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Tesis Doctoral - Carlos Lara Romero

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Evaluación de los factores que determinan la respuesta de las plantas de alta montaña mediterránea ante el cambio global. Una perspectiva integradora.

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A mis padres.
Gracias por vuestro amor, dedicación y apoyo incondicional.

Dr. José María Iriondo Alegría, Catedrático del Departamento de Biología y Geología, Física y Química Inorgánica y Analítica de la Universidad Rey Juan Carlos,

CERTIFICA:

Que los trabajos de investigación desarrollados en la memoria de tesis doctoral: “Evaluación de los factores que determinan la respuesta de las plantas de alta montaña mediterránea ante el cambio global. Una perspectiva integradora", han sido realizados bajo su supervisión y son aptos para ser presentados por el licenciado Carlos Lara Romero ante el tribunal que en su día se consigne, para aspirar al Grado de Doctor en el Programa de Doctorado de Conservación de Recursos Naturales por la Universidad Rey Juan Carlos.

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Tesis Doctoral

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Director: José María Iriondo Alegría

“*Many of my colleagues in other fields are surprised to learn that the study of biodiversity is still largely in a Linnaean phase of discovering and naming new species. Although our tools are more advanced, in many ways the science of biodiversity is not much farther along than medicine was in the Middle Ages. We are still at the state, as it were, of cutting open bodies to find out what organs are inside. The low investment in and slow pace of biodiversity research might be tolerable were it not for the overwhelmingly rapid destruction of the natural world. Without hyperbole we can truthfully say that we are almost out of time to save much of the diversity of life on Earth*”.

Stephen P. Hubbell, 2001
The Unified Neutral Theory of Biodiversity and Biogeography

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RESUMEN

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SÍNTESIS

Dada la elevada vulnerabilidad de los ecosistemas de montaña mediterráneos al cambio climático y otros motores de cambio global, resulta de primordial interés evaluar su capacidad de respuesta en el contexto actual y futuro de cambio ambiental. Existen, sin embargo, lagunas significativas en el conocimiento disponible sobre algunos de los mecanismos que están gobernando las respuestas que permiten su supervivencia en el actual escenario de cambio. Teniendo en cuenta estas consideraciones el objetivo general de la tesis doctoral es completar el conocimiento sobre los principales factores que determinan la respuesta de las plantas de alta montaña mediterránea al cambio global. Para ello, se ha seleccionado la comunidad de pastos psicroxerófilos de la Sierra de Guadarrama, de la cual se conocen numerosos aspectos de la biología, estado demográfico y éxito reproductivo de dos de sus especies más representativas: *Silene ciliata* y *Armeria caespitosa*. La tesis ha seguido un enfoque integral que contemple conjuntamente el patrón espacial del hábitat junto a la capacidad de dispersión de las especies, las redes de interacciones bióticas, la estructura genética espacial y la base genética de la variación fenotípica. Todos estos factores se evaluaron a lo largo de gradientes ambientales para recoger la elevada variabilidad ambiental a escala local que caracteriza a los ambientes de alta montaña y que puede afectar de manera significativa a los factores estudiados en la tesis.

En el **capítulo 1** hemos utilizado una aproximación de modelización inversa para evaluar los patrones de reclutamiento en cinco poblaciones de *Silene ciliata* y *Armeria caespitosa* a lo largo de un gradiente altitudinal. La distancia media de dispersión efectiva fue corta en ambas especies (rango: 0,23 – 0,71m), probablemente debido a que procesos post-dispersivos relacionados con la sequía estival reducen su distancia de dispersión primaria. Ambas especies mostraron variación interpoblacional en el tipo de kernel que proporciona mejores ajustes y en el parámetro de fecundidad. Sin embargo, solo *A. caespitosa* exhibió diferencias significativas entre poblaciones en la distancia media de dispersión. La variación ambiental altitudinal condicionó la fecundidad de los individuos en ambas especies pero no afectó a la escala espacial de la dispersión. Estos resultados resaltan la complejidad de la variación intraespecífica en los procesos que controlan el reclutamiento y sugieren la necesidad de análisis específicos en un amplio espectro de especies y ambientes para evaluar la prevalencia y magnitud de la variación intraespecífica de la dispersión. Otra conclusión significativa es que la baja distancia de dispersión de estas especies podría restringir su capacidad migratoria frente al cambio climático y limitar el flujo genético entre poblaciones.

En el **capítulo 2** aplicamos herramientas de análisis de autocorrelación espacial para cuantificar la estructura espacial demográfica y genética en cinco poblaciones de *Silene ciliata* estables demográficamente pero espacialmente heterogéneas. Encontramos estructura espacial genética a escala local (FSGS por sus siglas en inglés) significativa en tres de las cinco poblaciones estudiadas, confirmando la existencia de variación intra-poblacional. Se detectó una asociación positiva entre la intensidad de la FSGS y el nivel de agregación espacial de las plantas. Los cambios en las condiciones ambientales debidos a variaciones en la cantidad de hábitat adecuado y la heterogeneidad del hábitat afectaron parcialmente a la agregación de las plantas, y por lo tanto, podrían ser un factor clave a la hora de explicar los niveles de FSGS estimados. Los índices de diversidad genética y endogamia observados en las poblaciones estudiadas fueron homogéneos. Consecuentemente, no se encontró relación entre la intensidad de la FSGS y dichos parámetros. Esto sugiere que la variabilidad ambiental a escala local no está afectando a la variabilidad genética y endogamia de las poblaciones estudiadas.

El objetivo del **capítulo 3** fue evaluar si la variación fenotípica observada en un conjunto de rasgos reproductivos y vegetativos en las poblaciones naturales de la especie tiene una base genética. Con este propósito utilizamos una aproximación de jardín común (*Common garden*) para cuantificar la variación en este conjunto de rasgos en cuatro poblaciones situadas en los límites elevacionales superior e inferior del rango de distribución de la especie. Las plantas procedentes del límite de elevación superior florecieron antes y produjeron más frutos viables, pero crecieron menos en comparación con las plantas procedentes del límite de elevación inferior. Estos resultados confirman que la variación inter-poblacional observada en algunos de los rasgos ligados a la fenología y el éxito reproductivo tiene base genética. El estudio también mostró que la respuesta selectiva en favor de una floración temprana detectada en el límite de elevación inferior, no ha generado una mayor proporción de fenotipos de floración temprana. Sin embargo, sugerimos que la variabilidad genética asociada al inicio de la floración, podría ser relevante para hacer frente al impacto ocasionado por el cambio climático en curso.

En el **capítulo 4** hemos utilizado técnicas de análisis redes complejas para cuantificar el efecto de la matorralización de los pastos de alta montaña en la red de interacciones planta-polinizador. Construimos matrices de interacción cuantitativas para comparar parcelas matorralizadas frente a parcelas en las que el matorral no estaba presente. La matorralización de los pastos estudiados tuvo un efecto positivo sobre la riqueza y la frecuencia de visitas de los polinizadores a las plantas, pero incrementó la competencia entre plantas por los servicios de polinización. Esta última situación podría reducir el éxito reproductivo en alguna de las especies de plantas estudiadas. Un amplio número de grupo funcionales de polinizadores visitaron a las especies de matorral en mayor proporción que a la mayoría de las especies de plantas de la comunidad, lo que otorgó a los matorrales un rol de super-generalista. Pese a ello, la estructura de la red de interacciones planta-polinizador apenas mostró cambios significativos en sus propiedades estructurales. El efecto diferencial del matorral en las plantas y los polizadores resalta la necesidad de utilizar un enfoque integrador para evaluar el efecto de los motores de cambio global sobre las interacciones interespecíficas y la estructura y el funcionamiento de las comunidades ecológicas.

SUMMARY

Global change drivers are causing relevant alterations in Mediterranean high-mountain ecosystems. Although some first attempts to evaluate the effects of these modifications have been made, we are still far from understanding in detail the factors and processes that determine how Mediterranean high-mountain plants respond to accelerated climate change and other important drivers of global change. Thus, in this doctoral dissertation we followed an integrative approach to study three large groups of factors which *a priori* are determinant: a) the spatial pattern of the habitat together with the dispersal capacity of each species, b) the networks of biotic interactions and c) the spatial genetic structure and the genetic basis of the phenotypic variation. The study site chosen was high-mountain psicroxerophilous pastures of the Central System (Spain), as this ecosystem is one of the most vulnerable to climate change and other important global change drivers. Most studies were performed on five populations of each of two representative species of the community: *Silene ciliata* Poiret and *Armeria caespitosa* (Gómez Ortega) Boiss. i*n* DC.

In **chapter 1** we applied an inverse modeling approach to measure the net reproductive rate and effective dispersal patterns in five populations of both *S. ciliata* and *A. caespitosa* along an altitudinal gradient. Results showed that no single kernel function provides the best fit across all populations, although estimated mean dispersal distances were short in all cases (range: 0.23 – 0.71 m). Post-dispersal factors related to summer drought appeared to restrict effective seed dispersal in these two species. The altitudinal gradient appears to influence effective seed dispersal patterns in *S. ciliata* and *A. caespitosa* through adult plant fecundity and seedling density, but not through the effective seed dispersal range. There were significant differences in the fecundity parameter among populations in both species and in the mean dispersal distance among populations of *A. caespitosa*, while differences in the mean dispersal distance among *S. ciliata* populations were only marginally significant. These results illustrate the complexity of intraspecific

variation in the processes underlying recruitment and call for a case-by-case analysis in a wider range of plant taxa and environments to assess the prevalence and magnitude of intraspecific dispersal variation. Additionally, we suggested that the limited dispersal range of the two studied species raises uncertainty on their ability to track their climatic niche through migration under ongoing rapid global warming.

In **chapter 2** we used spatial autocorrelation analysis to assess the spatial demographic and genetic structure of five demographically stable but spatially heterogeneous populations of *S. ciliata*. We found significant FSGS in three of the five populations studied, and therefore, revealed among-population variation in the intensity of FSGS. There was a significant positive association between the spatial demographic and genetic structure measured in each population, probably as a result of changes in environmental conditions due to variation in suitable habitat availability and microhabitat heterogeneity. Finally, we did not detect any relationship between population genetic diversity and inbreeding with FSGS, suggesting that population genetic diversity and inbreeding are not related with fine-scale environmental variation.

In **chapter 3** we focused on *A. caespitosa* to assess whether the observed phenotypic variation in a suite of reproductive and vegetative traits in natural population has a genetic basis or is the result of plastic responses shaped by heterogeneous environmental conditions. For this purposes we used a common garden approach to study the variation of such traits in four *A. caespitosa* populations at the high and low edges of the species distribution range. Our results confirmed that among-population variation in flowering phenology and some relevant traits related to reproductive performance is genetically based. The later flowering onset found in the plants of the low-edge of the species distribution did not correspond with the stronger selection response in favor of early-flowering individuals previously reported in natural populations. Finally, we suggested that the inherited capacity of the species to shift its flowering phenology coupled with the observed genetic variation within populations indicates that there is adaptive potential to respond to ongoing global warming.

In **chapter 4** we used network analysis tools to investigate the effects of shrub encroachment on plant-pollinator interactions at the community level. We constructed quantitative visitation networks to compare replicated shrub encroached and un-encroached plots. Results showed distinctive effects of shrub encroachment on plant and pollinator species because it increased richness and visitation patterns of pollinators, but also increased pollinator-mediated competition among plant species, which can potentially result in reduced reproductive success. In spite of the central role of shrub species the structure of the networks remained relatively unchanged. This result is congruent with previous findings in the literature suggesting that plant-pollinator mutualism may be robust to the effects of global change.

ANTECEDENTES

Motores de cambio global y respuestas de las comunidades vegetales.

El concepto de cambio global incluye todos aquellos impactos originados por actividades antrópicas que modifican la composición y funcionamiento de los ecosistemas (Vitousek, 1992). Como consecuencia de estas alteraciones, la biodiversidad mundial está cambiando a un ritmo sin precedentes (Sala *et al.,* 2000; Thuiller *et al.,* 2005; Parmesan *et al.,* 2006), lo que se ha convertido en una cuestión importante tanto para la investigación ecológica como para los programas gubernamentales sobre cambio global (Sala *et al.,* 2000; MEA, 2005; IPCC, 2007; Thuiller *et al.,* 2008; Thomas *et al.,* 2010; Pauli *et al.,* 2012). Los cambios en los usos del suelo y el cambio climático acelerado son probablemente los motores de cambio global que tienen mayor incidencia sobre los ecosistemas terrestres (Vitousek *et al.,* 1992; Sala *et al.,* 2000). De hecho, existen considerables evidencias sobre su efecto directo en alteraciones del rango geográfico de las especies y en su presencia y abundancia en un conjunto amplio de ecosistemas (Tilman *et al.,* 2002; Parmesan *et al.,* 2006; Reidsma *et al.,* 2006; Thomas, 2010; Eldridge *et al.,* 2011; Pauli *et al.,* 2012).

Un gran número de estudios empíricos y teóricos indican que la principal respuesta de las plantas al actual escenario de cambio global es modificar su distribución geográfica desplazándose a latitudes o altitudes mayores siguiendo su actual nicho ambiental, lo que en muchos casos implica una disminución del área de ocupación (Thuiller *et al.,* 2004; Thomas *et al.,* 2006; Thuiller *et al.,* 2008; Thomas, 2010; Gottfried *et al.,* 2012; Pauli *et al.,* 2012). Sin embargo, las respuestas específicas de las especies al cambio global son complicadas de predecir, ya que las diferentes especies no responden de igual manera a los motores de cambio y los factores que las condicionan se encuentran estrechamente relacionados (Figura 1). Enfrentarse al cambio desplazándose puede no ser factible para algunas especies porque la tasa de cambio ambiental será más rápida que su capacidad de dispersarse siguiendo su nicho ambiental (Davis y Shaw *et al.,* 2001; Thuiller *et al.,* 2004; Thuiller *et al.,* 2005; Thomas *et al.,* 2006; Dullinger *et al.,* 2012b). Además, aunque la especie tenga capacidad para alcanzar un hábitat compatible con sus necesidades, los cambios no tienen por qué ocurrir de forma coordinada con otras especies (Memmot *et al.,* 2007; Tylianakis *et al.,* 2008; Hegland *et al.,* 2009; Svenning *et al.,* 2014). La viabilidad de las especies vegetales se encuentra condicionada en gran medida por la estructura y composición de las redes de interacciones bióticas de la comunidad a la que pertenecen (Tylianakis *et al.,* 2008; Hegland *et al.,* 2009; Svenning *et al.,* 2014). Por ello, otros factores relacionados con la interacción entre especies y sus efectos sobre parámetros demográficos y la configuración espacial de las comunidades vegetales podrían limitar su establecimiento en el nuevo ambiente (Brooker *et al.,* 2007; Svenning *et al.,* 2014). A consecuencia de estas limitaciones, existen diversos modelos predictivos que prevén un rápido incremento del riesgo de extinción a nivel mundial durante las próximas décadas (Tilman *et al.,* 2002; Thomas *et al.,* 2004*;* Thuiller *et al.,* 2005; Dullinger *et al.,* 2012a). En estos casos, y si la opción de desplazamiento es inviable, otra alternativa a la extinción es permanecer en el lugar ajustando el fenotipo a las nuevas condiciones ambientales mediante procesos de adaptación evolutiva o plasticidad fenotípica (Davis *et al.,* 2005; Miner *et al.,* 2005; Jump y Peñuelas, 2005; Gienapp *et al.,* 2008; Chuine *et al.,* 2010).

El concepto de adaptación evolutiva implica la actuación de una fuerza de selección que permite la evolución de un rasgo concreto, proporcionando una ventaja en un hábitat determinado (Reeve y Sherman, 1993; Kawecki y Ebert, 2004). La respuesta adaptativa a presiones selectivas direccionales derivadas de la aparición de cambios ambientales ha sido demostrada en un amplio rango de especies y sistemas, incluso en aquellos casos en los que los cambios ambientales ejercieron una presión selectiva muy intensa en poblaciones naturales (Turesson 1925; Clausen *et al.,* 1940; Linhart y Grant, 1996; Jump y Peñuelas, 2005; Parmesan 2006). La evolución implica por definición un cambio en las frecuencias alélicas, y por lo tanto, resulta necesaria una diversidad genética adecuada y heredable, sobre la que puedan actuar las presiones selectivas (Kawecki y Ebert, 2004). La configuración espacial de los fragmentos de hábitat que contienen poblaciones de una especie y la acción de los agentes polinizadores y dispersores que residan en la comunidad determinan en gran medida los flujos genéticos entre poblaciones; lo que en última instancia, configura la estructura genética espacial de la especie y su variabilidad genética (Slatkin, 1987; Linhart y Grant, 1996; Hamrick y Trappnel, 2011). Además, la existencia de una elevada heterogeneidad ambiental en el nivel local puede impedir la existencia de presiones selectivas direccionales y dificultar la aparición de adaptaciones (Kawecki y Ebert, 2004).

La plasticidad fenotípica es la capacidad de un organismo de producir fenotipos diferentes en respuesta a cambios en su ambiente (Schlichting, 1986; Ghalambor *et al.,* 2007). Esta respuesta permite ajustes más rápidos y flexibles en los individuos, cuando la respuesta por una vía genética resulta insuficiente (Schlichting, 1986; Miner et al., 2005). A largo plazo, las respuestas plásticas a cambios ambientales extremos tienen sus límites (Bradshaw y McNeilly, 1991; Jump y Peñuelas, 2005; Gienapp et al., 2008). Sin embargo, en estos escenarios extremos, pueden promover el establecimiento y la persistencia en el nuevo ambiente situando a la especie cerca del nuevo fenotipo óptimo, lo que facilita que la evolución adaptativa ocurra en una escala de tiempo ecológica (Van Tienderen, 1997; Ghalambor *et al.*, 2007). La capacidad de ajustar el fenotipo a las nuevas condiciones ambientales está intrínsecamente ligada a una serie de procesos, tales como la tolerancia fisiológica, mecanismos a nivel demográfico u otros que implican cambios en las interacciones entre especies, y que por sí mismos, también pueden permitir a las poblaciones ser viables localmente sin necesidad de desplazarse (Doak y Morris, 2010; García-Camacho *et al.,* 2012; Lloret *et al.,* 2012; Svenning *et al.,* 2014).

