as a refugium of genetic diversity. In addition, La Gomera is the island sharing more alleles exclusively with the rest of the islands, which suggests that either (1) it represented a significant source of genetic variation for other islands, based on its geographical intermediate position within the *J. cedrus* range of distribution, or (2) felling has more dramatically affected the larger islands of Gran Canaria and Tenerife, so that the genetic distinctness of La Gomera may be attributed to the preservation of mostly relictual variation, once present in the very large populations that previously existed on those two islands (Francisco-Ortega *et al.* 2009, Hollermann 1978, Leuschner 1996). The lack of historical information in this regard prevents us from testing this 'genetic diversity sanctuary' hypothesis.

Isolation by human-mediated fragmentation

The dispersal attributes displayed by *J. cedrus* (particularly anemophily and ornithochory) have been described as most favourable for long-distance dispersal in Macaronesia (Vargas 2007), and several studies furnish robust evidence that plant species displaying these features are capable of maintaining substantial gene flow between islands (Ferreira *et al.* 2011, García-Verdugo *et al.* 2010). Besides this, the strong human-mediated fragmentation in the natural populations of the Canarian juniper appears to have played a key role in their present genetic impoverishment and isolation. The greater genetic variation within populations than among islands (Table 4), and the weak isolation by distance detected by the Mantel test, both suggest the existence of relatively dynamic gene flow among the islands. In sharp contrast, interception distances in the correlograms (Fig. 4) do not exceed ca. 30 km, which is lower than any inter-island distance. In the case of La Gomera, despite some fluctuations, the interception distance is ca. 500 m. The Bayesian clustering analysis also reflected a remarkable insular isolation, since populations on Tenerife, Gran Canaria and La Gomera formed independent clusters. On the whole, these results indicate that gene dispersal via pollen and seed between islands may have been spatially much more restricted, whereas levels of effective intra-insular gene flow are still high. A recent study of *J. macrocarpa* (Juan *et al.* 2011) based on AFLPs and plastid markers shows that this juniper species has not lost much of its genetic variation despite severe population fragmentation, as inferred from high levels of genetic variability and lack of geographical structure. However, the lower levels for *J. cedrus* and its moderate population structure, strongly suggest that its genetic composition has been outstandingly influenced by the dramatic reduction in population sizes after the European human colonization of the islands in the 15th century.

The combination of individual longevity, high intra-population genetic diversity and the potential for high rates of pollen and seed gene flow should make *J. cedrus* resistant to extinction until individuals and populations recover in suitable habitats (Hamrick 2004). Although all *J. cedrus* populations are located in protected areas at present, their severe degree of fragmentation, the low number of mature individuals within populations, the intolerance of the species to fire (B. Rumeu, unpubl. data), and its dependence on migrant thrushes for long-distance seed dispersal (Rumeu *et al.* 2011b), frame the Canarian juniper in a fragile conservation status.

This study points to the National Park of Garajonay in La Gomera as a sanctuary of genetic diversity for *J. cedrus*, so management efforts prioritizing this island are necessary. As in other juniper species (Chambers *et al.* 1999), the seed-cones of *J. cedrus* are able to persist on the plant for a long period of time before dispersal (months and probably years, (Rumeu *et al.* 2009a), which means that embryos can remain viable for long periods. This is an advantageous feature that supports the ex situ conservation strategy of seed collection. In a spatial autocorrelation analysis, the first x-axis intercept is an operational estimate of the average length of the shortest side of true genetic patches that contain individuals related by descent (Sokal 1979). Although Epperson (1990) criticizes this calculation because it is liable to slight stochastic and statistical biases, it is useful for conservation because it provides an operative estimate of the areas where individuals are more likely to be inbred. Therefore, in the case of *J. cedrus*, our autocorrelation results indicate that the seed collection to avoid sampling inbred seeds should be separated by more than 500 m in La Gomera, or more than 30 km considering the whole distribution range of *J. cedrus*.

Management efforts should also avoid undesired genetic admixture that would prevent the preservation of lineages (e.g. paying especial attention to possible anthropic introductions of the Madeiran juniper, until recently considered the same species as *J. cedrus*).

Finally, it is worth remarking that the conservation of *J. cedrus* in the Canary Islands not only depends on the implementation of proper management strategies focussed on this species, but they also should encompass its ecological interactions. Measures promoting the recovery of natural and effective long-seed dispersal agents such as ravens *Corvus corax* (Nogales *et al.* 1999, Rumeu *et al.* 2011b) –in dramatic decline in the Canaries (Nogales 1992, Siverio *et al.* 2010)– or the wintering ring ouzels *Turdus torquatus* (Rumeu *et al.* 2011b, Rumeu *et al.* 2009b) –listed as a species deserving high conservation concern in Britain (Sim *et al.*. 2007)–, should be combined with actions to avoid grazing by introduced mammals, in order to preserve and improve natural populations of *J. cedrus* in the Canaries.

Supporting information

Table S1 The GeneBank accession numbers, locality, and voucher information for the taxa included in the phylogenetic study. *No* is the code for sample identification in Fig. 2. Voucher abbreviations: *BR*:

Beatriz Rumeu collection numbers with the code given at the DNA Bank of the Jardín Botánico Canario 'Viera y Clavijo' – Unidad Asociada CSIC; *MA*: herbarium of the Royal Botanic Garden of Madrid, *JM*: Jorge Martínez collection numbers. Taxonomy follows that of Adams *et al.* (2011).

Table S2 The 86 samples of *Juniperus* used for *pet*N-*psb*M and *trn*T-*trn*L sequencing. *N* is the number of individuals sampled in each locality. Voucher abbreviations: *BR*: Beatriz Rumeu collection numbers, with the code given at the DNA Bank of the Jardín Botánico Canario 'Viera y Clavijo' – Unidad Asociada CSIC; *MA*: herbarium of the Royal Botanic Garden of Madrid, *JM*: Jorge Martínez collection numbers. *H* is the corresponding haplotype according to Fig. 3. Sequences of each haplotype were deposited in the GenBank.

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Table S1 The GeneBank accession numbers, locality, and voucher information for the taxa included in this study. *No* is the code for sample identification in Fig. 2. Voucher abbreviations: *BR*: Beatriz Rumeu collection numbers with the code given at the DNA Bank of the Jardín Botánico Canario 'Viera y Clavijo' – Unidad Asociada CSIC; *MA*: herbarium of the Royal Botanic Garden of Madrid, *JM*: Jorge Martínez collection numbers. Taxonomy follows that of Adams *et al.* (2011).

No	Taxon	**Geographical area/locality/voucher-source**	**GenBank**
1	*J. brevifolia*	Portugal, Azores, Santa Maria, Almagreira, BR4710	JF950948
2	*J. brevifolia*	Portugal, Azores, Terceira, Malha Grande, BR4546	JF950950
3	*J. cedrus*	Spain, Canary Islands, La Palma, JM2E6	JN845578
4	*J. cedrus*	Spain, Canary Islands, La Palma, Pared de Roberto, BR5000	JF950959
5	*J. cedrus*	Spain, Canary Islands, La Gomera, P. N. Garajonay, BR4109	JN845579
6	*J. cedrus*	Spain, Canary Islands, Tenerife, P. N. del Teide, BR5486	JN845580
7	*J. cedrus*	Spain, Canary Islands, Gran Canaria, Mña. del Cedro, BR4998	JN845581
8	*J. communis* var. *communis*	France, WO-2111 (Mao *et al.* 2010)	HM024557
9	*J. communis* var. *communis*	Spain, Granada, Pico Trevenque, MA612184 (Martínez & Vargas, in prep.)	JF950961
10	*J. communis* var. *depressa*	USA, New Mexico, BU-10936 (Mao *et al.* 2010)	HM024558
11	*J. communis* var. *saxatilis*	Pakistan, G.S.Miehe-3011 (Mao *et al.* 2010)	HM024559
12	*J. communis* var. *saxatilis*	Georgia, Caucasus, MA576696 (Martínez & Vargas, in prep.)	JF950963
13	*J. deltoides*	Turkey, WO-2002 (Mao *et al.* 2010)	HM024561
14	*J. formosana* var. *mairei*	China, Gansu, J.Q.Liu-1469 (Mao *et al.* 2010)	HM024568
15	*J. macrocarpa*	Spain, Cádiz, Barbate, Trafalgar Cape, Nieto, G.& Fuertes, J. (Martínez & Vargas, in prep.)	JF950964
16	*J. macrocarpa*	Spain, Cádiz, Tarifa, Martínez, J., JM5E8	JF950965
17	*J. macrocarpa*	Spain, Valencia, El Saler, Martínez, J. (Martínez & Vargas, in prep.)	JF950966
18	*J. macrocarpa*	Spain, Cádiz, Chiclana, Eliçabe, L.M. (Martínez & Vargas, in prep.)	JF950967
19	*J. macrocarpa*	Italy, Sardinia, Santa Teresa Gallura, Nieto, G.& Fuertes, J. (Martínez & Vargas, in prep.)	JF950968
20	*J. macrocarpa*	Italy, Sicily, Ragusa, MA645785 (Martínez & Vargas, in prep.)	JF950969
21	*J. maderensis*	Portugal, Madeira, Fajã da Nogueira, BR6174	JF950970
22	*J. maderensis*	Portugal, Madeira, road Ribeira Brava-Paul da Serra (cultivated), BR5205	JN845582
23	*J. maderensis*	Portugal, Madeira, Jardim Botânico (cultivated), JM1E20	JN845583
24	*J. navicularis*	Portugal, Apostiça, (Martínez & Vargas, in prep.)	JF950971
25	*J. navicularis*	Portugal, Apostiça, (Martínez & Vargas, in prep.)	JF950972

Table S1 (Continued)

No	Taxon	**Geographical area/locality/voucher-source**	**GenBank**
26	*J. navicularis*	Portugal, Estremadura, MA565189 (Martínez & Vargas, in prep.)	JF950973
27	*J. oxycedrus* var. *badia*	Spain, Huesca, Sierra de Guara, Vargas, P (Martínez & Vargas, in prep.)	JF950974
28	*J. oxycedrus* var. *badia*	Spain, Jaén, (Martínez & Vargas, in prep.)	JF950978
29	*J. oxycedrus* var. *badia*	Spain, Jaén, (Martínez & Vargas, in prep.)	JF950977
30	*J. oxycedrus* var. *badia*	Morocco, El Ksiba, MA616313 (Martínez & Vargas, in prep.)	JN845584
31	*J. oxycedrus* var. *badia*	Morocco, Kjbel Kelti, Pérez, R. (Martínez & Vargas, in prep.)	JF950980
32	*J. oxycedrus* var. *badia*	Morocco, Kjbel Kelti, Pérez, R. (Martínez & Vargas, in prep.)	JN845585
33	*J. oxycedrus* var. *badia*	Morocco, Kjbel Kelti, Pérez, R. (Martínez & Vargas, in prep.)	JF950979
34	*J. oxycedrus* var. *badia*	Morocco, Marrakech, MA391342 (Martínez & Vargas, in prep.)	JN845586
35	*J. oxycedrus* var. *badia*	Turkey, Gümüshane, Torul, Herrero, A. *et al.*, (Martínez & Vargas, in prep.)	JF950981
36	*J. oxycedrus* var. *oxycedrus*	Spain, Granada, El Peñón, Martínez, J., JM1E7	JF950982
37	*J. oxycedrus* var. *oxycedrus*	Spain, Granada, El Peñón, Martínez, J., JM4E8	JF950983
38	*J. oxycedrus* var. *oxycedrus*	Spain, Balearic Islands, Menorca, Martínez, J. (Martínez & Vargas, in prep.)	JF950984
39	*J. oxycedrus* var. *oxycedrus*	Greece, Lemo, (Martínez & Vargas, in prep.)	JF950985
40	*J. oxycedrus* var. *oxycedrus*	Greece, Lemo, (Martínez & Vargas, in prep.)	JF950986
41	*J. oxycedrus*	Turkey, Istanbul, Kartal, EOO138135 (Martínez & Vargas, in prep.)	JF950987
42	*J. oxycedrus*	Greece, Kalavryta, Diakoftó, Vargas, P. (Martínez & Vargas, in prep.)	JF950988
43	*J. oxycedrus*	Tunisia, Cap Bon, Sidi Daoud, Aldasoro *et al.* (Martínez & Vargas, in prep.)	JF950989
44	*J. oxycedrus*	France, WO-2113 (Mao *et al.* 2010)	HM024581
45	*J. oxycedrus*	Morocco, Jebel Lekst, MA786340	JN845587
46	*J. oxycedrus*	Morocco, Jebel Imzi, MA785987	JN845588
47	*J. oxycedrus*	Morocco, Beni-Mellal, Taghzour, MA745601	JN845589
48	*J. oxycedrus*	Morocco, High Atlas mountains, Aït el Qâq – Oukaïmedene, MA746811	JN845590
49	*J. oxycedrus*	Morocco, Asni, Tizi n’Test, MA760217	JN845591
50	*J. oxycedrus*	Morocco, Agadir, Imouzzer Ida Ou Tane, MA785633	JN845592
51	*J. rigida* var. *conferta*	Cultivated (Spain, Pontevedra, Lourizán), MA547007 (Martínez & Vargas, in prep.)	JF950990

Table S1 (Continued)

No	Taxon	**Geographical area/locality/voucher-source**	**GenBank**
52	*J. rigida* var. *conferta*	Japan, J.Q.Liu-TBG-JRIC (Mao *et al.* 2010)	HM024591
53	*J. rigida* var. *rigida*	Japan, (Martínez & Vargas, in prep.)	JF950991
54	*J. rigida* var. *rigida*	Japan, J.Q.Liu-TBG-JRIR (Mao *et al.* 2010)	HM024592
55	*J. taxifolia*	Japan, J.Q.Liu-TBG-JTA (Mao *et al.* 2010)	HM024602
56	*J. taxifolia*	Japan, Bonin Island (Martínez & Vargas, in prep.)	JF950992
57	*J. taxifolia* var. *lutchuensis*	Japan, (Martínez & Vargas, in prep.)	JF950993
	OUTGROUP		
58	*J. drupacea*	Greece, (Martínez & Vargas, in prep.)	JF950994
59	*J. drupacea*	Greece, (Martínez & Vargas, in prep.)	JF950995

Table 2 The 86 samples of *Juniperus* used for *pet*N-*psb*M and *trn*T-*trn*L sequencing. *N* is the number of individuals sampled in each locality. Voucher abbreviations: *BR*: Beatriz Rumeu collection numbers, with the code given at the DNA Bank of the Jardín Botánico Canario 'Viera y Clavijo' – Unidad Asociada CSIC; *MA*: herbarium of the Royal Botanic Garden of Madrid, *JM*: Jorge Martínez collection numbers. *H* is the corresponding haplotype according to Fig. 3. Sequences of each haplotype were deposited in the GenBank.

Taxon	Geographical area/locality	N	Voucher-source	H	GenBank accession no. (*pet*N-*psb*M/*trn*T-*trn*L)	
J. cedrus	Spain, Canary Islands, La Palma, Piedra Llana	4	BR4096, BR4099	10	JN845605	JN845593
			BR4097, BR4098	9	JN845606	JN845594
J. cedrus	Spain, Canary Islands, La Palma, Pared de Roberto	9	BR4100, BR4101, BR4999, BR5001, BR5004, BR5005	10	–	–
			BR5000	11	JN845607	JN845595
			BR5002	12	JN845608	JN845596
			BR5003	9	–	–
J. cedrus	Spain, Canary Islands, La Gomera, P. N. Garajonay	17	BR4102–BR4106, BR4109, BR5006, BR5007, BR5009, BR5012, BR5013, BR5016, BR5018, BR5020, BR5023, BR5024	10	–	–
			BR5025	9	–	–
J. cedrus	Spain, Canary Islands, Tenerife, Riscos de La Fortaleza	6	BR5260	11	–	–
			BR5292, BR5296, BR5307, BR5336, BR5352	12	–	–
J. cedrus	Spain, Canary Islands, Tenerife, Siete Cañadas	6	BR5426, BR5432, BR5450, BR5486	10	–	–
			BR5438	11	–	–
			BR5462	9	–	–
J. cedrus	Spain, Canary Islands, Tenerife, Mña. del Cedro	3	BR5517, BR5519, BR5520	10	–	–
J. cedrus	Spain, Canary Islands, Tenerife, Ucanca	2	BR5522, BR5523	10	–	–
J. cedrus	Spain, Canary Islands, Gran Canaria, Mña. del Cedro	5	BR4110–BR4113, BR4998	10	–	–
J. macrocarpa	Spain, Cádiz, Tarifa	1	JM5E8	5	JN845609	JN845597
J. macrocarpa	Italy, Sardinia, Santa Teresa Gallura	1	(Martínez & Vargas, in prep.)	5	–	–
J. maderensis	Portugal, Madeira, road Ribeira Brava-Paul da Serra	3	BR5205, BR5207	6	JN845610	JN845598
			BR5206	8	JN845611	JN845599
J. maderensis	Portugal, Madeira, road to Pico Arieiro (cultivated)	1	BR5208	6	–	–

Table S2 (Continued)

Taxon	Geographical area/locality	N	Voucher-source	H	**GenBank accession no. (*pet*N-*psb*M/*trn*T-*trn*L)**	
J. maderensis	Portugal, Madeira, Fajã da Nogueira	1	BR6174	6	–	–
J. maderensis	Portugal, Madeira, Jardim Botânico (cultivated)	5	BR6175-BR6177 BR6178 BR6179	6 7 8	– JN845612 –	– JN845600 –
J. maderensis	Portugal, Madeira, road Santa-Achadas da Cruz (cultivated)	4	BR6180, BR6182 BR6181 BR6183	6 7 8	– – –	– – –
J. oxycedrus	Tunisia, Cape Bon, Sidi Daoud	1	(Martínez & Vargas, in prep.)	5	–	–
J. oxycedrus	Morocco, Anti-Atlas	2	MA786340, MA785987	2	JN845613	JN845601
J. oxycedrus	Morocco, Beni-Mellal, Taghzour	1	MA745601	2	–	–
J. oxycedrus	Morocco, High Atlas mountains, Aït el Qâq – Oukaïmedene	1	MA746811	2	–	–
J. oxycedrus	Morocco, Asni, Tizi n'Test	1	MA760217	2	–	–
J. oxycedrus	Morocco, Agadir, Imouzzer Ida Ou Tane	1	MA785633	1	JN845614	JN845602
J. oxycedrus var. *badia*	Morocco, Marrakech	1	MA391342	1	–	–
J. oxycedrus var. *badia*	Morocco, Jebel Kelti	3	JM1E19,JM4E19 JM3E19	2 3	– JN845615	– JN845603
J. oxycedrus var. *badia*	Morocco, El Ksiba	1	MA616313	2	–	–
J. oxycedrus var. *badia*	Spain, Jaén	2	JM3E7 JM6E8	2 3	– –	– –
J. oxycedrus var. *badia*	Spain, Huesca, Sierra de Guara	1	JM7E14	3	–	–
J. oxycedrus var. *oxycedrus*	Spain, Granada, El Peñón	2	JM4E8 JM1E7	3 4	– JN845616	– JN845604
J. oxycedrus var. *oxycedrus*	Spain, Balearic Islands, Menorca	1	JM9E22	3	–	–

Resumen Global: objetivos, metodología, resultados, discusión y conclusiones finales

Objetivos

En general, los ecosistemas insulares oceánicos ofrecen un marco ideal para el estudio de procesos ecológico-evolutivos, los cuales presentan una menor complejidad que en los ecosistemas continentales, fundamentalmente debido a la menor variedad de especies, y a su composición dis-armónica (p. ej. frecuente ausencia de grupos principales como mamíferos). Además, las islas también constituyen un escenario ideal para el estudio de los patrones de variación genética asociados a la especiación (Carlquist 1965, Emerson 2002, Whittaker & Fernández-Palacios 2007). Dentro de este marco conceptual, cada uno de los estudios aquí presentados (I–V) ha tratado distintos aspectos ecológicos y evolutivos de los cedros endémicos de las islas macaronésicas. En concreto, a través de ellos, se ha pretendido alcanzar los siguientes objetivos:

I. *Patrones fenológicos y características de las gálbulas de las dos especies de cedro endémicas de las islas macaronésicas (Juniperus cedrus y J. brevifolia).*

El objetivo de este estudio fue el de recopilar información reproductiva básica que permitiera obtener una primera impresión sobre la dinámica poblacional de los cedros macaronésicos. Además, el conocimiento adquirido resultaría imprescindible para realizar un diseño apropiado del estudio de dispersión de semillas, y para interpretar adecuadamente los resultados que se fueran obteniendo con posterioridad. En concreto, a través de este trabajo se pretendía conocer:

– La fenología de las gálbulas, principalmente su disponibilidad para los dispersores de semillas.
– El efecto de la planta-madre en la viabilidad de las semillas.
– Las características principales de las gálbulas (tamaño, peso, contenido hídrico y número de semillas).
– Las características básicas de las semillas (dimensiones, peso y viabilidad).