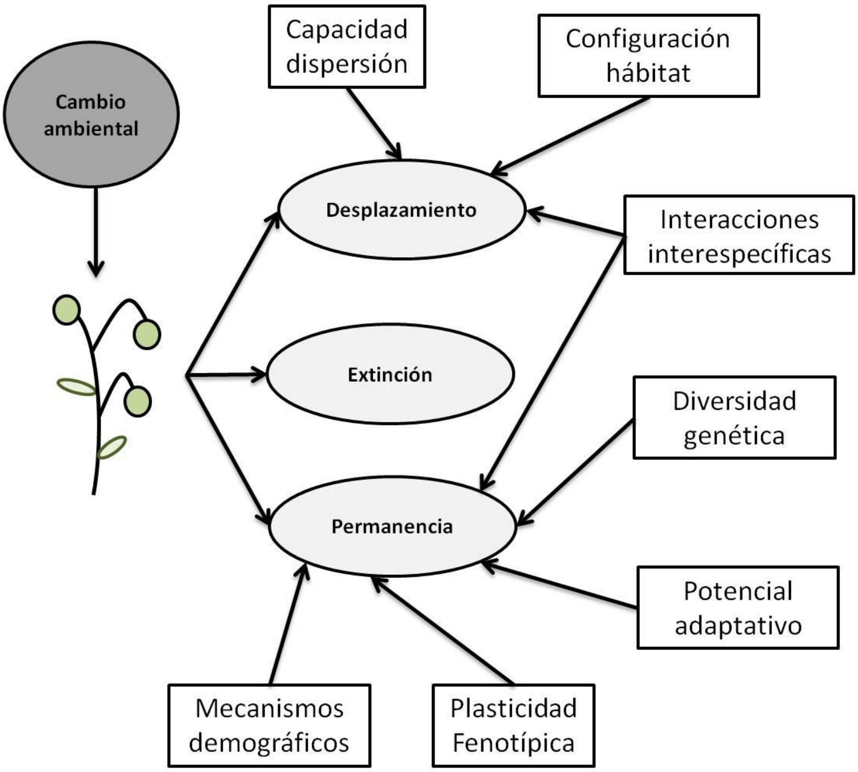

Figura 1. Modelo conceptual de las respuestas específicas de las especies vegetales (elipses de color gris claro) a cambios ambientales derivados de los motores de cambio global. Los factores que inciden de forma más relevante sobre las respuestas aparecen representados por cuadrados blancos. Algunas especies permanecerán en sus localidades originales adaptándose a las nuevas condiciones ambientales mediante procesos de adaptación evolutiva o plasticidad fenotípica. Otras modificarán su distribución geográfica al desplazarse a latitudes o altitudes mayores siguiendo el clima al cual están adaptadas, mientras que otras no podrán responder lo suficientemente rápido como para escapar de la extinción.

En definitiva, son numerosos los factores que pueden influir en la respuesta de las especies a los motores cambio global. Todos estos factores pueden actuar en la misma dirección o en direcciones opuestas y también pueden ver sus efectos potenciados o aminorados entre sí. Todo ello ocasiona que la respuesta de las especies a los cambios ambientales sea específica de cada especie, lo que dificulta enormemente pronosticar el estado y la funcionalidad de los ecosistemas bajo los cambios físicos, biológicos y químicos que se están produciendo en la actualidad (Clark *et al.,* 2001; Higgins *et al.,* 2003; Svenning *et al.,* 2014). Es necesario, por tanto, adquirir una aproximación multidisciplinar para estudiar los principales determinantes de las respuestas de las plantas a los cambios ambientales acusados y evaluar su capacidad de respuesta ante un escenario de cambio global.

Vulnerabilidad de los ecosistemas de alta montaña mediterránea al cambio global.

Las comunidades vegetales de alta montaña se encuentran entre los organismos más vulnerables al cambio global y particularmente a uno de sus motores más notables, el cambio climático (Thuiller *et al.,* 2005; Parmesan 2006; Nogues-Bravo *et al.,* 2007; Engler *et al.,* 2011). Pese a su escasa superficie, estos ecosistemas tiene un valor desproporcionado en términos de diversidad biológica y de servicios ecosistémicos (Körner *et al.,* 2007; Nogues-Bravo et al 2008; Nagy y Grabherr 2009; Barrio *et al.,* 2013), y es por ello que constituyen una prioridad de investigación. Su distribución se ve afectada de manera natural por la existencia de un gradiente altitudinal que pone gradualmente fin a las condiciones ambientales que permiten su existencia y marca los límites altitudinales de distribución. Esto otorga al ecosistema un carácter insular al configurar en el espacio una red de fragmentos de hábitat por encima de una determinada altitud, que supone el aislamiento de las poblaciones por encima del límite del bosque (Escudero *et al.,* 2004; Körner *et al.,* 2007; Nagy y Grabherr 2009). Esta situación restringe la capacidad de respuesta frente al cambio climático mediante migración latitudinal, y condiciona el flujo genético entre fragmentos, especialmente si la capacidad de dispersión es baja, lo que indirectamente también condiciona su respuesta mediante tolerancia o adaptación (Engler *et al.,* 2011; Dullinger *et al.,* 2012b).

Las evidencias de los cambios originados por el clima en las comunidades de alta montaña provienen principalmente de las regiones boreales y templadas. El incremento de temperatura y precipitación asociado a la retirada de la nieve de forma temprana incrementa la producción primaria (Körner, 2003) y, en definitiva, el periodo de actividad vegetativa y la cantidad de recursos para poder invertir en reproducción. En general, la mayoría de los estudios han observado un patrón general de ascenso en altitud de especies de carácter más termófilo o peor adaptadas a los ambientes de alta montaña que implica incremento en la diversidad alfa acompañado de descensos en la diversidad beta. Esto ha supuesto una mayor homogeneidad de la comunidad en paralelo con un ascenso de los límites altitudinales superiores de las especies más orófilas de la comunidad (Paulí *et al.,* 2007; Jurasinski y Kreyling, 2007; Frei *et al.,* 2010; Gottfried *et al.,* 2012; Pauli *et al.,* 2012).

Las condiciones experimentadas por las comunidades vegetales en las montañas de la región mediterránea son marcadamente diferentes respecto a las de las regiones templadas y boreales, lo que provoca que el marco general de afección del cambio global sea diferente. Como en las formaciones de regiones más septentrionales, las montañas de gran altitud de la región mediterránea se ven sometidas a fríos inviernos que las cubren de una capa de nieve espesa que impide la actividad vegetativa en la época invernal (Körner *et al.,* 2007; Nagy y Grabherr 2009). Sin embargo, la sequía estival característica de climas mediterráneos, también se manifiesta en estas montañas. Esta situación reduce la ventaja asociada a un deshielo temprano para muchas especies tanto de carácter termófilo como orófilo, al restringir el periodo de crecimiento y reproducción al final del periodo estival (Escudero *et al.,* 2004; Giménez-Benavides *et al.,* 2007, 2011). Esta característica climática, ubicua a la región mediterránea, se puede ver agravada en el actual y futuro escenario de cambio climático, ya que la mayor parte de los modelos predictivos de clima pronostican que los cambios de temperatura y precipitación más intensos de Europa van a tener lugar en las montañas de clima mediterráneo (Nogues-Bravo *et al.,* 2008). Esto puede significar que las respuestas al cambio climático tengan que ser más intensas y rápidas que en otros sistemas montañosos situados en regiones más septentrionales. A este efecto, además hay que asociarle los desequilibrios causados por la modificación de los usos del suelo motivada por el abandono de las prácticas ganaderas tradicionales. Este proceso está interactuando con el cambio climático para beneficiar el ascenso de las plantas leñosas por encima de sus límites altitudinales actuales (Wookey *et al.,* 2009; Brandt *et al.,* 2013*)*. Un proceso de alcance global, pero que está afectando de manera significativa a las montañas mediterráneas (Peñuelas y Boada, 2003; Sanz-Elorza, 2003; García-Romero *et al.,* 2010). Finalmente, cabe destacar que las especies de carácter más orófilo ya habitan las cumbres más altas (Escudero *et al.,* 2004), por lo que resulta físicamente imposible responder mediante el desplazamiento de su límite superior (Marris, 2007). El resultado previsible es la extinción local de las poblaciones que habitan las alturas inferiores con la reducción del área de ocupación total de la especie (Marris, 2007). Existen estudios recientes que parecen confirmar esta idea, ya que han observado un patrón general de contracción del límite inferior de distribución y una reducción general de la riqueza de especies en las montañas mediterráneas del sur de Europa (Gottfried *et al.,* 2012; Paulí *et al.,* 2012).

Silene ciliata **y** ***Armeria caespitosa*** **como especies de estudio representativas de las comunidades vegetales de alta montaña mediterránea.**

Dada la elevada vulnerabilidad de los ecosistemas de montaña mediterráneos al cambio climático y otros motores de cambio global, resulta de primordial interés evaluar su capacidad de respuesta frente al actual y futuro escenario de cambio global (Regato y Salman 2008). Sin embargo, existen pocos proyectos de investigación ecológica y conservación a largo plazo en estas zonas que incrementen el conocimiento sobre las especies que las habitan y los mecanismos que están gobernando las respuestas que permiten su supervivencia en el medio (Barrio *et al.,* 2013). Esta información es imprescindible para poder diseñar e interpretar estudios específicos sobre las respuestas a nivel de especie y/o comunidad al cambio global (Clark *et al.,* 2001; Thuiller *et al.,* 2004; Thuiller *et al.,* 2008; Svenning *et al.,* 2014).

Una excepción son los trabajos desarrollados durante la última década en la comunidad de pastos psicroxerófilos de alta montaña de la Sierra de Guadarrama (Sistema Central, España). Estos estudios se han centrado principalmente en dos especies representativas de la comunidad: *Armeria caespitosa* (Gómez Ortega) Boiss. *In* DC. y *Silene ciliata* Poiret, en las que se ha evaluado el cambio en las respuestas individuales (en términos vegetativos, reproductivos, demográficos y genéticos) a lo largo de gradientes altitudinales como un subrogado de lo que puede ocurrir cuando las condiciones climáticas cambian (revisado en Escudero *et al.,* 2012). *Silene ciliata* presenta dinámicas demográficas conducentes a la extinción en su límite inferior de distribución, dónde también se han detectado evidencias de una mayor intensidad de la depresión endogámica (Giménez-Benavides *et al.,* 2008, 2011a; García-Fernández *et al.,* 2012a). Paralelamente, en estas mismas poblaciones se han identificado procesos de adaptación local que permiten aumentar el éxito en el establecimiento de plántulas, uno de los cuellos de botella demográficos de la especie, lo que sin duda, contribuye a aumentar su éxito demográfico (Giménez-Benavides *et al.,* 2007a). También se han encontrado evidencias de adaptación local en individuos adultos según su población de origen frente a un periodo de sequía extrema y relativa tolerancia en la fase de plántula a la reducción del periodo de cobertura nival (García-Fernández *et al.,* 2013; García-Fernández *et al.,* 2014). Ambos resultados sugieren cierto nivel de tolerancia fisiológica a eventos climáticos extremos. Por otro lado, *Armeria caespitosa* parece estar respondiendo variando algunos rasgos relacionadas con el éxito reproductivo entre poblaciones (García-Camacho y Escudero, 2009; García-Camacho *et al.,* 2010), mediante un mecanismo de “compensación demográfica” que implica procesos de tolerancia fisiológica y morfológica o adaptativos (Doak y Morris, 2010, García-Camacho *et al.,* 2012). Finalmente, se han detectado presiones selectivas significativas hacia una floración más temprana en algunas poblaciones de ambas especies, aunque no se pudo determinar su potencial adaptativo (Giménez-Benavides *et al.*, 2011b).

De estos estudios, se deduce que *S. ciliata* y *A. caespitosa* podrían mostrar respuestas divergentes a las condiciones impuestas por el cambio climático (Escudero *et al.*, 2012). Existen, sin embargo, lagunas significativas en el conocimiento disponible sobre algunos de los factores que inciden en las respuestas de estas especies a los motores de cambio global. Alguno de estos factores serían los relacionados con las interacciones bióticas, la capacidad de dispersión o el potencial evolutivo de la variación genotípica y fenotípica encontrada en ambas especies. Resultan necesarias por tanto investigaciones adicionales que aborden estas lagunas y faciliten el pronóstico del estado y funcionalidad de las comunidades vegetales de alta montaña mediterránea en el contexto actual y futuro de cambio ambiental (Clark *et al.*, 2001; Higgins *et al.*, 2003; Svenning *et al.*, 2014).

OBJETIVOS

El objetivo general de esta tesis es completar el conocimiento sobre los principales factores que determinan la respuesta de las plantas de alta montaña mediterránea al cambio global. Para ello, se ha seleccionado la comunidad de pastos psicroxerófilos de la Sierra de Guadarrama, de la cual se conocen numerosos aspectos de la biología, estado demográfico y éxito reproductivo de dos de sus especies más representativas: *Silene ciliata* y *Armeria caespitosa*. La tesis ha seguido un enfoque integral que contemple conjuntamente el patrón espacial del hábitat junto a la capacidad de dispersión de las especies, las redes de interacciones bióticas, la estructura genética espacial y la base genética de la variación fenotípica. Todos estos factores se evaluaron a lo largo de gradientes ambientales para recoger la elevada variabilidad ambiental a escala local que caracteriza a los ambientes de alta montaña y que puede afectar de manera significativa a los factores estudiados en la tesis.

Más concretamente, para alcanzar el objetivo general anteriormente citado, se han planteado siete objetivos específicos que se abordan en los cuatro capítulos de esta tesis doctoral:

a) Evaluar la capacidad de dispersión de *Silene ciliata* y *Armeria caespitosa* cuantificando la fecundidad y el kernel de dispersión efectiva a lo largo de un gradiente altitudinal (Capítulo 1).

b) Estimar la variación intraespecífica de la dispersión efectiva bajo las distintas condiciones ambientales impuestas por el gradiente altitudinal (Capítulo 1).

c) Cuantificar la estructura genética espacial en cinco poblaciones de *Silene ciliata* y evaluar si su intensidad está relacionada con las distintas condiciones de heterogeneidad espacial existentes o con su capacidad de dispersión (Capítulo 2).

d) Determinar si existe relación entre la intensidad de la estructura genética espacial y los índices de diversidad genética y endogamia de las poblaciones estudiadas (Capítulo 2).

e) Estudiar si la variación fenotípica observada en un conjunto de rasgos reproductivos y vegetativos en las poblaciones naturales de *Armeria caespitosa* tiene base genética (Capítulo 3).

f) Evaluar si las presiones selectivas hacia una floración más temprana detectadas en las poblaciones del límite inferior de distribución de *A. caespitosa* están promoviendo la predominancia de fenotipos de floración temprana (Capítulo 3).

g) Evaluar el efecto de la matorralización de los pastos de alta montaña en la red de interacción mutualista planta-polinizador (Capítulo 4).

METODOLOGÍA GENERAL

Área de estudio.

Las localidades en las que se ha realizado el trabajo de campo, así como la totalidad de los individuos y especies vegetales de la presente tesis doctoral forman parte de las comunidades de pastizales psicroxerófilos de alta montaña de la Sierra de Guadarrama, una de las más importantes del Sistema Central (Figura 2).

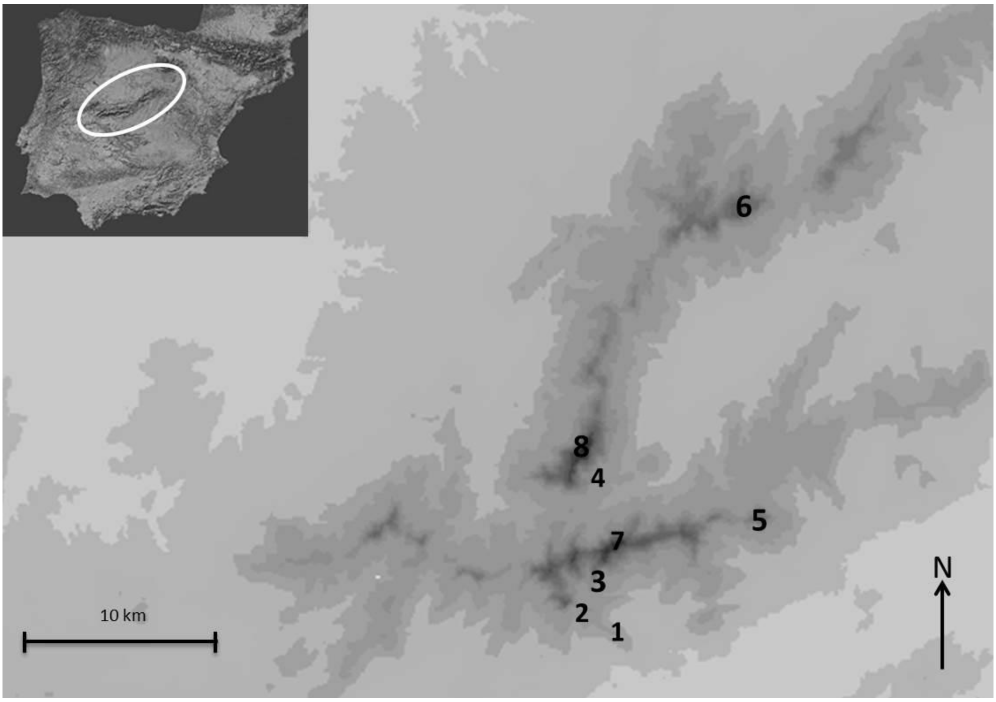

Figura 2. Localización de las localidades de estudio en la Sierra de Guadarrama (España). Las áreas sombreadas con tonos más oscuros indican mayor altitud. El mapa de la esquina superior izquierda indica la localización de la Sierra de Guadarrama en la Península Ibérica. Los números indican la posición de cada localidad y las clasifica de menor a mayor altitud (Ver tabla 1): (1) Sierra de los Porrones, (2) Collado de las Vacas, (3) Loma de Cabezas, (4) Laguna, (5) Najarra, (6) Nevero, (7) Cabezas de Hierro, (8) Peñalara.

La citada comunidad vegetal constituye la vegetación potencial de las cumbres de la Sierra de Guadarrama por encima de los 2100-2200 metros. La comunidad está dominada por la gramínea *Festuca curvifolia* Lag. Ex Lange y un importante número de nanocaméfitos almohadillados o pulviformes (Figura 3 y 4). Desde un punto de vista fitosociológico la comunidad ha sido denominada como *Hieracio myriadeni-Festucetum curvifoliae* (Rivas-Martinez, 1963) y es endémica de las cumbres de la Sierra de Guadarrama (Rivas-Martinez *et al.*, 1990). La comunidad es particularmente rica en endemismos, como *Festuca curvifolia* Lag. ex Lange, *Pilosella vahlii* (Froel.) F.W. Schultz & Sch.Bip., *Armeria caespitosa* (Gómez Ortega) Boiss. *in* DC. o *Minuartia recurva* (All.) Schinz & Thell. Algunas especies relictas de origen artico-pirenaicas como *Agrostis rupestris* All. y *Phynteuma hemisphaericum* L. enriquecen aún más la composición de especies (Escudero *et al.*, 2004). La comunidad convive intercalada en una matriz arbustiva más o menos densa caracterizada por *Cytisus oromediterraneus* Rivas Mart. e*t al.*, *Adenocarpus hispanicus* (Lam. DC.) y *Juniperus communis subsp. alpina* (Suter) Celak. Se diferencia de las comunidades genuinamente alpinas por presentar un periodo de sequía estival intensa característico de la alta montaña mediterránea, lo que, unido a la fuerte insolación recibida, confiere a las especies que los habitan caracteres propios de la vegetación xerofítica: cutículas gruesas, colores glaucos, abundancia de especies leñosas (Rivas-Martinez *et al.*, 1990). A consecuencia de su singularidad y riqueza florística, así como por el reducido área de distribución esta comunidad se encuentra incluida dentro de la Directiva Habitats de la Unión Europea (Directiva 92/43/CEE). Más información sobre la composición florística de esta comunidad puede consultarse en Escudero *et al.*, (2004).

Figura 3. Aspecto del pastizal psicroxerófilo en .las proximidades del Alto de las Guarramillas (2.200 m). Los procesos de crioturbación y las condiciones extremas confieren escasa cobertura y porte al pastizal (Fotografía: Carlos Lara).

Figura 4. Ejemplares de *Armeria caespitosa* y *Festuca curvifolia* en las proximidades de la cumbre de Peñalara (2.430 m) (Fotografía: Alfredo García).

La estación meteorológica más próxima a la zona se encuentra en el Puerto de Navacerrada (40º 46’N, 4º19’O; 1890 msnm). La precipitación media anual registrada es de 1330 mm y la temperatura media anual es de 6,3ºC (http://aemet.es). La temperatura media mensual oscila desde -1ºC en Enero hasta 16ºC en Julio. Existe un acusado periodo seco en verano (Junio – Septiembre), con menos del 10% de las precipitaciones, que reduce drásticamente el periodo vegetativo (Giménez-Benavides *et al.,* 2007b). La caracterización ecogeográfica de las localidades de estudio se incluye en la tabla 1 y figura 2. Todas las localidades se encuentran incluidas dentro del Parque Nacional de la Sierra de Guadarrama.

Tabla 1. Caracterización ecogeográfica de las localidades de estudio.

Localidad de estudio	Coordinadas geográficas	Altitud (m)	Pm (mm)	Tm (ºC)
Porrones	40° 45′N, 3° 56′O	1750	1133 (25 – 130)	8,2 (5,2 – 18,3)
Collado de las Vacas	40º 6’N, 3º 56’O	1880	1210 (28 – 142)	7,3 (-0,9 – 18,9)
Loma de Cabezas	40° 46’N, 3° 56′O	1950	1351 (30 – 153)	5,7 (-0,7 – 15,1)
Laguna	40º 50’N, 3º 57’O	1980	1347 (31 – 179)	6,6 (-1,1 – 14,8)
Najarra	40º 49’N, 3º 49’O	2080	1335 (27 – 181)	7,3 (-0,8 – 15,7)
Nevero	40º 58’N, 3º 50’O	2190	1245 (30 -144)	6,6 (-1,3 – 14,6)
Cabezas de Hierro	40° 48′N, 3° 55′O	2305	1594 (34 – 193)	4,2 (-2,8 – 13,7)
Peñalara	40º 51’N, 3º 57’O	2405	1457 (32 – 183)	4,9 (-2,6 – 13,8)

Pm: precipitación anual media, *Tm:* temperatura anual media. Las temperaturas y precipitaciones mínimas y máximas mensuales se muestran entre paréntesis. Datos climáticos obtenidos de Gonzalo-Jiménez (2011).

Especies de estudio: *Silene ciliata* y *Armeria caespitosa*.

Silene ciliata Poiret (Caryophyllaceae) es un caméfito pulviniforme perene que se distribuye en los sistemas de montaña mediterráneos del sur de Europa, desde la Península Ibérica hasta los Balcanes (Tutin, 1995). La especie alcanza uno de sus límites de distribución más meridionales en las cumbres de la Sierra de Gudarrama, donde habita un área restringida entre 1900 y 2430 m de altitud. Tiene un porte de pequeño tamaño, alcanzando los 25-30 cm de diámetro, mientras que las inflorescencias con 1 a 5 flores alcanzan hasta 15 cm de altura (Figura 5). Es una especie geitonogámica (en la planta aparecen flores hermafroditas y femeninas) con una fuerte protandria (estructuras femeninas se desarrollan posteriormente a las masculinas), lo cual limita la posibilidad de autocruzamientos, aunque la planta es autocompatible (Garcia-Fernández *et al.,* 2012b). Tiene síndrome de polinización nocturna, por lo que es polinizada principalmente por polillas (Lepidoptera: Noctuidae), aunque también puede ser polinizada por insectos diurnos, principalmente sírfidos (Himenoptera: Syrphidae). Florece a finales del verano, con un pico de floración en Agosto. El fruto es una cápsula dehiscente que contiene hasta 100 semillas dispersadas por viento entre agosto y septiembre. Las semillas, que no tienen ninguna estructura específica para promover su dispersión, tienen una masa media de 0,59 mg y su diámetro oscila entre 1,1 y 1,5 mm (Giménez-Benavides *et al.,* 2005). Su dotación cromómica en la Sierra de Gudarrama es diploide (2n=24, Garcia-Fernández *et al.,* 2012b).

Figura 5. Ejemplar de *Silene ciliata* con las flores abiertas durante la noche. Debido a su sidrome de polinización nocturna, la especie es polinizada principalmente por polillas (Fotoragía: Luis Giménez).

Armeria caespitosa (Gómez Ortega) Boiss. *in* DC. es un caméfito pulviniforme de escaso porte que pertenece a la familia Plumbaginaceae. Es una planta endémica del Sistema Central Ibérico (Sierra de Guadarrama, Ayllón y Este de Gredos), apareciendo entre los 1600 y 2430 m de altitud (Nieto-Feliner, 1996). Se desarrolla en dos hábitats bien diferenciados, pero que coexisten: en pastos psicroxerófilos sobre litosuelos pobres, poco desarrollados, y en fisuras y pequeñas repisas de afloramientos rocosos de granito y gneis (Figura 6). Sus flores rosadas se agrupan en pequeños capítulos cimosos (19 ± 8 flores por capítulo) sustentados por escapos cortos. Cada flor contiene un único ovulo, que produce una única semilla que se dispersa entre junio y agosto dentro del cáliz, engrosado durante el proceso de maduración. El peso medio de la semilla es de 1.18 mg y su diámetro oscila entre 2 y 4,7 mm (Giménez-Benavides *et al.*, 2005). Es una de las especies de floración más temprana de la comunidad, abarcando desde finales de abril en las poblaciones de menor altitud hasta finales de junio en las poblaciones más elevadas (García-Camacho y Escudero, 2009). Es polinizada por insectos generalistas, principalmente abejas (Himenoptera: Apoidea) y sírfidos (Himenoptera: Syrphidae). Tiene dotación diploide y, como la mayoría de las especies del genero *Armeria* es autoincompatible estricta, debido a un sistema dimórfico de autoincompatiblidad (García-Camacho y Escudero, 2009).

Figura 6. Principales microhábitats que ocupa *A. caespitosa*. Foto superior: individuos en afloramiento rocoso. Foto inferior: individuos en pasto psicroxerófilo de *Festuca curvifolia* (Fotografía: Raúl García Camacho).

Técnicas de análisis estadístico.

En cada capítulo de esta memoria se incluye información detallada sobre la metodología y técnicas de análisis estadístico utilizadas para responder a los objetivos concretos planteados. Este apartado incluye una breve descripción de las principales técnicas utilizadas.

Modelización inversa y funciones kernel de dispersión.

La fecundidad de las planta adultas reproductoras y la distribución de las distancias de dispersión son características fundamentales del proceso de dispersión (Ribbens *et al.,* 1994; Clark *et al.,* 1999; Nathan y Muller-Landau, 2000; Bullock *et al.,* 2006). Sin embargo, en muchos sistemas es un reto estimar la producción de semillas y las distancias de dispersión porque la trayectoria de las semillas dispersadas no puede ser establecida, y como consecuencia no se puede determinar la planta de origen (Bullock *et al.,* 2006). La modelización inversa aplica métodos de máxima verosimilitud para estimar la fecundidad de las plantas adultas y la función kernel de dispersión sin identificar la fuente exacta de la que procede cada semilla o plántula (Ribbens *et al.,* 1994; Clark *et al.,* 1999). Esta técnica asume que la contribución de cada planta adulta a la lluvia de semillas en un área dada depende sólo de la fecundidad de la planta y de su distancia a dicha área (Ribbens *et al.,* 1994; Clark *et al.,* 1999, Nathan *et al.,* 2012).

La curva o “kernel” de dispersión es una función de probabilidad de densidad que caracteriza la distribución espacial de los propágulos (semillas o plántulas) dispersados desde una fuente común (Nathan *et al.,* 2012). La mayoría de las funciones kernel de dispersión que se aplican en ecología se definen en base al parámetro de forma u y de escala a. u describe la forma de la curva del kernel de dispersión; mientras que a proporciona la extensión espacial a la que se producen los eventos de dispersión (Nathan *et al.,* 2012).

Herramientas de análisis basados en patrones de puntos

El patrón espacial de organización de las comunidades vegetales está recibiendo un interés creciente en ecología en los últimos 20 años (Wiegand *et al.*, 2012). Este interés se debe principalmente a que a través del análisis de estos patrones y de su dinámica podemos entender los procesos subyacentes que los están generando y testar hipótesis ecológicas (Legendre y Fortin 1989; Legendre *et al.*, 2002; Wiegand *et al.*, 2012). Los patrones de puntos no son más que un tipo básico de dato espacial, definidos como la posición geográfica del conjunto individuos de una especie en un área (Wiegand *et al*., 2012). El fundamento que subyace detrás de las técnicas de análisis de patrones de puntos es que algunos procesos ecológicos tienen un fuerte componente espacial que crea patrones espaciales identificables (Brown *et al.,* 2011; Wiegand *et al.,* 2012). El estadístico espacial más utilizado es la función K de Ripley (*K(r),* Ripley, 1976), que es el número de puntos (*i.e.*, plantas) dentro de un circulo de radio *r* alrededor de cualquier punto del patrón. Otros estadísticos básicos ampliamente usados en ecología son la función de correlación de par *g(r),* relacionada con la derivada de *K(r)* (Stoyan y Stoyan, 1994); y la función de la distancia al vecino más cercano *D (r),* que estima la probabilidad de tener la planta más próxima dentro de una distancia *r* (Loosmore y Ford, 2006).

Análisis de autocorrelación espacial genética.

Los análisis de autocorrelación espacial genética permiten analizar si la diversidad genética se encuentra espacialmente estructurada (*i.e.*, distribución no aleatoria de los genotipos en el espacio). El interés de la estructura espacial de la diversidad genética se debe a que la distribución no aleatoria de genotipos en el espacio influencia varios procesos evolutivos como la pérdida de diversidad genética por endogamia, el tamaño efectivo poblacional o los procesos de evolución adaptativa. Como consecuencia, puede tener un efecto significativo en la eficacia biológica de los individuos y las poblaciones (Linhart y Grant, 1996; Escudero *et* *al.,* 2004). Los análisis de autocorrelación espacial genética permiten evaluar la existencia de la estructura espacial genética y cuantificarla (Smouse y Peakall, 1999). El estadístico utilizado para establecer el grado de emparentamiento genético en los análisis de autocorrelación espacial ha sido el coeficiente de parentesco genético, $F_{ij,}$ propuesto por Loiselle *et al.,* (1995). Se define como la probabilidad de que un gen muestreado al azar de un individuo *i* sea idéntico a un gen muestreado al azar del individuo *j* (Vekemans y Hardy, 2004).

Experimentos de jardín común.

Cultivar las plantas en un único ambiente permite controlar el efecto de factores ambientales en la expresión de los caracteres de interés, asegurando que cualquier diferencia observada entre los tratamientos experimentales tiene base genética (Turesson, 1922; Cluasen *et al.,* 1948; Kawecki y Ebert, 2004). Los experimentos de jardín común (*common garden*, en inglés) utilizan esta técnica para detectar diferenciación genética en rasgos fenotípicos de plantas procedentes de diferentes regiones o poblaciones (Turesson, 1922; Cluasen *et al.,* 1948). No son adecuados para establecer sin incertidumbre el origen de la variación genética, por lo que no permiten establecer si las diferencias en la expresión de los caracteres estudiados han sido causadas por fuerzas evolutivas neutrales (*e.g.,* deriva genética) o por procesos adaptativos (Kawecki & Ebert 2004). Sin embargo, una respuesta diferencial en función de la población de origen a diferentes condiciones experimentales (*e.g.,* diferentes tratamientos de riego), o una relación estadística significativa entre los rasgos fenotípicos medidos en el jardín común y las condiciones ambientales de los sitios de origen pueden indicar adaptación (Linhart y Grant 1996).

Redes complejas de interacciones mutualistas

El enfoque de redes complejas al estudio de interacciones ecológicas parte del supuesto de que no es posible comprender el funcionamiento de las comunidades ecológicas a partir del estudio individualizado de las especies que lo componen, sino que es necesario estudiar la manera en que se organizan las interacciones entre las especies (Jordano *et al.,* 2003). Las redes de interacciones mutualistas pueden ser representadas mediante la utilización de matrices de interacción (Jordano, 1987). En ellas, los vectores fila representan especies del nivel trófico superior (*e.g.,* polinizadores o frugívoros) y los vectores columna representan especies del nivel trófico inferior (*e.g.,* plantas con flores o frutos carnosos). Para cada especie animal o de planta, podemos mantener un registro de las especies con las que interactúa y la intensidad con la que se produce la interacción. A partir de la información contenida en estas matrices, podemos describir las redes de interacción mediante un conjunto de métricas y estadísticos que aportan información sobre la estructura, funcionamiento y estabilidad de la comunidad estudiada (revisado en Bascompte y Jordano, 2007). La tesis doctoral se ha centrado en las redes de interacción planta-polinizador. Como consecuencia de su potencial, estas redes han sido ampliamente utilizadas para analizar la estabilidad de los ecosistemas (Bascompte *et al.*, 2006), las consecuencias de las perturbaciones originadas por el cambio climático (Hegland *et al.*, 2009), el efecto de la introducción de especies invasoras en los ecosistemas (Aizen *et al.,* 2008, Bartomeus *et al.,* 2008; Traveset *et al.,* 2013) y el éxito de la restauración ecológica de ecosistema degradados (Devoto *et al.,* 2012).

ESTRUCTURA DE LA TESIS

La tesis se estructura en cuatro capítulos que han sido escritos en inglés para su publicación en revistas científicas de ámbito internacional. Por ello, se presentan los manuscritos originales de dichos artículos. A continuación se incluye la traducción del título al español, la lista de coautores y el estado de publicación de cada capítulo y una breve síntesis de cada uno de ellos.

Capítulo 1. Evaluación de la variación intraespecífica de la dispersión efectiva a lo largo de un gradiente altitudinal: un análisis en dos especies de alta montaña mediterránea.