II. *Papel básico de la población invernante de Mirlo capiblanco Turdus torquatus en la dispersión de semillas del endemismo amenazado Juniperus cedrus en un ambiente insular.*

El objetivo principal de este trabajo fue el de evaluar el papel de la población invernante de mirlo capiblanco como dispersor de semillas del cedro canario en la alta montaña de Tenerife. Específicamente, este estudio trató de:

- Conocer la composición de la dieta del mirlo capiblanco durante su invernada.
- Evaluar su efectividad como dispersor de semillas del cedro canario.

III. *Los sistemas de dispersión de semillas de los cedros endémicos de las islas macaronésicas: influencia de las características biogeográficas y biológicas.*

Este estudio tuvo como objetivo general, conocer el funcionamiento de los sistemas de dispersión de semillas de *Juniperus cedrus* y *J. brevifolia*, valorando sus diferencias y similitudes en función de las particularidades biogeográficas de cada uno de los archipiélagos donde se distribuyen en la actualidad, así como de las características reproductivas previamente investigadas. Los objetivos concretos estuvieron centrados en el conocimiento de los siguientes aspectos:

- El conjunto de dispersores que intervienen en cada sistema de dispersión de semillas.
- La contribución cuantitativa de cada uno de los frugívoros implicados.
- El componente cualitativo de la efectividad de la dispersión, valorando las consecuencias del paso de las semillas a través del tracto digestivo de los frugívoros, tanto respecto al daño morfológico de la semilla como a su capacidad para germinar.
- La variación estacional, cuando fuera posible, en el sistema de

dispersión de semillas.

IV. *Historia de la colonización de Juniperus brevifolia (Cupressaceae) en las islas Azores*

Una vez sentadas las bases ecológicas del sistema de dispersión de semillas de los cedros macaronésicos, este trabajo abordó la historia colonizadora de *J. brevifolia* (cedro do mato) en el archipiélago de Azores. Se trató de evaluar la manera en que esta historia puede estar condicionada por los síndromes de dispersión, y de estimar los niveles de diferenciación genética que ha adquirido esta especie endémica en las islas. Los objetivos concretos de este estudio fueron:

- inferir el origen espacio-temporal de *J. brevifolia*.
- estimar los niveles de diversidad genética en cada isla.
- reconstruir la historia filogeográfica del *J. brevifolia* en Azores.

V. *Origen y diferenciación genética del endemismo canario amenazado Juniperus cedrus (Cupressaceae).*

Al igual que el trabajo anterior, el objetivo de este estudio fue el de reconstruir la historia filogeográfica del cedro canario *J. cedrus*. Sin embargo, dado el grado de fragmentación de las poblaciones y su estado de conservación, mucho más precario que el de *J. brevifolia* en Azores, también se intentó evaluar de qué modo la historia evolutiva de esta especie se ha visto condicionada por las actividades antrópicas de los últimos siglos. Los objetivos específicos de este estudio fueron:

- inferir el origen espacio-temporal de *J. cedrus*.
- Conocer los patrones de diversidad y diferenciación en cada isla.
- Contribuir al diseño de medidas de conservación basadas en un conocimiento más amplio de la biología de esta especie, que tenga en cuenta la estructura espacial de su diversidad genética.

Material y métodos

Cada uno de los cinco trabajos de investigación aquí presentados (I–V) consta de una metodología específica. Sin embargo, a grandes rasgos, estos trabajos pueden agruparse en dos bloques. El primero de ellos trata sobre la investigación de los sistemas de dispersión de semillas de los cedros macaronésicos, y estaría conformado por los trabajos I, II y III. El segundo, integrado por los trabajos IV y V, se centra en dilucidar, mediante una aproximación molecular, la historia evolutiva de los cedros endémicos de las islas macaronésicas.

I, II, III. Los sistemas de dispersión de semillas de los cedros macaronésicos

Área de estudio

Formando parte del conjunto de islas macaronésicas, los archipiélagos de Canarias (27–29°N, 13–18°O) y Azores (36–40°N, 24–32°O) constituyen el área de distribución de *Juniperus cedrus* y *J. brevifolia*, respectivamente. Estas islas de origen volcánico difieren considerablemente, entre muchos otros aspectos, en cuanto a su distancia al continente. Así, mientras la más oriental de las Islas Canarias (Fuerteventura) se encuentra tan solo a 96 km de la costa africana, la isla azoriana más cercana al continente europeo (Santa Maria) se encuentra a unos 1.400 km de distancia. Los trabajos relacionados con la dispersión de semillas se llevaron a cabo en poblaciones representativas de las islas de Tenerife (en Canarias), y Terceira (en Azores).

Tenerife, con una edad geológica que oscila los 4–12 Ma y un área de 2.058 km^2, es la mayor de las islas Canarias (Carracedo & Day 2002) (Fig 1). Exceptuando algunos individuos aislados en otras zonas, las poblaciones naturales de *J. cedrus* se encuentran confinadas a la alta montaña, en el Parque Nacional del Teide. Este espacio protegido posee una superficie de 190 km^2 y se encuentra situado a unos 2.000 m s.n.m. Se encuentra influenciado por un clima típico de alta montaña, con grandes oscilaciones térmicas a lo largo del año (diferencias de unos 10°C entre las temperaturas medias mensuales máximas y mínimas), inviernos fríos con presencia de nieve, y veranos secos y calurosos (Bustos & Delgado 2004). La temperatura y precipitación media anual es de 10,7°C y 367,5 mm, respectivamente. La vegetación es fundamentalmente de tipo arbustivo, con un alto componente endémico (p. ej. *Spartocytisus supranubius*, *Pterocephalus lasiospermus*, *Adenocarpus viscosus*). Con el fin de evaluar la influencia de las condiciones del hábitat en los parámetros estudiados, la población de *J. cedrus* seleccionada para el estudio de investigación I (*Riscos de La Fortaleza*) fue dividida en dos subpoblaciones, en función de su localización: (1) "*cresta del risco*", con árboles de porte más pequeño y localizados en una zona expuesta a vientos relativamente frecuentes del NO y con un escaso desarrollo del suelo, y (2) "*base del risco*", con árboles de mayor tamaño, más resguardados del viento y en una zona con mayor desarrollo del suelo.

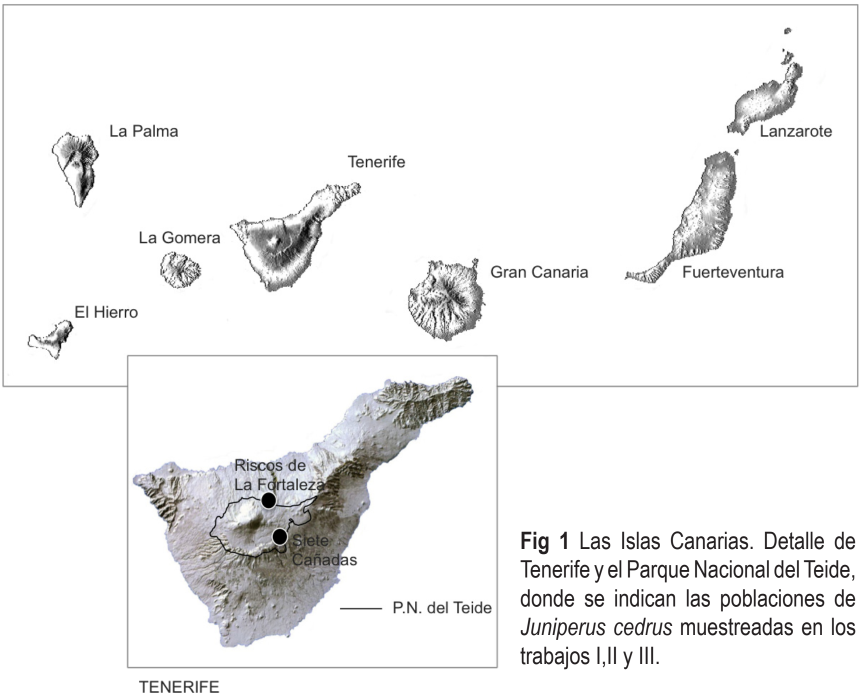

Fig 1 Las Islas Canarias. Detalle de Tenerife y el Parque Nacional del Teide, donde se indican las poblaciones de *Juniperus cedrus* muestreadas en los trabajos I,II y III.

La isla de Terceira tiene una superficie de 402,2 km^2 y su edad geológica se estima en 3,52 Ma (França *et al.* 2003) (Fig 2). A pesar de la fragmentación de las poblaciones naturales de *J. brevifolia* en Azores, en esta isla aún es posible encontrar algunas zonas bien conservadas, donde la comunidad vegetal está dominada por esta especie (Dias *et al.* 2004, Elias 2007, Elias & Dias 2009). *Juniperus brevifolia* presenta una distribución muy amplia, pudiendo crecer desde zonas costeras hasta las partes más altas de la isla (Serra de Santa Bárbara, 986 m s.n.m). Las poblaciones seleccionadas para el desarrollo de los estudios I y III se encuentran localizadas entre los 500–980 m de altitud. La temperatura y precipitación media anual oscila entre los 11,1–14,0°C y los 2.100–3.000 mm, respectivamente, según localidades. La vegetación en todos los casos se encuentra dominada por *J. brevifolia* y otras especies que varían en abundancia en función de la altitud, como son *Erica azorica*, *Laurus azorica* o *Calluna vulgaris*.