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Las condiciones ambientales y su variación espacial controlan en gran medida los patrones de reclutamiento de las comunidades vegetales. Para poder entender cómo esta variación ambiental afecta a los procesos que controlan el reclutamiento es necesario adoptar un enfoque multi-poblacional. Hemos utilizado una aproximación de modelización inversa para evaluar los patrones de reclutamiento en cinco poblaciones de *Silene ciliata* y *Armeria caespitosa* a lo largo de un gradiente altitudinal. Ambas especies son representativas de uno de los ecosistemas ibéricos más vulnerables al cambio climático: la comunidad de pastos psicroxerófilos de alta montaña mediterránea. Consideramos cuatro kernels de dispersión de semilla diferentes: Exponencial-Potencial, 2Dt, WALD y Log-normal. Los resultados mostraron una distancia media de dispersión reducida (<1m) en todas las poblaciones. Ambas especies mostraron variación interpoblacional en el tipo de kernel que proporciona mejores ajustes y en el parámetro de fecundidad. Sin embargo, solo *A. caespitosa* exhibió diferencias significativas entre poblaciones en la distancia media de dispersión. La variación ambiental altitudinal condicionó la fecundidad de los individuos en ambas especies. Junto a la limitación por dispersión primaria, la dispersión efectiva podría estar limitada por procesos post-dispersivos relacionados con la sequía estival. Estos resultados resaltan la complejidad de la variación intraespecífica en los procesos que controlan el reclutamiento, mostrando que el kernel de dispersión puede permanecer relativamente invariante entre poblaciones de una misma especie, incluso si existe fuerte variación en la estructura demográfica y/o el ambiente físico entre poblaciones; mientras que la asunción de invariancia intraespecífica del kernel de dispersión podrían no mantenerse para otras especies en el mismo ambiente. Por lo tanto, los resultados de este estudio sugieren la necesidad de análisis específicos en un amplio espectro de especies y ambientes para evaluar la prevalencia y magnitud de la variación intraespecífica de la dispersión. La baja distancia de dispersión de estas especies podría restringir la capacidad migratoria frente al cambio climático y limitar el flujo genético entre poblaciones.

Capítulo 2. Variación de la estructura genética espacial a escala local entre poblaciones de *Silene ciliata* (Caryophyllaceae) con diferente grado de agregación espacial.

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Manuscrito en preparación.

El patrón y la intensidad de la estructura espacial de la variación genética a escala local (FSGS por sus siglas en inglés) pueden variar entre poblaciones de una especie como consecuencia de la interacción entre diferentes factores demográficos y ambientales. Los modelos teóricos predicen que en hábitats o ambientes heterogéneos, la intensidad de la FSGS se incrementará como consecuencia de la variación local de la densidad. Sin embargo, existen pocos trabajos empíricos que hayan examinado las diferencias en la intensidad de la FSGS entre poblaciones con diferente grado de heterogeneidad espacial. En este estudio usamos métodos de autocorrelación espacial para cuantificar la estructura espacial demográfica y genética en cinco poblaciones estables demográficamente pero espacialmente heterogéneas de *Silene ciliata*. Encontramos FSGS significativa en tres de las cinco poblaciones estudiadas, confirmando la existencia de variación intra-poblacional en la intensidad de la estructura espacial genética. Se detectó una asociación positiva entre la intensidad de la FSGS y el nivel de agregación espacial de las plantas. Los cambios en las condiciones ambientales debidos a variaciones en la cantidad de hábitat adecuado y la heterogeneidad del hábitat afectaron parcialmente a la agregación de las plantas, y por lo tanto, podrían ser un factor clave a la hora de explicar los niveles de FSGS observados. Contrariamente a lo observado en otras especies, la población con mayor densidad de plantas fue también la población con mayor intensidad de FSGS, probablemente debido a una reducida distancia de dispersión de polen. Estos resultados muestran el potencial que tiene incorporar explícitamente información sobre la distribución espacial a escala local de los individuos y el hábitat en estudios de la variación espacial de la diversidad genética.

Capítulo 3. Variación genética en la fenología y el éxito reproductivo en un especialista de alta montaña mediterránea.

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Manuscrito en 2º revisión en *Botanical Journal of the Linnean Society*.

Las respuestas adaptativas al clima pasado podrían ser importantes para la persistencia de las plantas de alta montaña en el actual escenario de cambio climático acelerado. *Armeria caespitosa* es una planta de alta montaña, endémica del Sistema Central Ibérico. Las diferencias encontradas en el ambiente abiótico a lo largo del gradiente altitudinal someten a la especie a dos gradientes de estrés opuestos relacionados con el estrés hídrico y la duración de la temporada de crecimiento vegetativo. Además, la especie tiene la capacidad de subsistir en dos microhabitats contrastados (afloramientos rocosos y pastos psicroxerófilos) que, dependiendo de la altitud, tienen diferentes efectos sobre su dinámica poblacional. Esta variación ambiental provoca que la especie muestre gran diferenciación inter-poblacional en un conjunto de rasgos reproductivos y vegetativos. En este estudio, utilizamos una aproximación de jardín común para evaluar si la variación fenotípica observada en las poblaciones naturales tiene una base genética, o si por el contrario, es el resultado de respuestas plásticas moduladas por el conjunto condiciones ambientales heterogéneas descrito anteriormente. Las plantas procedentes del límite de elevación superior y de los pastos psicroxerofilos florecieron antes y produjeron más frutos viables, pero crecieron menos en comparación con las plantas procedentes del límite de elevación inferior y de los afloramientos rocosos. Estos resultados confirman que la variación inter-poblacional observada en algunos de los rasgos ligados a la fenología y el éxito reproductivo tiene base genética. El estudio también mostró que la respuesta selectiva en favor de una floración temprana detectada en el límite de elevación inferior, no ha generado una mayor proporción de fenotipos de floración temprana. Sin embargo, la variabilidad genética asociada al inicio de la floración, podría ser relevante para hacer frente al impacto ocasionado por el cambio climático en curso.

Capítulo 4. Efectos complejos y opuestos de la matorralización de los pastos de alta montaña sobre la red de interacciones planta-polinizador.

Carlos Lara-Romero, Cristina García, Javier Morente-López, José M. Iriondo.
Manuscrito en preparación.

Las interacciones mutualistas son claves para la estructura y el funcionamiento de las comunidades ecológicas y son especialmente sensibles a los motores de cambio global. En este estudio utilizamos técnicas de análisis redes complejas para

cuantificar el efecto de la matorralización de los pastos de alta montaña en la red de interacciones planta-polinizador. Realizamos el estudio en los pastos psicroxerófilos de la Sierra de Guadarrama porque el ascenso del matorral motivado por el cambio global está ocasionando una reducción del hábitat disponible para la comunidad que los habita, lo que constituye una de las mayores amenazas para su persistencia. Construimos matrices de interacción cuantitativas para comparar parcelas matorralizadas frente a parcelas en las que el matorral no estaba presente. La matorralización de los pastos estudiados tuvo un efecto positivo sobre la riqueza y la frecuencia de visitas de los polinizadores a las plantas, pero incrementó la competencia entre plantas por los servicios de polinización. Esta última situación podría reducir el éxito reproductivo en alguna de las especies de plantas estudiadas. Un amplio número de grupo funcionales de polinizadores visitaron a las especies de matorral en mayor proporción que a la mayoría de las especies de plantas de la comunidad, lo que otorgó a los matorrales un rol de super-generalista. Pese a ello, la estructura de la red de interacciones planta-polinizador apenas mostró cambios significativos en sus propiedades estructurales, aunque la red de los pastos matorralizados fue ligeramente más generalista. Este resultado coincide con estudios anteriores y sugiere que el mutualismo planta-polinizador podría ser relativamente permeable y robusto a las modificaciones provocadas por los motores de cambio global. Este estudio resalta la necesidad de utilizar un enfoque integrador en los estudios del efecto de los motores de cambio global sobre las interacciones interespecíficas y la estructura y el funcionamiento de las comunidades ecológicas.

CONCLUSIONES

De los cuatro capítulos en los que se ha estructurado esta tesis doctoral se pueden extraer las siguientes conclusiones:

1) La distancia media de dispersión efectiva de *Silene ciliata* y *Armeria caespitosa* es reducida (0,23 – 0,71 m). Junto a la limitación por dispersión primaria, la dispersión efectiva podría estar limitada por procesos post-dispersivos relacionados con la sequía estival. Esta situación podría restringir la capacidad migratoria frente al cambio climático y limitar el flujo genético entre poblaciones.

2) La variación ambiental altitudinal condicionó la fecundidad de los individuos *S. ciliata* y *A. caespitosa* pero no afectó a la escala espacial a la que se produce la dispersión. Ambas especies mostraron variación inter-poblacional en el tipo de kernel que proporciona mejores ajustes y en el parámetro de fecundidad. Sin embargo, solo *A. caespitosa* exhibió diferencias significativas entre poblaciones en la distancia media de dispersión. Estos resultados sugieren la necesidad de análisis específicos en un amplio espectro de especies y ambientes para evaluar la prevalencia y magnitud de la variación intraespecífica de la dispersión.

3) Se ha encontrado una estructura genética espacial (EGS) significativa en tres de las cinco poblaciones estudiadas de *Silene ciliata*, confirmando la existencia de variación intra-poblacional en la intensidad de la EGS.

4) Se ha detectado una asociación positiva entre la intensidad de la EGS y el nivel de agregación espacial de las poblaciones de *Silene ciliata*. Los cambios en las condiciones ambientales debidos a variaciones en la heterogeneidad del hábitat afectaron parcialmente a la agregación de las plantas, y por lo tanto, podrían ser un factor clave explicativo de los niveles de EGS observados.

5) Los índices de diversidad genética y endogamia observados en las poblaciones estudiadas de *Silene ciliata* fueron homogéneos. Consecuentemente, no se encontró relación entre la intensidad de la EGS y dichos parámetros. Esto sugiere

que la variabilidad ambiental a escala local no está afectando a la variabilidad genética y endogamia de las poblaciones estudiadas.

6) La variación inter-poblacional observada en el inicio de la floración y en el número de inflorescencias y de frutos viables en poblaciones naturales de *Armeria caespitosa* tiene base genética.

7) La respuesta selectiva en favor de una floración temprana detectada en el límite de elevación inferior de distribución de *A. caespitosa* no ha generado una mayor proporción de fenotipos de floración temprana. Sin embargo, la variabilidad genética asociada al inicio de la floración, podría ser relevante para hacer frente al impacto ocasionado por el cambio climático en curso debido a su potencial adaptativo.

8) La matorralización de los pastos estudiados tuvo un efecto positivo sobre la riqueza y la frecuencia de visitas de los polinizadores a las plantas, pero incrementó la competencia entre plantas por los servicios de polinización. Esta última situación podría reducir el éxito reproductivo en alguna de las especies de plantas estudiadas.

9) Un amplio número de grupo funcionales de polinizadores visitaron a las especies de matorral en mayor proporción que a la mayoría de las especies de plantas de la comunidad, lo que otorgó a los matorrales un rol de super-generalista. Pese a ello, la estructura de la red de interacciones planta-polinizador apenas mostró cambios significativos en sus propiedades estructurales. Este resultado coincide con estudios anteriores y sugiere que el mutualismo planta-polinizador podría ser relativamente robusto a las modificaciones provocadas por los motores de cambio global.

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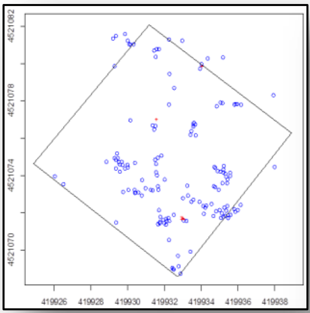
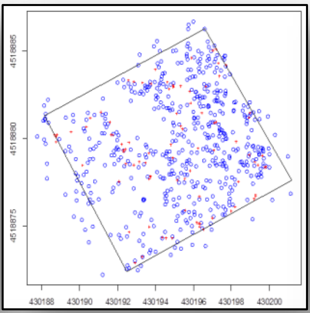
CAPÍTULOS

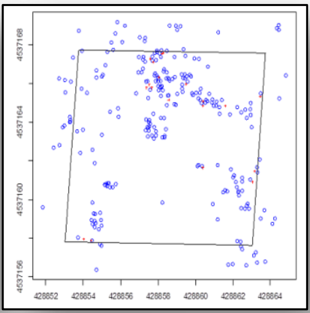
CHAPTERS

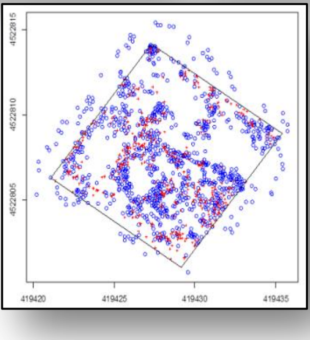
CAPÍTULO 1/CHAPTER 1

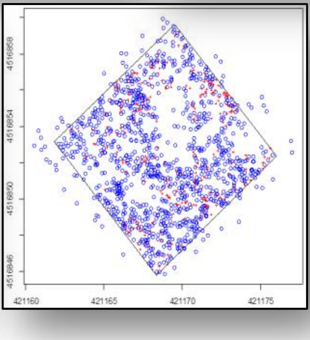
Assessing intraspecific variation in effective dispersal along an altitudinal gradient: a test in two Mediterranean high-mountain plants.











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1. ABSTRACT

Background: Plant recruitment depends among other factors on environmental conditions and their variation at different spatial scales. Characterizing dispersal in contrasting environments may thus be necessary to understand natural intraspecific variation in the processes underlying recruitment. *Silene ciliata* and *Armeria caespitosa* are two representative species of cryophilic pastures above the tree line in Mediterranean high mountains. No explicit estimations of dispersal kernels have been made so far for these or other high-mountain plants. Such data could help to predict their dispersal and recruitment patterns in a context of changing environments under ongoing global warming.

Methods: We used an inverse modelling approach to analyse effective seed dispersal patterns in five populations of both *Silene ciliata* and *Armeria caespitosa* along an altitudinal gradient in Sierra de Guadarrama (Madrid, Spain). We considered four commonly employed two-dimensional seedling dispersal kernels: exponential-power, 2Dt, WALD and log-normal.

Key results: No single kernel function provided the best fit across all populations, although estimated mean dispersal distances were short (< 1 m) in all cases. *S. ciliata* did not exhibit significant among-population variation in mean dispersal distance, whereas significant differences in mean dispersal distance were found in *A. caespitosa*. Both *S. ciliata* and *A. caespitosa* exhibited among-population variation in the fecundity parameter and lacked significant variation in kernel shape.

Conclusions: This study illustrates the complexity of intraspecific variation in the processes underlying recruitment, showing that effective dispersal kernels can remain relatively invariant across populations within particular species, even if there are strong variations in demographic structure and/or physical environment among populations, while the invariant dispersal assumption may not hold for other species in the same environment. Our results call for a case-by-case analysis in a wider range of plant taxa and environments to assess the prevalence and magnitude of intraspecific dispersal variation.

2. INTRODUCTION

Dispersal has important implications for plant species. It determines gene flow rates and the spatial distribution of genetic diversity (Govindaraju, 1988; Ouborg *et al.*, 1999; Moran and Clark, 2012), and it influences processes like colonization (Escribano-Avila *et al.*, 2012), range expansion (Clark *et al.*, 1998), local adaptation (Blanquart *et al.*, 2012) and recruitment patterns (Nathan and Muller-Landau, 2000). It also affects metapopulation dynamics and species coexistence and diversity (Hanski, 2001; Levine and Murrell, 2003).

Dispersal studies distinguish between primary and effective dispersal. Primary dispersal is the initial movement of seeds to the location where they are first deposited, whereas effective dispersal is the complex result of primary dispersal and the post-dispersal processes that take place after the seed is deposited on the soil surface until it is a successfully established seedling (Cousens *et al.*, 2008; Nathan and Muller-Landau, 2000). Effective dispersal is more relevant to population dynamics, because it comprises the environmental factors that are needed for seedling recruitment (Cousens *et al.*, 2008; Nathan and Muller-Landau, 2000; Nathan *et al.*, 2003).

The fecundity of reproductive adults and the distribution of dispersal distances are fundamental characteristics of the dispersal process which have been extensively used as basic descriptors of this process (Clark *et al.*, 1999b; Nathan and Muller-Landau, 2000; Ribbens *et al.*, 1994; Schurr *et al.*, 2008). However, in many systems, measuring seed production and dispersal distances poses a challenge, because dispersing seeds cannot be tracked easily, and seed or seedling shadows of neighboring plants typically overlap, making it difficult to identify mother plants (Bullock *et al.*, 2006). Inverse modelling is a useful methodology that allows the fecundity of adult plants and the shape of the dispersal kernel (the probability density function of propagule dispersal distances from an individual plant) to be estimated without identifying the exact source of each seed or seedling (Clark, 1998; Clark *et al.*, 1999b; Martinez and Gonzalez-Taboada, 2009; Ribbens *et al.*, 1994; Uriarte *et al.*, 2005); reviewed in (Nathan *et al.*, 2012).

Most seed dispersal research, employing inverse modelling or other methods, has been carried out in single populations, disregarding potentially relevant environmental variation across space that could affect the natural processes underlying the recruitment of particular species (Cousens *et al.*, 2008; Nathan *et al.*, 2012); but see (Clark *et al.*, 1998; LePage *et al.*, 2000). Heterogeneity in environmental conditions and landscape properties (e.g. intra and interspecific plant density, fragmentation, soil moisture, wind conditions) may indeed influence the dispersal kernel even over local geographic scales (Cousens *et al.*, 2008; Martinez and Gonzalez-Taboada, 2009; Nathan *et al.*, 2002; Nathan *et al.*, 2012). However, this variation is implicitly ignored in spatially unreplicated studies, which may lead to local results that are unrepresentative of the average dispersal pattern of the species. Hence, more comparative empirical studies are needed to test whether effective dispersal kernels exhibit intraspecific variation across contrasting environments and to find environmental correlates of potential variation (Cousens *et al.*, 2008; Klein and Oddou-Muratorio, 2011; Nathan *et al.*, 2012). Such studies could assess the validity of kernel-based approaches that assume a sole kernel for each species (Klein and Oddou-Muratorio, 2011).

High-mountain habitats have been identified as one of the most fragile environments in the world, and global warming is thought to be especially critical for plant populations in mountain systems (Nogues-Bravo *et al.*, 2007; Thuiller *et al.*, 2005). The movement of plants to higher elevations tracking their climatic niche seems to be one of the main responses to ongoing global warming (Gottfried *et al.*, 2012; Pauli *et al.*, 2012), although phenotypic plasticity and adaptation may also play an important role in their response (Davis *et al.*, 2005; Jump and Peñuelas, 2005; Miner *et al.*, 2005). Although all these processes are significantly conditioned by dispersal and recruitment patterns (Clark *et al.*, 1998; Cousens *et al.*, 2008; Davis *et al.*, 2005; Escribano-Avila *et al.*, 2012; Blanquart *et al.*, 2012), no explicit measurements of dispersal kernels have been made for high-mountain plants so far. A better understanding of dispersal and recruitment patterns of high-mountain plants, through the study of effective dispersal kernels, would, therefore, provide greater insight into high-mountain plant species response to climate warming.

We used an inverse modelling approach to measure the net reproductive rate and effective dispersal kernel parameters along an altitudinal gradient in a threatened Mediterranean high-mountain pasture community in central Spain. Analyses were performed on five populations of each of two representative species of the community: *Silene ciliata* Poiret and *Armeria caespitosa* (Gómez Ortega) Boiss. *in* DC. Previous studies carried out on these species showed that the altitudinal gradient is associated with an environmental stress gradient, with the lowest population experiencing the most stressful conditions, constraining seedling establishment and reproductive performance (Garcia-Camacho *et al.*, 2010; Garcia-Camacho and Escudero, 2009; Gimenez-Benavides *et al.*, 2007b; Gimenez-Benavides *et al.*, 2007c; Gimenez-Benavides *et al.*, 2008). We expected that variation in population structure and physical environment along the altitudinal gradient could modify the spatial recruitment pattern and dispersal distance of the species. Specifically, we addressed two main questions: (1) What is the spatial range of effective seed dispersal in the two high-mountain species? (2) Do effective seed dispersal and fecundity parameters vary among populations at different altitudes?

3. METHODS

Ethics Statement

All necessary permits were obtained for the described field studies. Mr. Juan Antonio Vielva from the Administration Bureau of the Natural Park of Cumbres, Circo y Lagunas de Peñalara and Mr. Antonio Sanz from the Administration Bureau of the Regional Park of Cuenca Alta del Manzanares gave their permission to work in the protected natural areas. Field studies did not involve any endangered or protected species.

Study site and species

The study was carried out in the orophyllous cryophilic pastures of Sierra de Guadarrama, a mountain range located in central Spain. Mean annual precipitation, measured at the Navacerrada Pass (40° 46′N, 4 ° 19′W; 1860 m a.s.l.), is 1330 mm with a pronounced dry season (< 10% of total annual rainfall) from May to October. Mean annual temperature is 6.3ºC, with mean monthly temperatures ranging from -1ºC in

January to 16ºC in July (www.aemet.es). Dry cryophilic pastures occur in the higher summits above the tree line between 1900 and 2430 m.a.s.l and are dominated by *Festuca curvifolia* Lag. ex Lange and other perennial plants interspersed in a shrub matrix characterized by *Cytisus oromediterraneus* Rivas Mart. & al. and *Juniperus communis* subsp. *alpina* (Suter) Čelak.

Silene ciliata Poiret (Caryophyllaceae) is a chamaephytic cushion perennial plant that occurs in the Mediterranean mountain ranges of southern Europe (Tutin *et al.*, 1995). One of its southernmost distribution limits is found in Sierra de Guadarrama at altitudes from 1900 to 2430 m, where it grows in dry cryophilic pastures dominated by *Festuca curvifolia*. It blooms in late summer, with a peak in early August (Gimenez-Benavides *et al.*, 2011b). Flowering stems reach 15 cm in height and have 1–5 flowers. Fruit capsules have up to 100 seeds which are wind dispersed in August-September. *Silene ciliata* seeds have an average mass of 0.59 mg and their diameter range between 1.1 and 1.5 mm (Gimenez-Benavides *et al.*, 2005). The species is essentially barochorous (seeds lack any specific structure to promote dispersal) and is pollinated by syrphid flies, bumblebees and moth species (Gimenez-Benavides *et al.*, 2007a). It is self-compatible, although autogamy is restricted by pronounced protandry (Garcia-Fernandez *et al.*, 2012a). In Sierra de Guadarrama, genetic diversity is quite homogeneous across *S. ciliata* populations, which show significant levels of inbreeding (Garcia-Fernandez *et al.*, 2012a).

Armeria caespitosa (Gómez Ortega) Boiss. in DC. is a high-mountain dwarf chamaephytic cushion plant, endemic to the mountains of central Spain (Sierra de Guadarrama, Ayllón and East Gredos), which occurs at altitudes from 1600 to 2430 m. It grows in the same dry cryophilic pastures dominated by *Festuca curvifolia*, although isolated individuals have also been found on the ledges of granite and gneiss rocks. Its flowers are grouped in short-scaped flowerheads with 19 ± 8 flowers each. Each flower has a single ovule, which yields one seed that remains enclosed in the papyraceous calyx. Average seed mass is 1.18 mg and its diameter ranges between 2.0 and 4.7 mm (Garcia-Camacho *et al.*, 2010). The seeds have a papyraceous calyx that may facilitate wind dispersal (Garcia-Camacho *et al.*, 2010). *A. caespitosa* is self-incompatible (Garcia-Camacho and Escudero, 2009) and pollinated by bees, bumblebees and syrphid flies

(Garcia-Camacho and Escudero, 2009). Previous studies on the population genetics of both species revealed relatively low genetic differentiation and a complex genetic structure among populations (García-Fernández *et al.*, 2013; Garcia-Fernandez *et al.*, 2012b).

Field data

In August and September 2010, we established 10x10m plots in five populations of both *S. ciliata* and *A. caespitosa* distributed along an altitudinal gradient in the study site (Table 1). For each plot, we estimated a set of climate variables for the growing season (April-September). We calculated the mean, minimum and maximum rainfall and temperature for each population using the Digital Climatic Atlas of Spain of the Spanish Ministry of Environment (Gonzalo-Jiménez, 2011) (Table 1), and the mean wind speed at 80 m height using the Spanish Wind Energy Atlas of the Spanish Ministry of Industry (http://atlaseolico.idae.es) (Table 1).

In each study plot, we mapped every *S. ciliata* and *A. caespitosa* reproductive plant and seedling using two high-resolution Differential Global Navigation Satellite System (DGNSS) receivers (Viva GS15, Leica, Switzerland) with an accuracy of 5 cm for *x* and *y* coordinates. We also measured plant diameter and the number of inflorescences of every reproductive plant. To account for potential seedling sources outside the study area, we also recorded the location, diameter and number of inflorescences of reproductive plants in a buffer zone of two meters around the plots. The seedling stage in *S. ciliata* and *A. caespitosa* includes up to one-year individuals (i.e. plants that had germinated in fall or spring and had survived their first full summer), because the seedlings that survive a second growth period grow into the reproductive stage (Garcia-Camacho *et al.*, 2012; Gimenez-Benavides *et al.*, 2011a). Summer is critical to the survival of Mediterranean plants due to the incidence of severe droughts (Beniston *et al.*, 2007; Larcher, 2000). This is also the case in Mediterranean high-mountain plants (Castro *et al.*, 2005) such as *S. ciliata* and *A. caespitosa* (Garcia-Camacho *et al.*, 2010; Gimenez-Benavides *et al.*, 2007c). With this framework, we included all critical stages of effective dispersal: seed dispersal, seed germination and seedling survival.

The dataset used for this study is available from the institutional repository of Rey Juan Carlos University (BURJC-Digital, http://eciencia.urjc.es/handle/10115/11835).

Table 1. Description of *A. caespitosa* and *S. ciliata* sampling sites.

Population	UTM Coordinates (x,y) m		Altitude m	*Pm* (min, max) mm	*Tm* (min, max) ºC	*Ws* m/s	Seedling	Reproductive
Silene ciliata								
Peñalara	419427.30	4522814.15	2405	82.50 (32, 149)	8.77 (0.2, 14.1)	10.62	469	1068
Cabezas de Hierro	421169.50	4516859.61	2305	85.17 (34, 151)	8.37 (0.2, 13.7)	10.87	243	1085
Nevero	428863.74	4537167.57	2190	71.00 (30, 121)	9.67 (2.1, 14.6)	8.97	19	245
Najarra	430196.59	4518886.29	2080	72.17 (27, 112)	10.45 (2.8, 15.7)	9	89	523
Laguna	419931.16	4521082.09	1946	77.00 (31, 134)	10.27 (2.8, 15.2)	7.17	6	147
Armeria caespitosa								
Cabezas de Hierro	420950.54	4516685.32	2336	85.2 (34, 151)	8.37 (0.2, 13.7)	10.87	145	318
Najarra	430196.79	4518886.33	2080	72.2 (27, 112)	10.45 (2.8, 15.7)	9	498	356
Loma de Cabezas	420013.12	4514794.49	1970	77.0 (30, 131)	9.78 (2.5, 14.8)	8.45	53	132
Collado de las Vacas	419168.95	4513371.38	1882	66.3 (25, 110)	11.80 (4.0, 17.2)	8.46	49	169
Sierra de los Porrones	420580.85	4512251.64	1647	64.5 (26, 102)	12.88 (4.4, 18.3)	7.4	32	52

Geographical coordinates, altitude, climatic variables for the growing season (April-September) and seedling and reproductive plant density (individuals per 10x10 m plot) in study populations. *Pm* mean monthly precipitation, *Tm* mean monthly temperature, *Ws* wind speed at 80 m height. Minimum and maximum monthly precipitation and temperatures are provided between brackets.

Statistical analysis

We used inverse modelling to estimate seedling dispersal kernel parameters for each population of each species and to test for dispersal parameter variation among populations within species. Following Ribbens *et al.*, (1994), the expected number of seedling recruits $\hat{s}_j$ at a given plot or trap *j* of area A_j ($\hat{s}_j$) equals the sum of the seedling shadows across this plot of all *m* maternal plants, in the form:

$$\hat{s}_j(\mathbf{b},\mathbf{r_j};\beta,\delta,u)=\sum_{i=1}^{m}Q(b_i;\beta)\int_{A_j} f\left(\sqrt{(x_i-x_j)^2+(y_i-y_j)^2};\delta,u\right)dx_j dy_j \qquad \textit{eqn. } 1a$$

where **b** and $\mathbf{r_j}$ are, respectively, *m*-length vectors of individual plant size measurements b_i and spatial distances r_{ij} between the spatial coordinates (x_j, y_j) of plant *i*, and the coordinates of plot *j* (x_i, y_i), *Q* is an allometric function with parameter β yielding the number of seedlings produced by a single plant, and *f* is a two-dimensional isotropic seedling dispersal probability density function (dispersal kernel) as a function of dispersal distance $r_{ij}=\sqrt{(x_i-x_j)^2+(y_i-y_j)^2}$ with mean δ and shape parameter *u*. The integral in *eqn.* 1*a* provides the exact probability of dispersal from plant *i* into the whole surface A_j of plot *j*. In practice, all studies of which we are aware have assumed that the probability of dispersal from any given plant into any of the points lying within the area A_j of each plot is constant, yielding the usual simplified expression:

$$\hat{s}_j(\mathbf{b},\mathbf{r_j};\beta,\delta,u)=\sum_{i=1}^{m}Q(b_i;\beta)\,f(r_{ij};\delta,u)A_j\,, \qquad \textit{eqn } 1b$$

where r_{ij} is the spatial distance between plant *i* and the centre of plot *j*. The approximation in *eqn* 1*b* will be closer to the exact values predicted by *eqn.* 1*a* the smaller the seedling plot areas and the larger the distances between maternal plants and seedling plots.

We assumed a linear relationship between seedling production and plant diameter, $Q_i = \beta b_i$, because previous studies showed that plant size is linearly related with seed production in both species (Garcia-Camacho and Escudero, 2009; Gimenez-Benavides *et al.*, 2007c). Furthermore, as found in previous studies (Clark *et al.*, 1999a; Schurr *et al.*, 2008), alternative allometric functions worsened inverse modelling fits, including those using the number of inflorescences (results not shown).

For every population of each species, we divided the 100-m^2 study area in $c = 100L^{-2}$ equal adjacent cells of area L^2, and for each cell j, we computed the observed (s_j) and predicted ($\hat{s}_j$) seedling densities. We used both equations 1*a* and 1*b* to estimate $\hat{s}_j$, given that in our case, with relatively large cell areas compared to some of the mother-cell pairwise distances (occasionally with maternal plants lying within the target cell), it was not obvious *a priori* that the approximation in equation 1*b* would be sufficiently good. We obtained however virtually identical dispersal and fecundity estimates for all populations and assumed kernels with both *equation* 1*a* or 1*b* (results not shown), so we only present the results for the latter, whose computational efficiency enabled calculating confidence intervals and conducting hypothesis tests (see below) in a reasonable number of CPU hours. We also tested the effect on parameter estimates of three different values for cell side length, L = 0.125, 0.25 and 0.50 m, with lower and upper values approximating DGNSS accuracy and dispersal range, respectively. Distribution of seedling densities across cells exhibited overdispersion relative to a Poisson distribution ($p<0.05$) in all populations excepting *S. ciliata*'s Nevero, and we thus modelled seedling counts using a negative binomial distribution (Clark *et al.*, 1998):

$$L(\mathbf{S} \mid \beta,\delta,u,\theta) = \prod_{j=1}^{c} \frac{\Gamma(s_j+\theta)}{\Gamma(s_j+1)\Gamma(\theta)} \frac{\hat{s}_j^{s_j}(\mathbf{b},\mathbf{r_j};\beta,\delta,u)\theta^{\theta}}{[\hat{s}_j(\mathbf{b},\mathbf{r_j};\beta,\delta,u)]^{s_j+\theta}}, \qquad \textit{eqn } 2$$

where $\mathbf{S}$ is the set of c observed seedling counts s_j, Γ is the gamma function, and θ is the negative binomial parameter, determining the distribution variance (values of $\theta<1$ indicate overdispersed distributions, while $\theta>>1$ tend to the Poisson distribution). Maximum-likelihood estimates for β, δ, u and θ were obtained for each population of each species by maximizing *eqn* 2. Confidence intervals were computed using the profile-likelihood method (Edwards, 1992).

As most studies show that no single dispersal function provides consistently superior fits across species (Greene *et al.*, 2004; Nathan *et al.*, 2012, Schurr *et al.*, 2008; Uriarte *et al.*, 2005), it is advisable to always compare the performance of alternative dispersal kernels (Martinez and Gonzalez-Taboada, 2009; Schurr *et al.*, 2008). We considered four commonly employed two-dimensional seedling dispersal kernels, with scale parameter *a* and shape parameter *u*: the 2Dt kernel (Clark *et al.,* 1999b); see (Austerlitz *et al.*, 2004) for the parameterization used here.

$$f_{2\mathrm{Dt}}(r_{ij} \mid a,u) = \frac{u-1}{\pi a^2}\left(1+\frac{r_{ij}^2}{a^2}\right)^{-u}, \qquad \textit{eqn } 3$$

the log-normal kernel (Stoyan and Wagner, 2001)

$$f_{LN}(r_{ij} \mid a,u) = \frac{1}{(2\pi)^{3/2} u r_{ij}^2}\exp\left[-\frac{(\ln(r_{ij})-a)^2}{2u^2}\right], \qquad \textit{eqn } 4$$

the WALD kernel (Katul *et al.*, 2005, Schurr *et al.*, 2008)

$$f_{WALD}(r_{ij} \mid a,u) = \left(\frac{u}{8\pi^3 r_{ij}^5}\right)^{1/2}\exp\left[-\frac{u(r_{ij}-a)^2}{2a^2 r_{ij}}\right], \qquad \textit{eqn } 5$$

and the exponential-power kernel (Clark *et al.,* 1998)

$$f_{EXPOW}(r_{ij} \mid a,u) = \frac{u}{2\pi a^2 \Gamma(2/u)}\exp\left[-\left(\frac{r_{ij}}{a}\right)^u\right]. \qquad \textit{eqn } 6$$

Mean seedling dispersal distances for each kernel are given by $\delta_{2\mathrm{Dt}} = a\Gamma(3/2)\,\Gamma(u\text{–}3/2)/\,\Gamma(u\text{–}1)$, $\delta_{LN} = \exp\,(a + 0.5u^2)$, $\delta_{\mathrm{WALD}} = a$ and $\delta_{\mathrm{EXPOW}} = a\Gamma(3/u)/\,\Gamma(2/u)$, respectively. Given that δ provides a more intuitive description of dispersal range than *a*, independently of the assumed kernel, we parameterized the model (*eqns* 1 and 2) in terms of δ and *u,* rather than in terms of *a* and *u*, facilitating estimation of profile-likelihood confidence intervals for δ (Clark *et al.,* 1998; Oddou-Muratorio *et al.,* 2005) and tests of its variation across populations (see below). We used the Akaike Information criterion (AIC) (Akaike, 1973) to assess method performance and calculated the goodness of fit of the most parsimonious model by measuring Pearson's product-moment correlation between observed vs. predicted seedling densities.