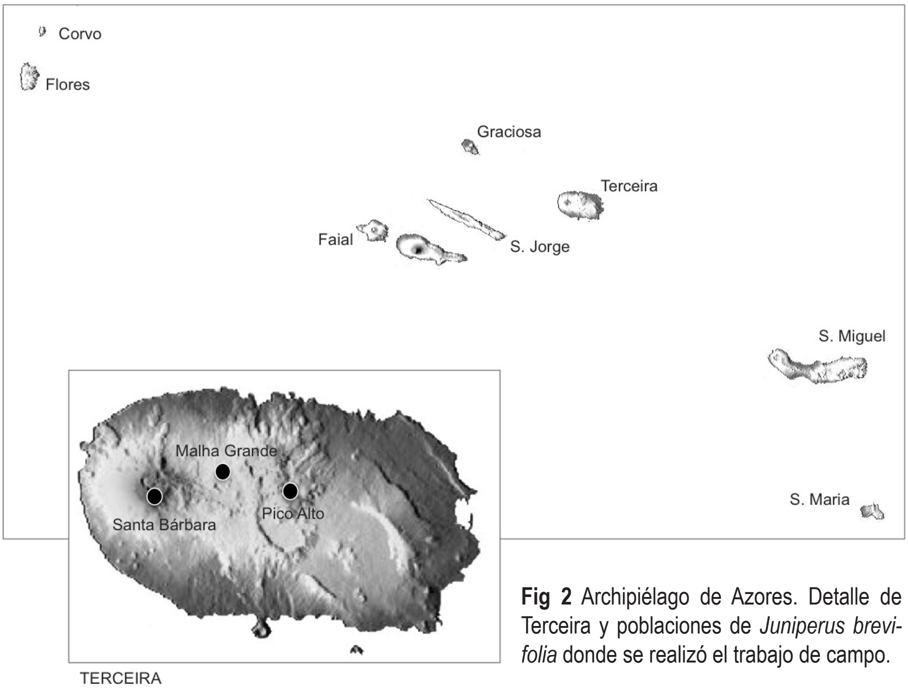

Fig 2 Archipiélago de Azores. Detalle de Terceira y poblaciones de *Juniperus brevifolia* donde se realizó el trabajo de campo.

Metodología

Para la realización de la primera investigación presentada (I), el trabajo de campo consistió, por un lado, en el seguimiento fenológico de las gálbulas de 15–20 plantas-madre por población. En cada planta se marcaron una serie de ramas para el contaje de las gálbulas maduras e inmaduras, que se extendió durante uno o dos años para *J. brevifolia* y *J. cedrus*, respectivamente. Pese a las pequeñas variaciones en la metodología, el porcentaje de gálbulas de cada tipo fue calculado para ambas especies durante las cuatro estaciones, lo que permitió la comparación de los datos fenológicos. Por otro lado, para caracterizar las gálbulas maduras, se colectaron unas 90 por especie y se tomaron medidas de su tamaño, peso y contenido hídrico; también se midieron y pesaron 180 semillas de cada especie. Para testar la viabilidad de las semillas y valorar el efecto de las plantas-madre sobre esta última característica, se abrieron más de 800 semillas de cada especie (procedentes de 20 plantas-madre diferentes en cada caso) para examinar el estado morfológico de los embriones. Aquéllos en aparente buen estado, fueron sometidos al test del tetrazolio (*2,3,5–triphenil-tetrazolium chloride*; TTC). Mediante este test, las células vivas se tiñen de rojo al reducirse el terazolio por las enzimas deshidrogenasas, formando el triphenil formazano, un compuesto de color rojo e insoluble en agua (Tanaka 1984).

En el caso de los trabajos II y III, que analizan directamente la efectividad de la dispersión de semillas, el trabajo de campo consistió en la colecta de excrementos de los distintos frugívoros con el fin de obtener las semillas dispersadas. Puesto que el proceso dispersivo depende de la disponibilidad temporal de gálbulas maduras, los datos fenológicos recopilados en el trabajo I resultaron esenciales para el diseño del muestreo. Así, en base a esta información previa, la colecta de excrementos se realizó a lo largo de las cuatro estaciones del año en el caso de *J. cedrus*, y durante el verano y el otoño en el caso de *J. brevifolia*. Los excrementos de las aves se colectaron mediante el trampeo de las mismas con redes de niebla; una vez marcadas las aves y colectado su excremento, fueron liberadas en el mismo lugar. Para aumentar el número de muestras, también se colectaron excrementos sobre plásticos colocados bajo algunas plantas-madre, y sobre rocas cercanas, usadas con frecuencia por los túrdidos como posaderos de descanso (Zamora 1990).

Con el fin de obtener un volumen considerable de semillas de *J. cedrus* dispersadas por lagartos *Gallotia galloti*, los excrementos se colectaron durante el verano, coincidiendo con la máxima actividad metabólica de estos reptiles. Además, también se hizo un seguimiento estacional de la dispersión de semillas por parte de los lagartos, colectando excrementos estacionalmente en las inmediaciones de 13 plantas-madre elegidas al azar.

Los excrementos de la especie de conejo *Oryctolagus cuniculus*, se colectaron en distintos sitios de las poblaciones estudiadas y, en ambos casos, las semillas control se extrajeron de gálbulas directamente colectadas de las plantas-madre.

En el caso particular de *J. cedrus*, donde la interacción con el cuervo *Corvus corax* era conocida pero imposible de testar en el campo (debido a la extinción de esta ave en el área de distribución del cedro canario en Tenerife), se llevó a cabo un experimento en cautividad que permitió valorar el efecto cualitativo de este dispersor en la germinación de las semillas.

Todos los excrementos colectados fueron analizados con el fin de extraer las semillas y contabilizarlas, valorando así el componente cuantitativo de la eficacia en la dispersión por parte de cada frugívoro. Los excrementos de mirlo capiblanco colectados en el Parque Nacional del Teide fueron examinados con mayor detalle, para así poder identificar todos los componentes de su dieta invernal. Las semillas extraídas de las diferentes muestras fueron clasificadas, en función de su aspecto externo, como semillas sanas o dañadas. Para poder evaluar el efecto del paso de las semillas por el tracto digestivo de los dispersores, aquellas que se encontraban en aparente buen estado fueron sometidas a experimentos de germinación, siguiendo las recomendaciones de Traveset & Verdú (2002), Nogales *et al.* (2005) y Rodríguez-Pérez *et al.* (2005).

Análisis de datos

A lo largo de estos tres trabajos (I–III) que conforman el primer bloque de la Tesis, se realizaron múltiples comparaciones de datos en relación, por ejemplo, al número de gálbulas maduras entre especies o entre las distintas estaciones, a las características de las gálbulas y semillas, a la frecuencia de aparición de semillas en los excrementos según tratamientos y localidades, al efecto de los tratamientos digestivos sobre las semillas…etc. Para la realización de estas comparaciones se emplearon diferentes test estadísticos, tanto paramétricos como no paramétricos (en el caso de datos que no se ajustaban a ninguna distribución). Estos test fueron los siguientes: *Razón de Verosimilitud* o *Test de la G*, *ANOVA de un factor* con pruebas a posteriori *HSD de Tukey*, *Test de Kruskal-Wallis*, *Test de la T de Student*, y *Test de Mann-Whitney*. En aquellos casos en que fue necesario utilizar el mismo set de datos para varias comparaciones, se tuvo en cuenta la corrección *a posteriori* de *Bonferroni*. Todos estos análisis se realizaron con el paquete estadístico SPSS 15.0.

IV y V. Historia evolutiva de los cedros endémicos de las islas macaronésicas

Área de estudio

Con el objetivo de realizar un estudio filogeográfico de *J. brevifolia* y *J. cedrus*, el área de estudio en este caso abarcó todas las islas donde es posible encontrar estas especies.

La distribución de *J. brevifolia* (cedro do mato) en el archipiélago de Azores comprende la totalidad de las islas con excepción de Graciosa, donde está extinto (Elias 2007). En función de su situación geográfica, las nueve islas que conforman el archipiélago de Azores pueden dividirse en tres grupos: occidental (Corvo y Flores), central (Faial, Pico, São Jorge, Graciosa y Terceira) y oriental (São Miguel y Santa Maria). El grado de fragmentación de las poblaciones de *J. brevifolia* es variable en función de las islas. Así, en Santa Maria el cedro do mato se encuentra al borde de la extinción (se calcula que hay menos de diez individuos en toda la isla), mientras que por ejemplo en Terceira y Flores aún es posible encontrar extensas áreas de vegetación dominadas por esta especie (Elias 2007, Elias & Dias 2009). Tal y como se ha comentado anteriormente, *J. brevifolia* presenta una gran amplitud de hábitat, pudiendo crecer desde zonas costeras hasta casi la cima de la montaña de Pico (2.351 m, el punto más alto de Portugal) (Dias *et al.* 2007, Elias 2007).

En el archipiélago canario, *J. cedrus* crece en las islas de La Palma, La Gomera, Tenerife y Gran Canaria (Acebes-Ginovés *et al.* 2004). Aquí, la fragmentación de las poblaciones ha sido más drástica que en el caso de *J. brevifolia*, y las poblaciones naturales han quedado relegadas en muchos casos a lugares escarpados y de difícil acceso. Sin embargo, su distribución actual también denota una gran amplitud ecológica, compartiendo hábitat con especies del bosque termófilo en Gran Canaria, de la laurisilva en La Gomera o del matorral de alta montaña en Tenerife y La Palma.

Metodología

El muestreo de hojas de ambas especies de cedro para la obtención de ADN se realizó en todas las islas del rango de distribución. En el caso de *J. brevifolia* en Azores, se muestrearon unos 50 árboles por isla a excepción de Santa Maria, donde sólo pudieron colectarse 2 muestras debido a su escasez. En Canarias, sin embargo, dada la severa fragmentación de las poblaciones y la inaccesibilidad de muchos árboles, se obtuvo un número variable de muestras en función de la isla: 13 en La Palma, 28 en La Gomera, 27 en Tenerife y 5 en Gran Canaria. En esta última isla, los individuos muestreados corresponden a la única población natural conocida (*Montaña del Cedro*), con muy pocos individuos y en clara regresión. Pese a la existencia de ejemplares plantados en otras localidades, sería interesante en un futuro aplicar alguna técnica genética para cerciorarse del origen de éstos. Con el fin de disponer de material que permitiera aportar más información a la relación filogenética entre *J. maderensis* y *J. cedrus*, se colectaron 14 muestras en la isla de Madeira, aunque 13 de ellas se obtuvieron de individuos cultivados. Las coordenadas geográficas de cada muestra fueron registradas con un GPS.