We tested for potential differences in dispersal and fecundity parameters across populations using likelihood ratio statistics as in Clark *et al.* (Clark *et al.*, 1998). Assuming that mean dispersal distance δ is constant across populations within species, we obtained parameter estimates based on the likelihood of the whole species data set $\mathbf{S_q}$, incorporating information from all q populations of the species:

$$L_{\delta}(\mathbf{S_q} \mid \boldsymbol{\beta}_\mathbf{q}, \delta, \mathbf{u_q}, \boldsymbol{\theta}_\mathbf{q}) = \prod_{k=1}^{q} L(\mathbf{S}_k \mid \beta_k, \delta, u_k, \theta_k), \qquad \textit{eqn } 7$$

where $\boldsymbol{\beta}_\mathbf{q}$, $\mathbf{u_q}$, and $\boldsymbol{\theta}_\mathbf{q}$ are q-length vectors of population-specific fecundity parameters β_k, kernel shape parameters u_k, and negative binomial parameters θ_k. This model has $3q$ degrees of freedom and provides an average species-level estimate of δ. Note that, unlike Clark *et al.* (1998), we allowed for different values of the negative binomial parameter (i.e. different degrees of spatial seedling clumping) across populations. We assumed the same kernel family for all populations for conducting this test, choosing the one yielding the best average fit over all populations (i.e. the one with the smallest average AIC across the population-level fits obtained using *eqn*. 2). We then obtained parameter estimates assuming that all parameters (including δ) are variable among populations within species (still assuming a single consensual kernel family), based on the likelihood

$$L(\mathbf{S_q} \mid \boldsymbol{\beta}_\mathbf{q}, \boldsymbol{\delta}_\mathbf{q}, \mathbf{u_q}, \boldsymbol{\theta}_\mathbf{q}) = \prod_{k=1}^{q} L(\mathbf{S}_k \mid b_k, \delta_k, u_k, \theta_k), \qquad \textit{eqn } 8$$

where $\boldsymbol{\delta}_\mathbf{q}$ is the q-length vector of population-specific mean dispersal distances δ_k. The model in *eqn* 8 has $4q–1$ degrees of freedom. The deviance

$$D = -2\ln\left[\frac{L_{\delta}(\mathbf{S_q} \mid \boldsymbol{\beta}_\mathbf{q}, \delta, \mathbf{u_q}, \boldsymbol{\theta}_\mathbf{q})}{L(\mathbf{S_q} \mid \boldsymbol{\beta}_\mathbf{q}, \boldsymbol{\delta}_\mathbf{q}, \mathbf{u_q}, \boldsymbol{\theta}_\mathbf{q})}\right] \qquad \textit{eqn } 9$$

is asymptotically distributed as χ^2 with $q–1$ degrees of freedom. Large deviances mean that stand-specific δ improves the likelihood of the data so substantially that the null hypothesis of invariant δ across populations can be rejected (Clark *et al.*, 1998). We first tested for significant differences in δ across all populations of each species. When the overall test was positive, we tested for specific populations with δ-values significantly different from the global species-average by subsequently conducting q deviance tests, one for each population i (χ^2 with 1 degree of freedom), using:

$$D_i = -2\ln\left[\frac{L_\delta(\mathbf{S_q} \mid \boldsymbol{\beta}_\mathbf{q}, \delta, \mathbf{u_q}, \boldsymbol{\theta}_\mathbf{q})}{L(\mathbf{S_q} \mid \boldsymbol{\beta}_\mathbf{q}, \delta_i, \delta_{-i}, \mathbf{u_q}, \boldsymbol{\theta}_\mathbf{q})}\right], \qquad \textit{eqn } 10$$

where the numerator is the same likelihood as before, assuming that the mean dispersal distance δ is constant across all populations, while the likelihood in the denominator assumes that the i-th population has a mean dispersal distance δ_i, while the remaining q–1 populations have a constant mean dispersal distance δ_{-i}. We applied the sequential Bonferroni correction to correct for multiple testing (Holm, 1979). Analogous tests were conducted to investigate among-population variation in the dispersal kernel’s shape parameter (assuming constant u and variable δ in *eqn* 7) and in the fecundity parameter β (assuming constant β and variable δ in *eqn* 7). Although the field survey was exhaustive in all study sites, the sample sizes were unbalanced and relatively small in some sites due to natural density variation (Table 1), which may compromise the large numbers approximation under which the χ^2 distribution is expected. It has been shown that small samples can indeed make likelihood ratio (LR) tests relatively lax in the case of unequal male reproductive success analysis (Elle and Meagher, 2000). Smouse *et al.* (1999), proposed the use of nonparametric (permutational) tests as an alternative to LR tests, showing that the former can perform better than LR tests when parent-offspring genealogical information is available. Similar non-parametric tests have not been developed so far in the different statistical framework of inverse modeling, for which LR tests, such as those used here, are still widely employed as feasible approximations for exploratory assessment of variation in dispersal and fecundity parameters (Caspersen and Saprunoff, 2005; Clark *et al.*, 1998; Debain *et al.*, 2007; Sagnard *et al.*, 2007). All statistical analyses were performed in the open source software package R (R Core Team, 2013).

4. RESULTS

We mapped a total of 4095 reproductive individuals and 1603 seedlings across all populations of the two species (Table 1; Figure S1 in Supporting Information). Reproductive plant and seedling densities varied between populations in both species (Table 1). We focused on the results obtained with a 0.125 m cell side length (Tables 2 and 3), because some of the *S. ciliata* populations did not converge when 0.25 or 0.5 m cell side lengths were used (Table S1 in Supporting Information). In any case, similar results were obtained for both species for all cell side lengths (Tables 2, 3, S1 and S2). As none of the four models converged for *A. caespitosa* in the Najarra population, this population was excluded from further analyses.

Differences in AIC values indicated that no single kernel function provided the best fit across all populations of any of the two species (Table 2 and 3). For *S. ciliata*, the WALD kernel was among the most parsimonious models (i.e. models whose AIC values differ ΔAIC < 2 from the model with the lowest AIC; Burnham & Anderson 2002) in all five populations, while the log-normal model was among the most parsimonious in four populations, the exponential-power model in three populations and the 2Dt model in two populations (Table 2). Results did not support a particular kernel shape in *S. ciliata*: the mesokurtic, thin-tailed exponential-power with $u > 1$ showed similarly good fits (ΔAIC < 2) as leptokurtic functions with either exponential (WALD) or fat (2Dt and log-normal) tails, within the same populations. Using the WALD kernel across all populations of *S. ciliata*, species-level estimates of the mean dispersal distance and the shape and fecundity parameters were $\delta = 0.39$ m (95% CI: 0.35 – 0.45 m), $u = 0.80$ (0.63 – 1.13) and $\beta = 0.0032$ seedling/cm (0.0003 - 0.0061). For *A. caespitosa*, it was the log-normal kernel that ranked among the best models in all four populations where estimation converged, whereas the WALD, 2Dt and exponential-power models showed a similarly good performance within each of three populations (Table 3). Supported models in *A. caespitosa* always included leptokurtic functions with exponential or fat tails (including the exponential-power with $u<1$). Using the log-normal model across all populations of *A.*

caespitosa, the species-level estimates kernel´s parameters were $\delta = 0.43$ m (0.32 – 0.60 m), $u = 0.98$ (0.87 – 1.14) and $\beta = 0.0032$ seedling/cm (0.0003 - 0.0061).

Table 2. Estimated parameters for the models fitted to the seedling recruitment data of *S. ciliata*.

Population	Kernel	Mean dispersal (δ) (m)		Shape parameter (u)		Fecundity parameter (β) (seedlings/cm)		Negative binomial parameter (θ)		- log$L$	ΔAIC	r^2
Peñalara	**Log-norm**	**0.32**	**(0.27 - 0.39)**	**0.64**	**(0.52 - 0.77)**	**0.009**	**(0.008 - 0.010)**	**0.359**	**(0.260 - 0.523)**	**1624.80**	-	**0.12**
	WALD	0.33	(0.28 - 0.41)	0.64	(0.44 - 0.99)	0.009	(0.008 - 0.010)	0.360	(0.260 - 0.525)	1624.82	0.04	
	2Dt	0.32	(0.27 - 0.46)	3.80	(1.99 - *na*)	0.009	(0.009 - 0.011)	0.351	(0.255 - 0.509)	1627.77	5.94	
	Exp-pow	0.31	(0.27 - 0.37)	1.51	(0.97 - 2.34)	0.009	(0.008 - 0.010)	0.349	(0.253 - 0.507)	1628.76	7.92	
Cabezas de Hierro	**Exp-pow**	**0.37**	**(0.32 - 0.45)**	**9.86**	**(2.34 - *na*)**	**0.003**	**(0.003 - 0.004)**	**0.449**	**(0.235 - 1.198)**	**1029.04**	-	**0.05**
	Log-norm	0.43	(0.35 - 0.60)	0.43	(0.23 - 0.70)	0.003	(0.003 - 0.004)	0.460	(0.240 - 1.246)	1029.04	0.01	
	WALD	0.43	(0.35 - 0.62)	2.26	(0.84 - 8.70)	0.003	(0.003 - 0.004)	0.459	(0.239 - 1.244)	1029.17	0.27	
	2Dt	0.43	(0.33 - 0.58)	172.54	(3.65 - *na*)	0.003	(0.003 - 0.004)	0.450	(0.235 - 1.205)	1031.17	4.25	
Nevero	**Exp-pow**	**0.23**	**(0.17 - 0.50)**	**2.84**	**(0.74 - 21.91)**	**0.001**	**(0.001 - 0.002)**	**142.062**	**(0.045 - *na*)**	**108.04**	-	**0.08**
	2Dt	0.23	(0.17 - 0.42)	171.06	(1.74 - *na*)	0.001	(0.001 - 0.002)	142.162	(0.082 - *na*)	108.12	0.17	
	WALD	0.30	(0.22 - 0.60)	1.23	(0.41 - 3.27)	0.001	(0.001 - 0.002)	141.865	(0.054 - *na*)	109.04	1.99	
	Log-norm	0.30	(0.21 - 0.86)	0.47	(0.29 - 1.35)	0.001	(0.001 - 0.002)	50.393	(0.053 - *na*)	109.14	2.19	
Najarra	**WALD**	**0.71**	**(0.37 - *na*)**	**0.55**	**(0.27 - 1.24)**	**0.002**	**(0.003 - 0.003)**	**0.280**	**(0.092 - 8.456)**	**461.85**	-	**0.04**
	Log-norm	0.62	(0.36 - 3.01)	0.86	(0.58 - 1.46)	0.002	(0.002 - 0.003)	0.280	(0.092 - 8.401)	462.14	0.59	
	Exp-pow	0.61	(0.36 – 2.45)	0.83	(0.27 - 2.49)	0.002	(0.002 - 0.003)	0.275	(0.091 - 7.395)	463.15	2.59	
	2Dt	*nc*		*nc*		*nc*		*nc*		*nc*		
Laguna	**Exp-pow**	**0.29**	**(0.17 - 0.40)**	**1411.75**	**(0.03 - *na*)**	**0.001**	**(0.0003 - 0.004)**	**0.003**	**(0.001 – 0.035)**	**38.74**	-	**0.01**
	WALD	0.24	(0.14 - *na*)	1.00	(*na* - *na*)	0.001	(0.0003 - 0.029)	0.004	(0.001 - 0.049)	38.91	0.34	
	Log-norm	0.24	(0.16 - *na*)	0.47	(0.23 - *na*)	0.001	(0.0003 - *na*)	0.004	(0.001 - 0.048)	38.95	0.42	
	2Dt	0.23	(0.15 - 0.86)	165.18	(1.00 - *na*)	0.001	(0.0003 - 0.006)	0.003	(0.001 - 0.035)	39.12	0.76	

For each parameter, 95% confidence intervals are presented along with the mean value. Log-norm, log-normal; Exp-pow, exponential-power. *na* denotes that the confidence interval limit is not available because of flat likelihood function. *nc* denotes models that did not converge.–log*L*, log-likelihood; AIC, Akaike's information criterion; r, Pearson correlation coefficient between observed and predicted seedling density values. Models with the lowest AIC are marked in bold for each population.

The fits of the most parsimonious models ranged from good in *A. caespitosa* populations ($r^2 = 0.17$-0.35) to fair or poor ($r^2 = 0.01$-0.12) in *S. ciliata* populations (Tables 2 and 3), and were highly significant ($p < 0.0001$) in all cases except in the population with the lowest seedling number ($p = 0.32$ for Laguna in *S. ciliata*, which had only 6 seedlings). Population estimates of mean dispersal distance (δ) and fecundity (β)

parameters were generally consistent when assuming different kernel families (Table 2 and 3). No significant correlation was found between log β and log δ (r = 0.35, p = 0.65).

Table 3. Estimated parameters for the models fitted to the seedling recruitment data of *A. caespitosa*.

Population	Kernel	Mean dispersal (δ) (m)		Shape parameter (u)		Fecundity parameter (β) (seedlings/cm)		Negative binomial parameter (θ)		- log*L*	ΔAIC	r^2
Cabezas de Hierro	**Log-norm**	**0.33**	**(0.24 - 0.56)**	**0.97**	**(0.78 - 1.29)**	**0.024**	**(0.019 - 0.029)**	**0.122**	**(0.076 - 0.208)**	**584.41**	-	**0.17**
	2Dt	0.80	(0.30 - *na*)	1.60	(1.24 - 2.48)	0.024	(0.020 - 0.030)	0.122	(0.076 - 0.207)	584.78	0.73	
	WALD	0.35	(0.24 - 1.44)	0.21	(0.13 - 0.35)	0.024	(0.020 - 0.030)	0.121	(0.076 - 0.206)	584.80	0.79	
	Exp-pow	0.29	(0.23 - 0.45)	0.60	(0.31 - 1.03)	0.024	(0.020 - 0.029)	0.120	(0.075 - 0.202)	585.01	1.19	
Loma de Cabezas	**Exp-pow**	**0.66**	**(0.46 - 1.39)**	**0.69**	**(0.24 - 2.37)**	**0.014**	**(0.010 - 0.019)**	**0.061**	**(0.028 - 0.162)**	**247.54**	-	**0.12**
	Log-norm	0.78	(0.48 - 2.57)	1.02	(0.69 - 1.64)	0.014	(0.010 - 0.019)	0.063	(0.028 - 0.169)	247.82	0.57	
	WALD	0.77	(0.46 - *na*)	0.44	(0.19 - 1.01)	0.014	(0.010 - 0.020)	0.064	(0.028 - 0.171)	247.96	0.85	
	2Dt	0.73	(0.05 - *na*)	2.15	(0.12 - *na*)	0.014	(0.010 - 0.020)	0.058	(0.026 - 0.158)	248.64	2.20	
Collado de las Vacas	**2Dt**	**0.30**	**(0.18 - *na*)**	**1.84**	**(1.36 - 2.85)**	**0.011**	**(0.008 - 0.014)**	**0.286**	**(0.107 - 1.330)**	**206.84**	-	**0.22**
	WALD	0.28	(0.19 - 0.58)	0.25	(0.13 - 0.44)	0.011	(0.008 - 0.014)	0.298	(0.109 - 1.444)	207.11	0.56	
	Log-norm	0.25	(0.17 - 0.43)	0.84	(0.63 - 1.18)	0.011	(0.008 - 0.014)	0.298	(0.109 - 1.442)	207.47	1.26	
	Exp-pow	0.27	(0.18 - 0.45)	0.56	(0.29 - 0.96)	0.011	(0.008 - 0.015)	0.249	(0.096 - 1.057)	209.86	6.05	
Sierra de los Porrones	**Log-norm**	**0.23**	**(0.13 - 0.50)**	**1.07**	**(0.84 - 1.56)**	**0.021**	**(0.013 - 0.033)**	**0.369**	**(0.118 - 2.966)**	**110.01**	-	**0.35**
	2Dt	0.28	(0.16 - *na*)	1.75	(1.27 - 3.12)	0.021	(0.013 - 0.033)	0.371	(0.117 - 3.291)	110.02	0.01	
	Exp-pow	0.21	(0.13 - 0.40)	0.48	(0.19 - 0.93)	0.020	(0.013 - 0.033)	0.370	(0.118 - 3.227)	110.15	0.27	
	WALD	0.20	(0.10 - *na*)	0.07	(0.03 - 0.12)	0.024	(0.015 - 0.043)	0.280	(0.098 - 1.231)	111.27	2.52	

For each parameter, 95% confidence intervals are presented along with the mean value. Log-norm, log-normal; Exp-pow, exponential-power. *na* denotes that the confidence interval limit is not available because of flat likelihood function. – *logL*, log-likelihood; AIC, Akaike's information criterion; r, Pearson correlation coefficient between observed and predicted seedling density values. Models with the lowest AIC are marked in bold for each population.

Focusing on the WALD dispersal model for *S. ciliata* cross-population comparisons, estimated mean dispersal distances ranged between δ = 0.23 and 0.71 m (Table 2), although differences were only marginally significant ($\chi^2_{4\,d.f.}$ = 8.846, p = 0.065). Estimated shape parameters ranged between u = 0.55 and 2.26, and were not significantly different from each other ($\chi^2_{4\,d.f.}$ = 7.022, p = 0.135). Fecundity parameters ranged between β = 0.001 and 0.009 seedlings/cm across *S. ciliata* populations, being significantly different ($\chi^2_{4\,d.f.}$ = 254.26, $p < 0.001$). Per-population tests indicated that all population estimates of β were significantly different from the species average (Table 4).

Table 4. Comparison of estimated fecundity parameters (β) among *S. ciliata* populations.

Population	β_i (seedlings/cm)	β_{-i} (seedlings/cm)	-log*L*	$\chi^2_{1\,d.f.}$ (*p-value)*
Peñalara	0.009	0.003	-3276.28	229.28 (0.0000)*
Cabezas de Hierro	0.003	0.007	-3365.25	51.35 (0.0000)*
Nevero	0.001	0.006	-3361.72	58.41 (0.0000)*
Najarra	0.002	0.006	-3366.10	49.64 (0.0000)*
Laguna	0.001	0.005	-3380.92	19.99 (0.0000)*
Average	0.005	-	-3390.92	-

β_i is the fecundity parameter for the corresponding population and β_{-i} is the average β across the remaining four populations. –log*L* is the log-likelihood of the model considering β_i and separately β_{-i}. Last column shows the deviance ($\chi^2_{1\,d.f.}$-distributed) of the model considering β_i and separately β_{-i} relative to the model (shown in the last row) assuming constant β across all five populations. Asterisks denote a significant test after sequential Bonferroni correction.

Assuming a log-normal dispersal model for *A. caespitosa*, population estimates of δ varied between 0.23 and 0.78 m (Table 3) and were significantly different ($\chi^2_{3\,d.f.}$ = 14.022, $p = 0.003$). Per-population tests showed that only the population with the largest δ (Loma de Cabezas with 0.78 m) differed significantly from the species average (Table 5). Estimated shape parameters ranged between $u = 0.84$ and 1.07 and were not significantly different from each other ($\chi^2_{3\,d.f.}$ = 1.426, $p = 0.699$). Fecundity parameter estimates exhibited significant differences in *A. caespitosa* ($\chi^2_{3\,d.f.}$ = 22.51, $p < 0.001$), ranging from

β = 0.011 to 0.024 seedlings/cm. Per-population tests indicated that the maximum (at Cabezas de Hierro) and minimum (at Collado de las Vacas) estimates of β were significantly larger and smaller than the species average, respectively (Table 6).

Table 5. Comparison of estimated mean dispersal distances (δ) among *A. caespitosa* populations.

Population	δ_i (m)	δ_{-i} (m)	-log$L$	$\chi^2_{1\,d.f.}$ (*p-value)*
Cabezas de Hierro	0.33	0.49	1155.80	1.84 (0.1744)
Loma de Cabezas	0.78	0.29	1150.46	12.53 (0.0003)*
Collado de las Vacas	0.25	0.50	1154.47	4.50 (0.0338)
Sierra de los Porrones	0.23	0.47	1155.20	3.04 (0.0809)
Average	0.43	-	1156.72	-

δ_i is the mean dispersal distance for the corresponding population and δ_{-i} is the average δ across the remaining three populations. –log$L$ is the log-likelihood of the model considering δ_i and separately δ_{-i}. Last column shows the deviance ($\chi^2_{1\,d.f.}$-distributed) of the model considering δ_i and separately δ_{-i} relative to the model (shown in the last row) assuming constant δ across all four populations. The asterisk denotes a significant test after sequential Bonferroni correction.

Table 6. Comparison of estimated fecundity parameters (β) among *A. caespitosa* populations.

Population	β_i (seedlings/cm)	β_{-i} (seedlings/cm)	$-\log L$	$\chi^2_{1\,d.f.}$ (*p-value)*
Cabezas de Hierro	0.024	0.013	1152.64	16.65 (0.0000)*
Loma de Cabezas	0.014	0.020	1159.56	2.82 (0.0932)
Collado de las Vacas	0.011	0.021	1153.55	14.84 (0.0001)*
Sierra de los Porrones	0.021	0.017	1160.66	0.61(0.4337)
Average	0.017	-	1160.97	-

β_i is the fecundity parameter for the corresponding population and β_{-i} is the average β across the remaining three populations. –log*L* is the log-likelihood of the model considering β_i and separately β_{-i}. Last column shows the deviance ($\chi^2_{1\,d.f.}$-distributed) of the model considering β_i and separately β_{-i} relative to the model (shown in the last row) assuming constant β across all four populations. Asterisks denote significant tests after sequential Bonferroni correction.

5. DISCUSSION

We used an inverse modelling approach to analyse the variation in effective seed dispersal patterns of two coexisting species along an altitudinal gradient in a high mountain pasture community of central Spain. Estimated mean dispersal distances were short in both species, with most seedlings established less than a meter from the source. There was not a single kernel function that provided the best fit across all populations. Furthermore, there were significant differences in the fecundity parameter among populations in both species and in the mean dispersal distance among populations of *A. caespitosa*, while differences in the mean dispersal distance among *S. ciliata* populations were only marginally significant. The kernel's shape parameter did not vary significantly in any of the two species.

The inverse modelling approach proved to be useful in characterizing effective seed dispersal patterns in both species. Parameter estimates were consistent among populations and species independently of the assumed kernel. In addition, estimates conformed to independent fecundity measures for the same species (Garcia-Camacho *et al.*, 2010; Gimenez-Benavides *et al.*, 2008), and to primary seed dispersal estimates obtained with direct and indirect methods in congeneric species (*Silene latifolia*: 0.17-0.85 m, (Jongejans and Schippers, 1999); *Armeria maritima*: 0.6 m, (Philipp *et al.*, 1992). Although r^2 values were slightly lower than those obtained in analogous studies (LePage *et al.*, 2000; Ribbens *et al.*, 1994; Uriarte *et al.*, 2005), they were highly significant (except in the population with the lowest seedling number). This suggests that inverse modelling was able to provide information about the scale of dispersal in the high mountain plants studied, even though as frequently found in heterogeneous environments there seemed to be substantial distance-independent fluctuations in dispersal and establishment probabilities that cannot be characterized by simple kernel fits (Nathan *et al.*, 2012).

Most effectively dispersed seeds were established near conspecific adult plants (Figure S1). Consequently, estimated mean dispersal distances were low, suggesting that both *A. caespitosa* and *S. ciliata* have low effective seed dispersal ranges. Effective dispersal scale is influenced by factors operating at the level of both primary seed dispersal and germination and establishment. According to mechanistic wind dispersal models based on the WALD kernel, the seeds of six high-mountain species are transported no farther than a few meters by primary wind dispersal, probably due to low seed release height (Dullinger *et al.*, 2011). Release height, determined by inflorescence length, is indeed low (about 5-25 cm) in both *A. caespitosa* and *S. ciliata* (see Castroviejo *et al.,* for morphological characteristics). However, according to the mechanistic simulations in Dullinger *et al.* (Dullinger *et al.*, 2011), the primary seed dispersal range of these two species should be larger than that estimated by our effective dispersal models. This suggests that effective seed dispersal is further limited by post-dispersal processes in our study species. Environmental harshness in summer might be the main factor in reducing primary seed dispersal distance. In the two species studied here, recruitment is mainly limited at the seedling emergence and survival stages by summer drought (Garcia-Camacho *et al.*, 2010; Gimenez-Benavides *et al.*, 2008). Furthermore, García-Camacho *et al.* (Garcia-Camacho *et al.*, 2010) found a positive effect of *A. caespitosa* adult cover on seedling emergence and survival in 0.5 x 0.5 m samplings subplots. This suggests that adults act as nurse plants, facilitating seedling emergence and survival along the altitudinal gradient (Garcia-Camacho *et al.*, 2010), as observed in other Mediterranean mountain regions (Cavieres *et al.*, 2006). The effect of adult cover on post-dispersal survival might lead to unrealistic conclusions of short effective dispersal distance because our modelling approach assumes a distance-invariant survival function. This assumption may be violated in the case of distance-dependent survival because the primary and effective seed dispersal kernel would attain different shapes (Tautenhahn *et al.*, 2012)**.** Hence, the modelling approach will implicitly assume that seedlings close to a conspecific adult originate from this adult, even though facilitation can also favour seeds from other adults, after more substantial dispersal (Tautenhahn *et al.*, 2012). Nevertheless, given the mean distances between adult plants recorded in our study plots (mean ± SD: 5.35 ± 0.38 m for *S. ciliata;* 5.21 ± 0.58 m for *A. caespitosa*) and the essentially barochorous seed dispersal mechanism, the ratio of seeds from other adults to seeds from the closest adult is likely to be very small, and, therefore, the effect of this bias negligible. Moreover, in *S. ciliata*, all the populations studied exhibited significant positive fine-scale spatial genetic structure (FSGS) at the 0-1 m distance class (Lara-Romero et al unpublished data), indicating that the spatial aggregation of genotypes is consistent with a low effective dispersal distance observed in our study.

Mean dispersal distance provides a limited characterization of dispersal range (Klein *et al.*, 2006; Nathan *et al.*, 2012). Other characteristics of the dispersal kernel such as the kurtosis and fatness of the distribution tail may have important demographic, ecological and genetic consequences (Clark *et al.*, 1999b; Klein *et al.*, 2006; Nathan *et al.*, 2011; Nathan *et al.*, 2012). Our results did not support a particular kernel shape in the case of *S. ciliata*. Although the behavior near the origin and the tail is not independent in the assumed phenomenological kernels (see (Clark, 1998) and references therein), the virtual absence of seedlings beyond the close proximity of *S. ciliata* adults (Figure S1) may result in similarly good fits for kernels with very different tails but with sufficiently fast probability decay near the origin. That is, fat-tailed kernels could fit well in *S. ciliata*, because the actual dispersal probability decreased fast near the origin and not necessarily because the actual process had a fat tail. In *A. caespitosa*, by contrast, results supported leptokurtic functions with exponential or fat tails, with consistently more abundant isolated seedlings than for *S. ciliata* (Figure S1). This would suggest a larger proportion of long-distance effective dispersal events in *A. caespitosa* than in *S. ciliata*.

The few studies that have previously used inverse modelling with a multi-population approach found local variation in dispersal parameter estimates within species (Clark *et al.*, 1998; LePage *et al.*, 2000). In Clark *et al.* (1998) and LePage *et al.* (2000) variation in dispersal patterns within species was found across tree stands of temperate forests that spanned gradients in moisture and canopy openness. Similarly, we expected higher effective dispersal range in high-altitude populations because less stressful conditions could reduce the intensity of the adult nurse effect on the survival of emerged seedlings. Furthermore, the lower encroachment by subalpine shrubs at high elevation sites (Garcia-Romero *et al.*, 2010) would provide a more open habitat with greater exposure to wind that might favour longer-distance seed dispersal. However, contrary to our expectations, there were not significant differences in the shape parameter value among-populations in any of the two species, while only the population with the largest mean dispersal distance in *A. caespitosa* differed significantly from the species average value for this parameter. Despite the strong variation in demographic structure and/or physical environment along the altitudinal gradient, the dispersal parameter estimates tended to be consistent among populations within the species. This implies that the inherent seed dispersal traits of the species (e.g., inflorescence length, fruit morphology, seed size and shape), which are essentially the same in all populations, were the most important factors determining the effective dispersal kernel in our study sites during the study period. Post-dispersal processes controlling seedling emergence and establishment seem to operate similarly across populations, regardless of evident variation in local demographic and environmental conditions (intra and interspecific plant density, local topography, soil moisture, temperature regime, etc.). This result provides a new insight into the prevalence and magnitude of intraspecific dispersal variation because, although consistently with previous studies (Clark *et al.*, 1998; LePage *et al.*, 2000) it shows that effective mean dispersal distances vary among populations of particular species, it also indicates that effective dispersal kernels can remain invariant across populations of other co-occurring species, even if there is significant variation in the demographic structure and the environment.

Effective fecundity did vary significantly across populations in both species, and per-population tests identified that these differences were due to the greater fecundity values found in the populations located at the highest altitude. As expected, the fecundity parameter was related with the ratio of seedlings to reproductive adults ($\tau = 1$, $p < 0.001$ for *S. ciliata*; and $\tau = 0.67$, $p = 0.167$ for *A. caespitosa*), which represents a measure of the average number of successfully established seedlings produced by each mother. By contrast, the fecundity parameter was not associated with mean seed production per adult plant (as estimated with a seed-crop sample from a small number of individuals of each population; data not shown). Furthermore, seedlings density was positively related with altitude ($\tau = 0.8$, $p < 0.05$ for both species). These results suggest different seedling mortality rates at different altitudes, which have probably influenced the observed differences in the fecundity parameter among populations and which are congruent with previous studies on the demography of both species (Garcia-Camacho *et al.*, 2010; Gimenez-Benavides *et al.*, 2007c), indicating that more benign conditions at the populations located at the highest altitudes allow the emergence and establishment of larger numbers of seedlings. Hence, the altitudinal gradient appears to influence effective seed dispersal patterns in *S. ciliata* and *A. caespitosa* through variation in effective seedling establishment probabilities, but not through variation in the effective seed dispersal range. The explicit incorporation of finer-scale environmental factors and landscape features (e.g. shrub cover) in our models (for instance through the application of the movement space concept; Schurr *et al.*, 2008) might help to estimate and model the potential effects of environmental variables on fine-scale spatial patterns of effective dispersal more accurately, and thus to increase the amount of explained variance in within-population recruitment patterns.

The estimates provided by empirical dispersal studies such as this one could help to explicitly incorporate real migration constraints in predictive species distribution models. This information is all the more relevant because accurate predictions about dispersal and migration capacities are considered to be among the most significant uncertainties in projecting climate impacts on plant species ranges (Thuiller *et al.*, 2008). Our results, together with previous seed dispersal mechanistic simulations of alpine plant species (Dullinger *et al.*, 2011) show that the majority of dispersal events occur within a few meters from the source. This is consistent with (Dullinger *et al.*, 2012), who found widespread post-glacial dispersal constraints on the current distribution of plants in the European Alps. These findings raise doubts about the capacity of high mountain plants to track their climatic niche under the rapid climate warming predicted for mountain systems during the 21st century (Nogues-Bravo *et al.*, 2007). However, spatial spread and colonization rates are not necessary governed by the mean seed dispersal distance but by the frequency of rare long-distance events (LDD) (Bullock and Clarke, 2000; Nathan *et al.*, 2003; Schurr *et al.*, 2009). LDD are usually caused by extreme events in terms of horizontal wind speed or turbulence (Bullock and Clarke, 2000; Nathan *et al.*, 2002). High-mountain habitats are exposed to frequent and strong updrafts (Jackson *et al.*, 2013; Whiteman, 2000). Consequently, LDD may occur frequently in high-mountain environments (Tackenberg and Stocklin, 2008). These events could greatly increase the chance of threatened populations to track the altitudinal-zone displacement induced by warming. Therefore, further research should accurately estimate the impact of LDD on seed dispersal patterns in mountain ecosystems using adequate methods (for instance through the application of recently developed genetic methods (Burczyk *et al.*, 2006; Moran and Clark, 2011; Robledo-Arnuncio and García, 2007).

Conclusions

This study shows that there is not a single kernel function that provides consistently the best fits across species and populations. More importantly, this study points out that effective dispersal kernels can remain invariant across populations of particular species under strong variation in demographic structure and/or physical environment, while they may vary among populations of other co-occurring species. These results call for a case-by-case analysis in a wider range of taxa and environments to assess the validity of approaches that assume invariant species-specific dispersal kernels (Klein and Oddou-Muratorio, 2011).

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SUPPORTING INFORMATION

Figure S1. Spatial distribution of adults (circles) and seedlings (crosses) in each study plot of *A. caespitosa* and *S. ciliata.*

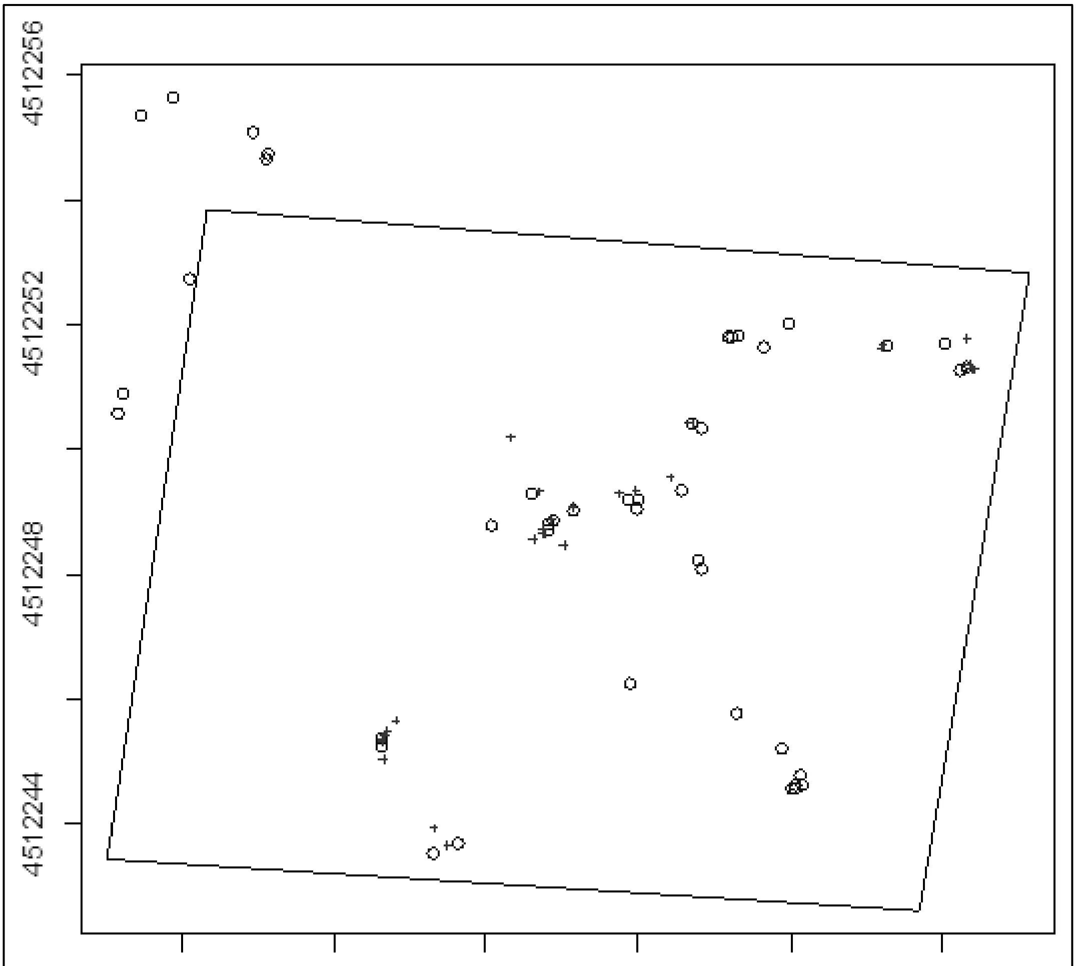

Figure S1. (A) Spatial distribution of adults (circles) and seedlings (crosses) in the Sierra de los Porrones population of *Armeria caespitosa*. Axes show UTM coordinates in meters.