Las hojas colectadas se almacenaron en bolsas de plástico herméticas con sílica-gel hasta su procesado en el laboratorio. El ADN total se extrajo mediante el método CTAB 2X (Doyle & Doyle 1987, Palmer *et al.* 1988). La concentración y calidad del ADN se midió mediante un biofotómetro y geles de agarosa al 1%, respectivamente. Las alícuotas del ADN extraído fueron depositadas en el Banco de ADN de la Flora canaria del Jardín Botánico "Viera y Clavijo"-Unidad Asociada CSIC (JBCVCSIC).

En ambos trabajos (IV y V), se realizaron análisis filogenéticos y filogeográficos basados en regiones del ADN plastidial. Las regiones usadas en la aproximación filogenética de la sección *Juniperus* (el intrón *trn*L y el espaciador intergénico *trn*L-*trn*F; Taberlet *et al.* 1991) fueron seleccionadas en base a estudios previos (Mao *et al.* 2010, Martínez & Vargas en prep.). Usando secuencias de estos estudios, se construyó una matriz que fue ampliada con secuencias de las especies macaronésicas, así como con algunos otros taxones geográficamente próximos a la especie focal en cada caso (*J. brevifolia* en el trabajo IV, y *J. cedrus* en el trabajo V).

Para la realización de los análisis filogeográficos se llevó a cabo un estudio piloto en el que se testaron varios individuos de cada especie procedentes de diferentes islas, para 19 regiones de ADN plastidial previamente usadas en otros estudios filogenéticos y filogeográficos (Adams *et al.* 2009, Fazekas *et al.* 2008, Hamilton 1999, Hwang *et al.* 2000, Kress & Erickson 2007, Provan *et al.* 2008, Sang *et al.* 1997, Shaw *et al.* 2005, 2007, Taberlet *et al.* 1991). De entre estas regiones, se seleccionaron las más variables (*pet*N-*psb*M, Adams *et al.* 2010; *trn*S-*trn*G, Hamilton 1999; y *trn*T-*trn*L, Taberlet *et al.* 1991) para la secuenciación de unas 9–10 muestras por isla en el caso de *J. brevifolia*, y 5–17 muestras por isla en el caso de *J. cedrus* (para esta última especie, la región *trn*S-*trn*G no fue utilizada, al no detectarse variabilidad). Las secuencias se alinearon y se ajustaron manualmente usando MAFFT V6.814B desde el software GENEIOUS 5.1.7. Todas las secuencias nuevas se depositaron en el GenBank.

En el caso concreto de *J. brevifolia*, la región *psb*A-*trn*H también fue amplificada puesto que la presencia de una inserción/deleción de 58 pb permitió su chequeo en geles de agarosa al 1.5% para la totalidad de las muestras colectadas (n = 367), extendiendo así el análisis filogeográfico.

Dado que en el caso de *J. cedrus*, las secuencias de ADN plastidial no revelaron suficiente diversidad intraespecífica como para llevar a cabo una aproximación de genética poblacional, se optó por el uso de marcadores de tipo AFLP (*Amplified Fragment Lenght Polymorphism*) en base a su utilidad para evaluar la diversidad genética intraespecífica en un gran número de linajes de plantas (Nybom 2004). Tras una evaluación inicial de 18 pares de cebadores selectivos, se seleccionaron cuatro pares (*Eco*RI/*Mse*I–ACA/CTCG, ACT/CTCG, AGG/CTGA, AGG/CTCG) en función de su reproducibilidad, niveles de polimorfismo y análisis previos (Terrab *et al.* 2008). El software GENMAPPER V.4.0 se utilizó para la obtención de la matriz binaria, así como para la visualización y ajuste manual de los fragmentos.

Análisis de datos

Las relaciones filogenéticas dentro de la sección *Juniperus* fueron inferidas en base a análisis de Máxima Parsimonia (TNT 1.1, Goloboff *et al.* 2008), Máxima Verosimilitud (PHYML, Guindon & Gascuel 2003; RAXML Stamatakis 2006) e Inferencia Bayesiana (BEAST 1.6.0, Drummond *et al.* 2006, Drummond & Rambaut 2007), con la que se estimaron tiempos de divergencia.

Se llevaron a cabo diversos análisis filogeográficos para los que se usaron diferentes elementos de software. Las relaciones genealógicas entre haplotipos se estimaron a través de parsimonia estadística empleando el software TCS 1.21 (Clement *et al.* 2000). En el caso del trabajo IV centrado en la historia evolutiva de *J. brevifolia*, el análisis de diferenciación genética (*Snn*) atribuible a aspectos geográficos o temporales, se realizó con el software DNASP V5 (Librado & Rozas 2009). Siguiendo el método descrito en Lemey *et al.* (2009), para evaluar la distribución geo-espacial y el proceso de difusión de *J. brevifolia* a través del tiempo, se combinó un análisis de BEAST (en el que el archivo BEAUTI fue modificado para asignar la localización espacial de cada muestra), y un análisis filogeográfico discreto (DPA, *Discrete Phylogeographic Analysis*), el cual determina la probabilidad de distribución de las ocho posibles islas de origen de los haplotipos en los nodos del árbol de máxima credibilidad. Posteriormente, el test de *Bayes Factor* (BF) se utilizó para obtener los niveles de significación estadística de las tasas de los eventos de dispersión, y así representar aquéllos bien apoyados en Google Earth.

En el caso del análisis de los AFLPs para *J. cedrus*, la matriz binaria fue importada desde Excel (.xlsx) al software Transformer-4 (Caujapé-Castells *et al.* 2011), con el que se calculó el número de alelos exclusivos por población y se generaron automáticamente las matrices de entrada para los distintos software utilizados para estimar los niveles de variabilidad genética (Arlequin 3.5.1.2, Excoffier & Lischer 2010), la estructura espacial de la diversidad genética (Passage 2.0, Rosenberg & Anderson 2011); SPAGeDI 1.3, Hardy & Vekemans 2002) y la estructura poblacional (Structure 2.3.2, Pritchard *et al.* 2000). La matriz de genotipos utilizada está depositada permanentemente en el sistema de información Demiurge con el código D-AFLPS-27 (http://www.demiurge-project.org/matrix_digest/D-AFLPS-27).

En ambos trabajos (IV y V), el aislamiento por distancia, es decir, la relación existente entre la distancia genética (*Fst*) y geográfica (km), se estimó mediante el test de Mantel implementado en el software GenAlex 6.3 (Peakall & Smouse 2006).

Resultados

I. *Patrones fenológicos y características de las gálbulas de las dos especies de cedro endémicas de las islas macaronésicas (Juniperus cedrus y J. brevifolia).*

Este trabajo reveló una fenología desigual de las gálbulas de los cedros macaronésicos. Mientras *Juniperus cedrus* presentó gálbulas maduras a lo largo de todo el año, en el caso de *J. brevifolia* sólo se detectaron durante el verano y el otoño. Sin embargo, las gálbulas inmaduras estuvieron presentes a lo largo de todo el año en ambas especies, siendo muy abundantes durante el invierno y la primavera. En relación a las características morfológicas de las gálbulas maduras, las del cedro canario fueron significativamente mayores y más pesadas que en el caso del cedro do mato, pese a que estas últimas mostraron cuatro veces más cantidad de agua. También se encontraron diferencias en cuanto al número de semillas por gálbula; mientras *J. cedrus* presentó dos o tres semillas indistintamente, *J. brevifolia* mostró normalmente tres. De acuerdo con lo encontrado para las gálbulas maduras, las semillas del cedro canario fueron mayores y más pesadas en comparación con las del cedro de Azores. La viabilidad de las semillas fue significativamente superior en el caso de *J. brevifolia* y, aunque para esta especie no se encontró relación entre determinados caracteres morfológicos de las semillas (tamaño, peso) y la viabilidad de las mismas, en el caso de *J. cedrus* las semillas viables fueron significativamente más pesadas que las no viables. En ambas especies se encontraron diferencias significativas en relación a la viabilidad de las semillas de las diferentes plantas-madre, con valores que oscilaron entre 0–78% en función de la planta de origen.

Este estudio también puso de manifiesto la influencia de las condiciones del hábitat en los parámetros estudiados. Así, de las dos subpoblaciones en las que fue dividida la población de *Riscos de La Fortaleza*, la situada en la base del risco, con árboles de mayor tamaño, más resguardados del viento y sobre un suelo bien desarrollado, presentó mayor abundancia de gálbulas maduras respecto a la subpoblación situada en la cresta del risco. Estas gálbulas, además, fueron de mayor tamaño y presentaron valores más elevados de viabilidad de semillas.

II. *Papel básico de la población invernante de mirlo capiblanco Turdus torquatus en la dispersión de semillas del endemismo amenazado Juniperus cedrus en un ambiente insular.*

En este trabajo se analizó un total de 1.020 excrementos de mirlo capiblanco (498 en *Riscos de La Fortaleza* y 522 en *Siete Cañadas*). El 97% de las semillas identificadas correspondió a *J. cedrus*, mientras que el 3% perteneció a *Rhamnus integrifolia*. El 100% de las presas identificadas (n = 229) fueron invertebrados, dentro de los cuales los miriápodos (Diplopoda) y coleópteros (Scarabaeidae, Curculionidae y Dasytidae) constituyeron los principales grupos consumidos. En cuanto a la biomasa aportada, el 95% correspondió a la fracción vegetal (93% *J. cedrus*, 2% *R. integrifolia*), mientras que el componente animal de la dieta no llegó a alcanzar el 2%. No se observaron diferencias significativas en cuanto a la composición de la dieta en las dos localidades estudiadas (*Riscos de La Fortaleza* y *Siete Cañadas*), por lo que los datos de estos dos sitios se analizaron conjuntamente.

El resultado obtenido del experimento de germinación demostró la legitimidad del mirlo capiblanco como dispersor de semillas de *J. cedrus*, favoreciendo significativamente la germinación de las semillas consumidas respecto a las semillas control.

III. *Los sistemas de dispersión de semillas de los cedros endémicos de las islas macaronésicas: influencia de las características biogeográficas y biológicas.*

Con una frecuencia de aparición (F.O.) de semillas en los excrementos del 75% y un incremento del porcentaje de germinación respecto al tratamiento control del 12%, los resultados de este estudio situaron al mirlo capiblanco *Turdus torquatus* como el dispersor de semillas más efectivo para *J. cedrus*. El lagarto tizón *Gallotia galloti* fue cuantitativamente importante como dispersor de semillas del cedro canario. Sin embargo, su contribución cualitativa no parece ser beneficiosa, puesto que el paso por su tracto digestivo redujo significativamente el porcentaje y la tasa de germinación de las semillas. En el caso de *J. brevifolia*, fue el mirlo común *Turdus merula* (F.O.: 80%; incremento del porcentaje de germinación respecto al tratamiento control: 16%) el agente dispersante más efectivo. Para el cedro do mato, aunque con una F.O. de semillas del 6%, la curruca capirotada *Sylvia atricapilla* constituyó el segundo dispersor en importancia. En ambos sistemas de dispersión de semillas, el conejo *Oryctolagus cuniculus* (mamífero introducido tanto en las Islas Canarias como en Azores), presentó valores reducidos de número de semillas por excremento, frecuencia de aparición y porcentaje de semillas no dañadas.

El experimento llevado a cabo en cautividad con el cuervo *Corvus corax* reveló el efecto beneficioso del paso de las semillas de *J. cedrus* por el tracto digestivo de este dispersor, que aumentó significativamente el porcentaje de semillas germinadas.

En el caso del cedro canario, el análisis de la variación estacional demostró que la dispersión de semillas no ocurre de igual forma a lo largo del año. Así, mientras el mirlo capiblanco dispersó el 60.5% de las semillas durante los meses de invierno, el peso de la dispersión de semillas recayó sobre el lagarto *G. galloti* principalmente durante los meses de verano (56.4% de las semillas dispersadas por los lagartos en todo el año), coincidiendo con el período de máxima actividad metabólica de estos vertebrados ectotérmicos. De este modo, existe un reemplazamiento temporal de los principales dispersores de semillas de *J. cedrus* que, en concordancia con la disponibilidad de gálbulas maduras durante todo el año, permite que las semillas sean diseminadas a lo largo de las cuatro estaciones.

IV. *Historia de la colonización de Juniperus brevifolia (Cupressaceae) en las islas Azores.*

Los resultados de este estudio señalaron la monofilia de *J. brevifolia*, cuyo grupo hermano estaría formado por especies estrechamente relacionadas que se encuentran en Portugal continental (*J. navicularis*) y el Este de la cuenca mediterránea (*J. deltoides* y poblaciones de *J. oxycedrus*). Los datos obtenidos no permitieron la estimación del tiempo de colonización de las Azores, aunque la diversificación de *J. brevifolia* ocurrió con posterioridad a la emergencia de la isla más antigua del archipiélago (Santa Maria; 8,12 Ma).

Se detectó una elevada variación genética intraespecífica (16 haplotipos); las relaciones genealógicas entre los haplotipos mostraron una amplia distribución de los más frecuentes y ancestrales, mientras que la mayoría de los haplotipos más recientes se encontraron relegados a una única isla. El análisis de la región *psb*A-*trn*H en los 367 árboles muestreados reveló dos fragmentos de longitudes diferentes (408 y 466 bp). Las islas del grupo central fueron las que presentaron mayores niveles de variación en la longitud de los fragmentos amplificados.

La diferenciación genética asociada a las islas más antiguas y cercanas al continente fue significativamente superior a la de las islas más recientes y remotas. Por otro lado, el test de Mantel reveló un efecto de aislamiento por distancia débil pero significativo (R^2 = 0,38, p = 0,001).

El análisis filogeográfico discreto indicó un amplio período de diversificación de *J. brevifolia* (4,99–0,65 Ma) y reveló un origen probable en São Miguel y Terceira para 7 de los 16 haplotipos detectados. La combinación de este análisis con el test de *Bayes Factor* apoyó la importancia de Terceira como ‘stepping-stone’ en la colonización interinsular de *J. brevifolia*, así como una direccionalidad Este–Oeste en la colonización de algunos haplotipos (9 y 16).

V. *Origen y diferenciación genética del endemismo canario amenazado Juniperus cedrus (Cupressaceae).*

El análisis filogenético llevado a cabo en este trabajo apoyó la monofilia de *J. cedrus* y estimó un amplio periodo de diversificación de sus poblaciones, entre 5,3 y 0,18 Ma. La falta de apoyo en el árbol resultante no permitió generar una hipótesis clara acerca del origen espacio-temporal de *J. cedrus*, pese al elevado número de muestras procedentes de Marruecos y el Oeste de la cuenca mediterránea. Los resultados también señalaron la monofilia de *J. maderensis*, que parece tener un origen diferente y haber seguido una evolución independiente del cedro canario.

La red de haplotipos construida a partir de secuencias plastidiales tampoco arrojó mucha luz al origen espacial de *J. cedrus*, aunque el clado genealógicamente más próximo al haplotipo ancestral del cedro canario estuvo formado por muestras de *J. macrocarpa* y *J. oxycedrus*, principalmente distribuidos en el Oeste de la cuenca mediterránea. A nivel intraespecífico, *J. cedrus* tan sólo mostró cuatro haplotipos. Las poblaciones de La Palma y Tenerife, que presentaron estos cuatro haplotipos, mostraron los niveles más altos de diversificación, mientras que en La Gomera se detectaron dos haplotipos y en Gran Canaria tan sólo uno.

El análisis de los AFLPs indicó que las poblaciones con valores más altos de loci polimórficos se localizan en las islas de La Palma y La Gomera (43% y 74%, respectivamente). Esta última isla albergó la mayor proporción de alelos exclusivos. El análisis molecular de la varianza (AMOVA) señaló que la mayor parte de la variación genética es debida al componente intrapoblacional (79%); los valores entre poblaciones dentro de las islas, o entre las islas fueron mucho menores (4% y 17%, respectivamente). El test de Mantel detectó un débil aislamiento por distancia ($R^2 = 0,34$; $p = 0,004$). Por otro lado, el análisis de autocorrelación espacial mostró valores positivos de la I de Moran dentro de ≈ 30 km (distancia insuficiente para conectar poblaciones de diferentes islas). De igual manera, el análisis de

STRUCTURE registró cierto aislamiento insular, con las poblaciones de Tenerife y Gran Canaria bien diferenciadas del resto. En concordancia con los valores de polimorfismo y exclusividad de alelos obtenidos, el análisis de STRUCTURE también señaló a la población de La Gomera como la más diversa, con individuos pertenecientes a cinco grupos diferentes.

Discusión general

Fenología y características de las gálbulas de los cedros macaronésicos: evolución diferencial en sistemas de dispersión de semillas

La dispersión de semillas puede ser un proceso bastante complejo, en el que multitud de factores se encuentran implicados de un modo u otro. Sin embargo, la relativa simplicidad de las islas oceánicas con respecto a las zonas continentales, en términos de diversidad de especies e interacciones ecológicas (Carlquist 1974, Olesen & Jordano 2002), así como en relación a su clara acotación territorial, hacen de estos lugares escenarios idóneos para el estudio de fenómenos ecológico-evolutivos. La relación entre las plantas con síndromes endozoócoros y los animales frugívoros constituye un proceso de coevolución difusa (Janzen 1980) por lo que muchas de las características reproductivas de las especies vegetales se encuentran en íntima relación con las particularidades del proceso dispersivo. El primero de los estudios de investigación de esta Tesis Doctoral, pone de manifiesto contrastes importantes en diversas características de las gálbulas de los cedros macaronésicos, lo que indica la existencia de sistemas de dispersión de semillas diferentes. La fenología de los frutos determina la disponibilidad de alimento para los vertebrados frugívoros, que modifican la composición de la dieta en función de esta disponibilidad (Noma & Yumoto 1997). En el caso del cedro do mato, la disponibilidad de gálbulas maduras únicamente en verano y otoño contrasta con la fenología de otros de sus congéneres, que pueden mantenerlas en el árbol incluso durante años (Chambers *et al.* 1999). Sin embargo, una alta tasa de remoción, la depredación por ratas y la abscisión natural como consecuencia de la putrefacción en un ambiente de elevada humedad (R.B.E., pers. obs.) podrían estar detrás de este patrón fenológico. Por el contrario, la disponibilidad de gálbulas maduras durante las cuatro estaciones en el cedro canario, va a permitir que la dispersión de semillas ocurra durante todo el año, tal y como se pone de manifiesto en el trabajo III.

Dada la fuerte correlación existente entre el diámetro de los frutos y el tipo de dispersor de semillas (Jordano 1995), las diferencias entre los cedros macaronésicos en relación al tamaño de las gálbulas, indican dos sistemas de dispersión de semillas que han evolucionado en presencia de agentes dispersantes de distinta talla. Así, mientras *Juniperus cedrus* debe haber pasado largos periodos evolutivos interaccionando con aves de gran tamaño como el cuervo *Corvus corax* (Nogales *et al.* 1999), o diferentes especies de lagartos gigantes (g. *Gallotia*) que habitaron las islas, el cedro do mato debe haberlo hecho con aves de mediana y pequeña talla como sus principales dispersores de semillas actuales, el mirlo común *Turdus merula* y la curruca capirotada *Sylvia atricapilla*. Por otro lado, las enormes diferencias encontradas en el contenido hídrico de la pulpa deben ser una respuesta fenotípica a las condiciones climáticas durante el desarrollo de las gálbulas (Debussche *et al.* 1987), mucho más húmedas en el archipiélago de Azores.

La mayor viabilidad de semillas de *J. brevifolia* respecto a *J. cedrus*, ubica a la especie azoriana en una posición más favorable para la regeneración natural. Sin embargo, la situación para *J. cedrus* se ve bastante más comprometida, probablemente como consecuencia de la combinación de factores tales como la baja proporción de individuos sexualmente maduros, el estrés climático de la alta montaña y la importante fragmentación de las poblaciones (García *et al.* 2000), que puede provocar limitaciones en la polinización aumentando el porcentaje de embriones no desarrollados. Por otro lado, las enormes diferencias encontradas en cuanto a la viabilidad de semillas de las distintas plantas-madre en ambas especies constituyen uno de los resultados más llamativos de este primer estudio. Estas variaciones intraespecíficas muestran la importancia crucial que tiene la procedencia de las semillas en su consecuente éxito de reclutamiento, e indica que, en este aspecto, el efecto maternal podría cobrar una importancia muchas veces superior al efecto cualitativo que los dispersores ejercen sobre las semillas que dispersan.

El conjunto de dispersores de semillas y su papel en el sistema de dispersión de los cedros macaronésicos

Los trabajos II y III de esta Tesis Doctoral nos indican que el cedro canario presenta un sistema de dispersión de semillas de mayor complejidad en el que, además de las aves y los conejos introducidos, intervienen lagartos nativos (ausentes en el archipiélago de Azores). Sin embargo, a pesar de las particularidades biogeográficas de las islas donde habitan los cedros macaronésicos, en ambos casos estuvieron mejor adaptados a la ornitocoria, siendo el mirlo capiblanco *Turdus torquatus* y el mirlo común *T. merula* los dispersores más efectivos para *J. cedrus* y *J. brevifolia*, respectivamente. Este patrón coincide en gran medida con lo que ocurre en ambientes continentales, donde la dispersión de semillas de sabinas y enebros por parte de los túrdidos ha sido descrita en numerosas ocasiones (García 2001, Jordano 1993, Livingston 1972, Santos *et al.* 1999, Zamora 1990).

La saurocoria, sin embargo, es un proceso eminentemente insular (Olesen & Valido 2003). La gran cantidad de semillas de *J. cedrus* dispersadas por *Gallotia galloti* confirma que el papel de los lagartos como dispersores de corta distancia en las islas parece ser más importante y frecuente de lo que se pensaba anteriormente (p ej. Cooper & Vitt 2002, Olesen & Valido 2003, Pérez-Mellado & Corti 1993, Rodríguez *et al.* 2008, Valido 1999). Por otro lado, el efecto de la saurocoria sobre el porcentaje de germinación de semillas suele ser positivo (Traveset 1998), pero este patrón no se cumple en el caso del cedro canario. La reducción que produce *G. galloti* en la viabilidad de las semillas de *J. cedrus* podría deberse al efecto enzimático y al tiempo de retención en el tracto digestivo, bastante superior en los lagartos que en el caso de las aves (ver Traveset 1998 y referencias allí citadas). Este efecto negativo en la calidad de la dispersión de semillas merece ser estudiada en mayor profundidad, puesto que, tal y como se ha puesto de manifiesto en el trabajo I, el efecto de la planta-madre en la viabilidad de semillas podría estar afectando al resultado obtenido.

Aunque con una frecuencia de aparición de semillas baja en sus excrementos, el conejo introducido *Oryctolagus cuniculus* tuvo un efecto negativo en la calidad de la dispersión de semillas de los cedros macaronésicos, dado el elevado porcentaje de semillas dañadas (43,7 y 58,8% para *J. cedrus* y *J. brevifolia* respectivamente). Los lagomorfos pueden actuar como dispersores de semillas de plantas con fruto carnoso, entre las que se encuentran sabinas y enebros (Santos *et al.* 1999, Schupp *et al.* 1997). Sin embargo, nuestro resultado constituye un ejemplo más del impacto que las especies introducidas pueden tener en las interacciones mutualistas nativas (p ej. Nogales *et al.* 2005).

En resumen, *J. brevifolia* presenta un sistema de dispersión de semillas sólido, en el que el principal dispersor es el mirlo común, un ave nativa y muy abundante capaz del mantenimiento del flujo génico vía semilla entre poblaciones. *Juniperus cedrus*, por el contrario, pese a tener un sistema de dispersión de semillas más complejo, depende de un ave migratoria para la dispersión a larga distancia de sus semillas, lo que refleja una mayor fragilidad en su sistema. En este sentido, cabe destacar que, aunque no se encuentra globalmente amenazado (IUCN 2011), las poblaciones británicas de mirlo capiblanco han sido incluidas en la lista de especies amenazadas debido a la reducción en su rango de distribución y el acusado declive en el número de individuos reproductores durante los últimos 25 años (Burfield & Brook 2005, Sim *et al.* 2007, Sim *et al.* 2010). Por tanto, es importante tener en cuenta que la conservación del cedro canario depende a su vez de la conservación de sus interacciones mutualistas. La recuperación de las poblaciones de cuervo en las Islas Canarias y la conservación del mirlo capiblanco tanto en las áreas de cría como de invernada, podrían constituir la clave para la persistencia de *J. cedrus* en el archipiélago canario.

Patrones evolutivos de Juniperus brevifolia y J. cedrus

Las filogenias de la sección *Juniperus* realizadas en los trabajos IV y V indicaron un origen monofilético tanto para *J. brevifolia* como para *J. cedrus*. Los árboles obtenidos también apoyaron la monofilia de *J. maderensis*, que parece haber seguido una historia evolutiva distinta a la de *J. cedrus*. Este resultado coincide con Adams *et al.* (2010) que, en base a datos bioquímicos y de secuencias de ADN plastidial, propusieron al "cedro de Madeira" como una especie diferente al cedro canario. Por tanto, los resultados de la reconstrucción filogenética sugieren tres eventos de colonización independientes de los cedros macaronésicos a sus respectivos archipiélagos, y apoyan la predicción de múltiples eventos de colonización para especies con síndromes de dispersión endozoócoros (p ej. *Olea*, *Hedera*; Vargas 2007).

Mientras el origen de *J. brevifolia* parece estar en Europa, los datos obtenidos a partir de las secuencias de ADN plastidial no fueron lo suficientemente resolutivos en el caso de *J. cedrus*, a pesar del elevado número de muestras procedentes de Marruecos y el Oeste de la cuenca Mediterránea. Estos resultados poco concluyentes evidencian una historia de colonización compleja en torno al origen espacial de *J. cedrus*, que merece ser estudiada con mayor profundidad.

El análisis de la diversidad intraespecífica mediante secuencias de ADN plastidial mostró una diversidad de haplotipos bastante superior para el cedro do mato (16 haplotipos) en comparación con el cedro canario (4 haplotipos), lo que podría deberse a las drásticas reducciones de los tamaños poblacionales en Canarias. En ambos casos, se detectó una distribución amplia de los haplotipos más frecuentes y ancestrales, mientras que los haplotipos recientes mostraron una distribución más restringida, muchas veces relegados a una sola isla. Este patrón, indicaría un aislamiento significativo de los haplotipos recientes previo a la colonización interinsular, lo que podría interpretarse como algún tipo de restricción en la capacidad de dispersión. Sin embargo, dado el potencial dispersivo de los cedros macaronésicos (anemofilia, endozoocoria, gálbulas con varias semillas), las colonizaciones interinsulares recientes podrían estar siendo impedidas por la existencia de cedros previamente establecidos que presentaran haplotipos ancestrales. Esta teoría, tiene relación con la “Hipótesis de la naturalización de Darwin” (Darwin 1859) pero a nivel intraespecífico, y requiere ser investigada con mayor profundidad en la flora de Azores, ya que el mismo patrón de distribución de haplotipos ancestrales y recientes ha sido descrito para *Picconia azorica*. En el caso de *J. cedrus*, sin embargo, su distribución actual tan restringida (sólo cuatro de las siete Islas Canarias) y el bajo número de individuos, impiden el planteamiento de esta teoría de forma inequívoca.

En general, los resultados del trabajo IV indican una dispersión interinsular temprana y activa de *J. brevifolia* tras la colonización del archipiélago de Azores, a pesar de las distancias geográficas que separan los grupos de islas (> 100 km). La diferenciación genética encontrada parece estar en relación con la secuencia temporal de emergencia de las islas. De este modo, si dejamos de lado la isla de Santa Maria (que es la más antigua, pero también ha sido drásticamente deforestada y donde sólo se encontraron y muestrearon dos individuos de *J. brevifolia*), encontramos que las siguientes islas más antiguas (São Miguel y Terceira, ambas anteriores al Pleistoceno), han jugado un papel primordial en la diversificación genética y en la conectividad en el archipiélago. El dinamismo en la colonización de *J. brevifolia*, aunque seguido de un establecimiento más parsimonioso de los linajes, debe haber sido promovido por los síndromes de dispersión de este cedro, favorables para un flujo génico exitoso y dinámico. Además, la gran amplitud ecológica de *J. brevifolia* (Elias 2007) así como la elevada homogeneidad ambiental en el archipiélago de Azores (Carine & Schaefer 2010), constituyen factores sin duda favorables para el establecimiento y, por tanto, para el éxito de la colonización.

Por otro lado, los resultados obtenidos en el trabajo V indican una situación bien diferente para el cedro canario, cuya composición genética se ha visto empobrecida debido a la pérdida de variación por cuellos de botella muy acusados en las poblaciones. En este caso, pese a ser una especie con potencial dispersivo suficiente para mantener un flujo génico dinámico entre las islas, se detectó un aislamiento insular moderado, probablemente debido a la importante fragmentación poblacional sufrida tras la colonización europea de las islas en el s. XV. Sin embargo, dentro de este escenario, el análisis intraespecífico basado en AFLPs señaló a la isla de La Gomera como un “santuario” de diversidad genética en comparación con el resto de islas donde se distribuye *J. cedrus*, que presentaron niveles mucho menores de polimorfismo. Este patrón, por un lado, podría ser el resultado de talas menos intensas en las islas de menor tamaño que hubieran permitido conservar una mayor proporción de la diversidad genética que en algún momento pudo haber estado presente también en las islas de mayor tamaño. Por otro lado, La Gomera es una isla de gran antigüedad (11 Ma), estabilidad y complejidad orográfica, factores que podrían haber permitido la acumulación de variación genética durante largos periodos evolutivos. Aunque existen datos que apoyan la existencia de grandes poblaciones de *J. cedrus* en Gran Canaria y Tenerife (Hollermann 1978, Leuschner 1996), la falta de información histórica en este sentido impide decantarse por una u otra hipótesis. Independientemente de cuál sea la causa de los altos niveles de diversidad genética en La Gomera, esta isla merece especial atención desde el punto de vista del manejo de *J. cedrus*, cuya variación genética debe ser conservada para salvaguardar el éxito y la persistencia de sus poblaciones en las Islas Canarias.

Conclusiones Principales

1. Los cedros macaronésicos presentan notables diferencias en la fenología y las características morfológicas de las gálbulas maduras. Mientras *Juniperus cedrus* mantiene sus gálbulas maduras en la planta durante todo el año, *J. brevifolia* sólo muestra disponibilidad para los dispersores durante el verano y el otoño. Las diferencias morfológicas de las gálbulas maduras indican dos sistemas de dispersión de semillas que han evolucionado en presencia de agentes dispersantes de distinta talla, mayores en el caso del cedro canario.

2. *Juniperus brevifolia* muestra una viabilidad de semillas superior a la de *J. cedrus*, reflejando una situación más ventajosa para la regeneración natural. Las condiciones climáticas extremas de la alta montaña en el área de distribución de *J. cedrus* estudiada, así como limitaciones en la polinización debidas a la fragmentación de las poblaciones, constituyen factores que comprometen el éxito de reclutamiento del cedro canario.

3. La enorme variación en cuanto a la viabilidad de las semillas de las distintas plantas-madre de ambas especies de cedro evidencia la importancia del efecto maternal sobre el éxito en la germinación de las semillas, que podría ser muchas veces superior al efecto cualitativo ejercido por sus dispersores.

4. Las gálbulas de *J. cedrus* constituyen el componente principal de la dieta del mirlo capiblanco *Turdus torquatus* durante su invernada en la alta montaña de Tenerife. Tras la extinción del cuervo *Corvus corax* en esta área, el cedro canario depende de esta interacción no descrita anteriormente para la dispersión a larga distancia de sus semillas, lo que denota cierta fragilidad en el sistema.

5. Tanto *J. cedrus* como *J. brevifolia* demuestran ser especies adaptadas a la ornitocoria, siendo *T. torquatus* y *T. merula* los principales dispersores de semillas, respectivamente. *Juniperus cedrus* presenta un sistema de dispersión de semillas más complejo, en el que los lagartos nativos juegan un importante papel cuantitativo. A nivel cualitativo, sin embargo, su efecto no parece ser del todo beneficioso. El conejo *Oryctolagus cuniculus*, mamífero introducido tanto en Canarias como en Azores, actúa en ambos casos como disruptor del sistema natural de dispersión de semillas.

6. La dispersión de semillas de *J. cedrus* recae en los lagartos principalmente durante los meses más cálidos y en el mirlo capiblanco durante los meses de su invernada. Este reemplazamiento temporal de los principales frugívoros permite que, en concordancia con la fenología de las gálbulas maduras, la dispersión de sus semillas se lleve a cabo durante todo el año.

7. La reconstrucción filogenética de la sección *Juniperus* sugiere historias evolutivas independientes para los tres cedros macaronésicos, y apoya la propuesta del "cedro de Madeira" como una especie diferente a *J. cedrus*. Mientras el origen espacial de *J. brevifolia* parece estar en Europa, en el caso de *J. cedrus* los datos obtenidos fueron poco concluyentes.

8. *Juniperus brevifolia* presenta una elevada diversidad haplotípica. La amplia distribución de los haplotipos más frecuentes y ancestrales apunta a una diversificación y una colonización inter-insular temprana que debió estar favorecida por las características reproductivas de *J. brevifolia*, así como por factores favorables para el establecimiento. Por el contrario, la distribución restringida de los haplotipos más recientes denota un aislamiento significativo de estos haplotipos previo a la colonización interinsular.

9. La secuencia temporal de emergencia de las islas Azores ha sido fundamental en el patrón de diferenciación genética de *J. brevifolia.* Exceptuando Santa Maria, las islas de mayor antigüedad como São Miguel y Terceira han jugado un papel central en la diversificación genética y en la dispersión de los haplotipos a través del archipiélago.

10. La escasa diversidad haplotípica de *J. cedrus* evidencia una composición genética depauperada como consecuencia de la pérdida de variación por cuellos de botella muy acusados en las poblaciones. La drástica fragmentación sufrida por las intensas talas se ve reflejada en la estructura poblacional, que muestra un aislamiento insular moderado pese al potencial dispersivo del cedro canario.

11. De las cuatro islas canarias donde se distribuye *J. cedrus*, La Gomera presenta los mayores valores de diversidad y diferenciación genética, lo que hace que esta isla merezca especial atención desde el punto de vista de la conservación.

12. Con una mejor capacidad de regeneración natural, un sistema de dispersión de semillas más robusto y altos niveles de diversidad genética, el cedro do mato se encuentra en un mejor estado de conservación que el cedro canario. Sin embargo, numerosas perturbaciones antropogénicas continúan en la actualidad afectando a las poblaciones de *J. brevifolia*, que deben ser paliadas con el fin de evitar la extinción de esta especie en las islas donde se encuentra más amenazada. El cedro canario, pese al declive de las poblaciones de cuervo en las islas, parece mantener aún su capacidad para la dispersión a larga distancia. Su conservación se encuentra ligada en gran medida a la conservación de sus interacciones mutualistas, así como al mantenimiento de su diversidad genética, imprescindible para salvaguardar su potencial adaptativo y, por tanto, la perpetuación de sus poblaciones en Canarias.

Main Conclusions

1. Macaronesian junipers show notable differences in the phenology and morphological characteristics of mature female cones. While in *Juniperus cedrus* they remain available on the tree all year round, *J. brevifolia* can only be dispersed during summer and autumn. Morphological differences in mature female cones indicate two seed dispersal systems that have evolved in contact with different-sized dispersal agents, larger in the case of the Canarian juniper.

2. *Juniperus brevifolia* shows greater seed viability than *J. cedrus*, reflecting a more favourable situation for natural regeneration. The harsh high-mountain climatic conditions in the studied range of *J. cedrus* and pollination limitation due to the fragmented populations are liable to affect the successful recruitment of this juniper.

3. The enormous variation in seed viability among mother plants of the two juniper species provides evidence of the maternal effect on seed germination success. This may be many times greater than the qualitative effect of the dispersers.

4. *Juniperus cedrus* female cones are the principal component of the winter diet of the ring ouzel *Turdus torquatus* during its stay in the high mountain area of Tenerife. Since the extinction of the raven *Corvus corax* in that area, this juniper depends on this previously undescribed interaction for long-distance seed dispersal, which reflects a certain fragility in the system.

5. *Juniperus cedrus* and *J. brevifolia* are species well adapted to ornithochory, *T. torquatus* and *T. merula* being their main seed dispersers, respectively. *Juniperus cedrus* presents a more complex dispersal system in which native lizards play a major role quantitatively. However, their qualitative effect does not appear to be beneficial overall. The European rabbit *Oryctolagus cuniculus,* introduced in the Canaries and the Azores, acts in both cases as a disruptor of the natural seed dispersal system.

6. Seed dispersal of *J. cedrus* mainly depends on the lizards during the warmer months and on the ring ouzel during their wintering stay. So, in accordance with the female cone phenology, there is a replacement of the main frugivores that allows *J. cedrus* seed dispersal to occur all year round.

7. Phylogenetic reconstruction of the section *Juniperus* suggests independent evolutionary histories for the three Macaronesian junipers, supporting the proposal of the Madeiran juniper (*Juniperus maderensis*) as a different species from *J. cedrus*. While the geographical origin of *J. brevifolia* seems to be in Europe, the results for *J. cedrus* were not so conclusive.

8. *Juniperus brevifolia* shows a high diversity of haplotypes. The wide distribution of the most frequent ancestral ones reflects an early diversification and inter-insular colonization that was probably favoured by the reproductive traits of *J. brevifolia*, and factors aiding its establishment. In contrast, the restricted distribution of the most recent haplotypes points to a significant period of isolation prior to inter-island colonization.

9. The temporal sequence of island emergence in the Azores has played a key role in the genetic differentiation pattern of *J. brevifolia*. Except from Santa Maria, the other old islands, São Miguel and Terceira, have been fundamental in generating genetic variation and dispersal of the haplotypes across the archipelago.

10. The low haplotype diversity of *J. cedrus* is evidence of an impoverished genetic composition due to the loss of variation caused by very narrow bottlenecks in natural populations. The drastic fragmentation as a consequence of intense felling is also reflected in the population structure, which shows a moderate insular isolation despite the dispersal potential of *J. cedrus*.

11. Of the four Canary Islands where *J. cedrus* grows, La Gomera shows the highest values of genetic diversity and differentiation, which highlights this island's importance from the conservation point of view.

12. Given its better capacity for natural regeneration, its more robust seed dispersal system and higher levels of genetic diversity, *J. brevifolia* shows a better conservation status than *J. cedrus*. However, numerous anthropogenic disturbances nowadays continue to affect its populations. These deserve special management attention, to avoid the species becoming extinct on the islands where it is most threatened. *Juniperus cedrus*, however, despite declining raven populations in the Canaries, appears to retain its capacity for long-distance dispersal. Its conservation is strongly linked to the maintenance of its mutualistic interactions and genetic diversity, essential to safeguard its potential for adaptation and therefore the long-term continuance of the Canarian populations.

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Muy recientemente nos hemos enterado de que la naturaleza se cansa, como nosotros, sus hijos; y hemos sabido que, como nosotros, puede morir asesinada. Ya no se habla de someter a la naturaleza: ahora hasta sus verdugos dicen que hay que protegerla. Pero en uno u otro caso, naturaleza sometida o naturaleza protegida, ella está fuera de nosotros. La civilización que confunde a los relojes con el tiempo, al crecimiento con el desarrollo y a lo grandote con la grandeza, también confunde a la naturaleza con el paisaje, mientras el mundo, laberinto sin centro, se dedica a romper su propio cielo.

Eduardo Galeano

Cuatro frases que hacen crecer la nariz de Pinocho