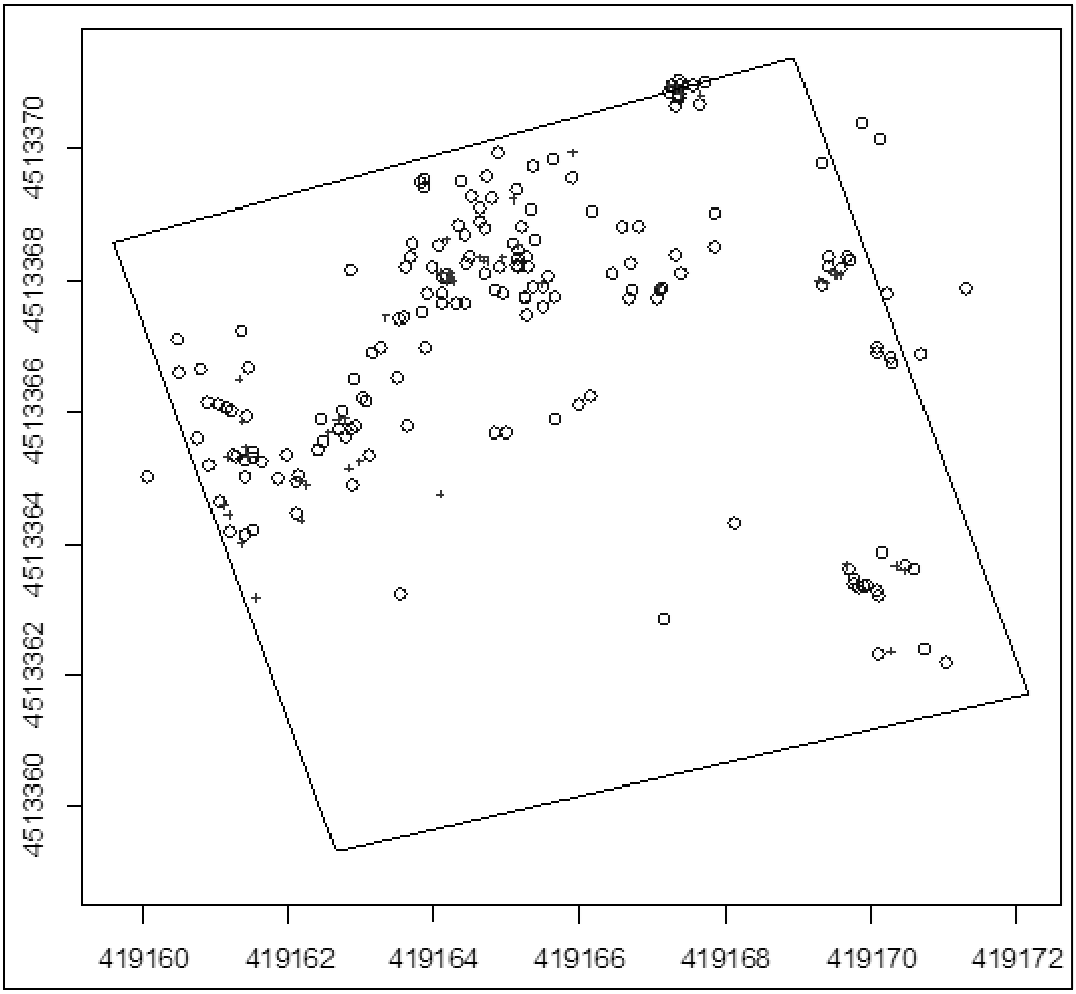

Figure S1. (B) Spatial distribution of adults (circles) and seedlings (crosses) in the Collado de las Vacas population of *Armeria caespitosa*. Axes show UTM coordinates in meters.

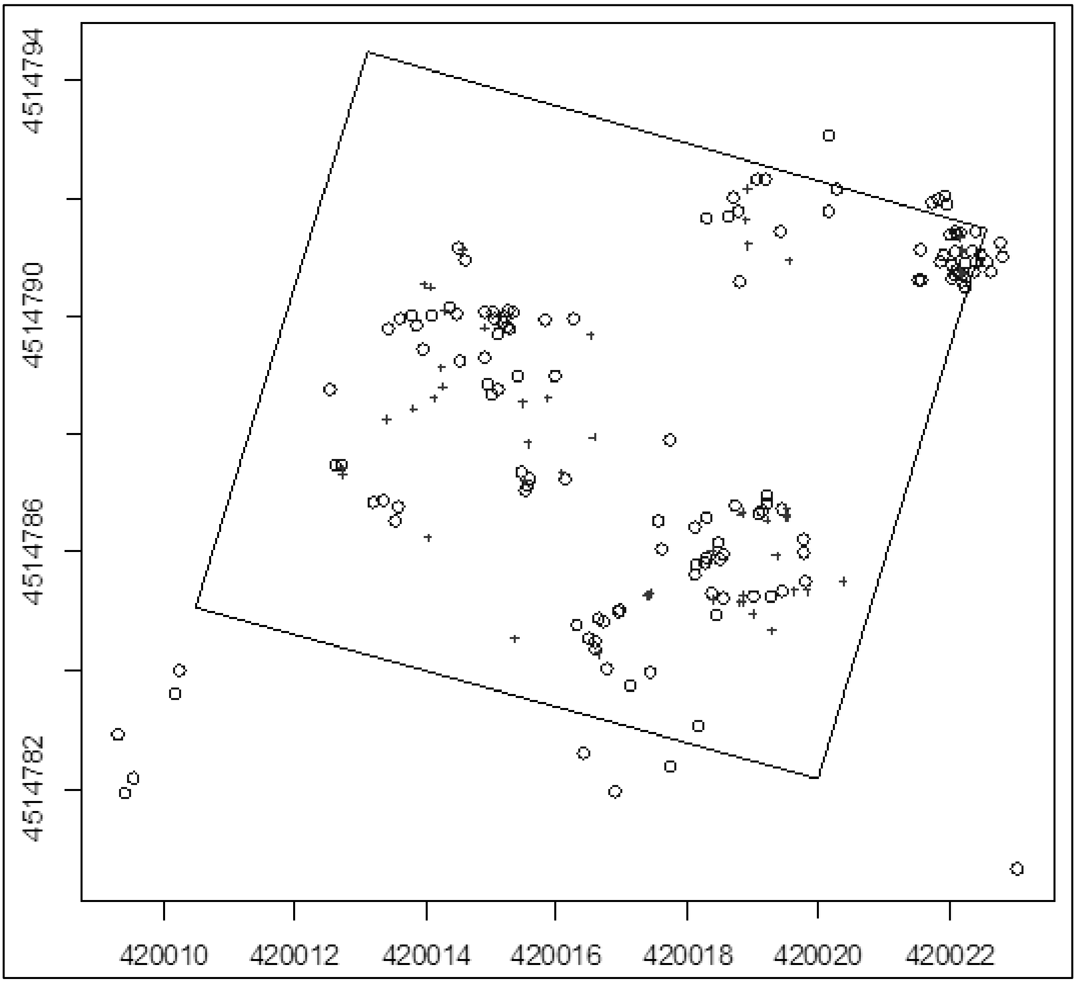

Figure S1. (C) Spatial distribution of adults (circles) and seedlings (crosses) in the Loma de Cabezas population of *Armeria caespitosa*. Axes show UTM coordinates in meters.

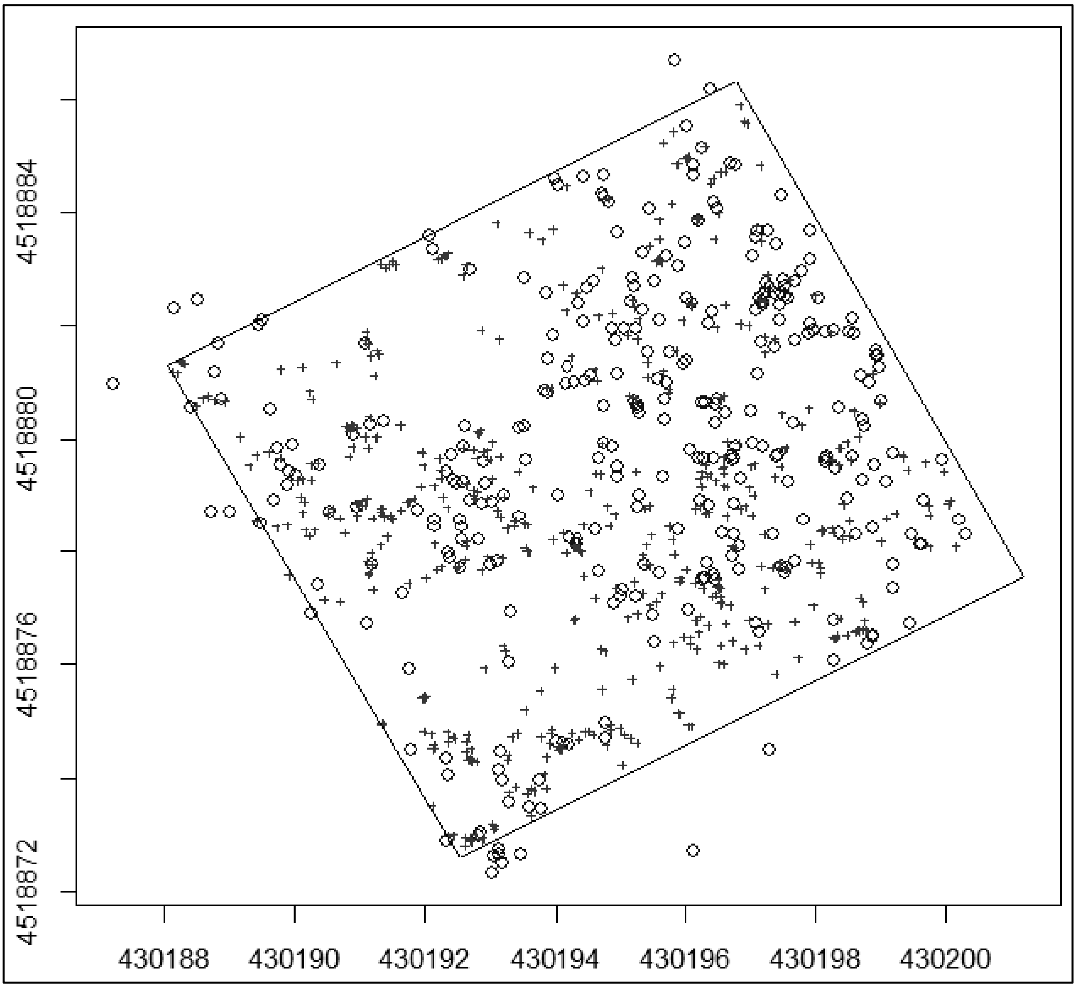

Figure S1. (D) Spatial distribution of adults (circles) and seedlings (crosses) in the Najarra population of *Armeria caespitosa*. Axes show UTM coordinates in meters.

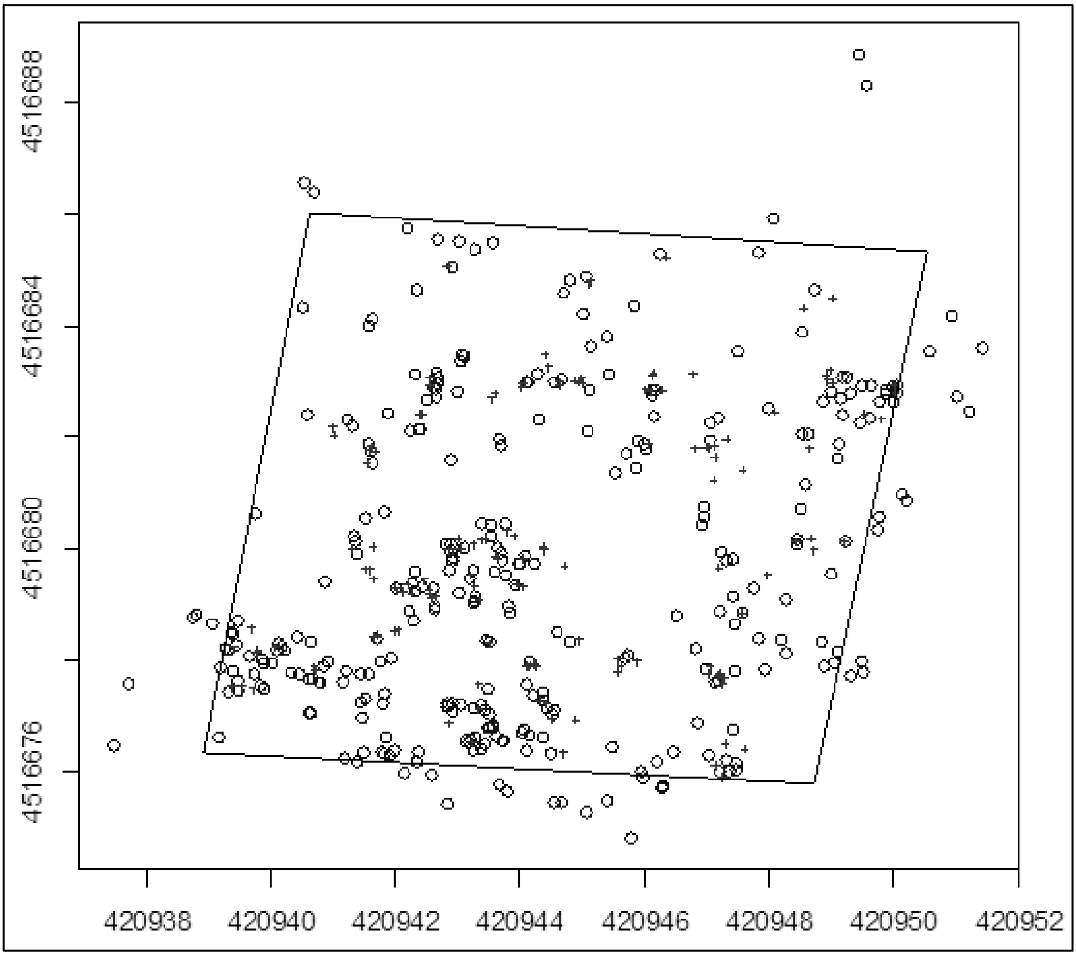

Figure S1. (E) Spatial distribution of adults (circles) and seedlings (crosses) in the Cabezas de Hierro population of *Armeria caespitosa*. Axes show UTM coordinates in meters.

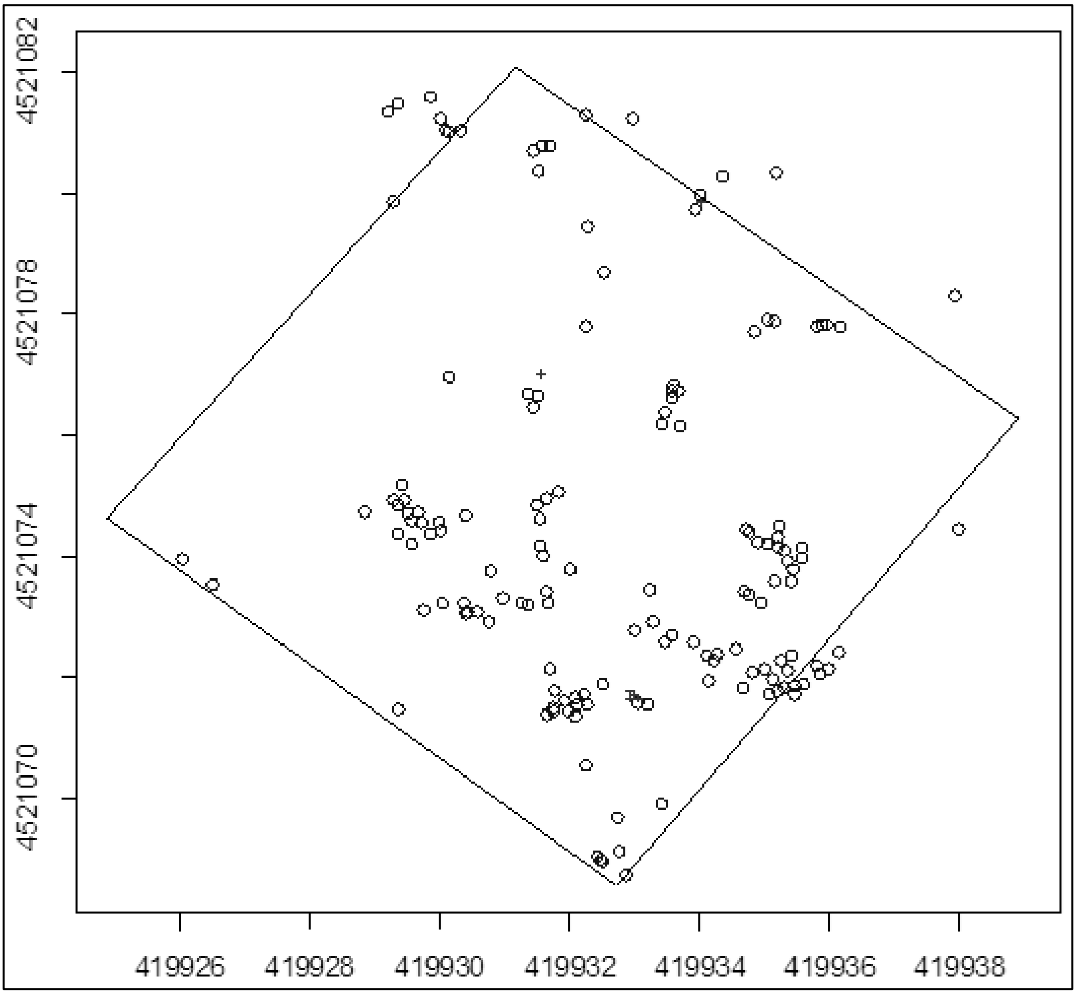

Figure S1. (F) Spatial distribution of adults (circles) and seedlings (crosses) in the Laguna population of *Silene ciliata*. Axes show UTM coordinates in meters.

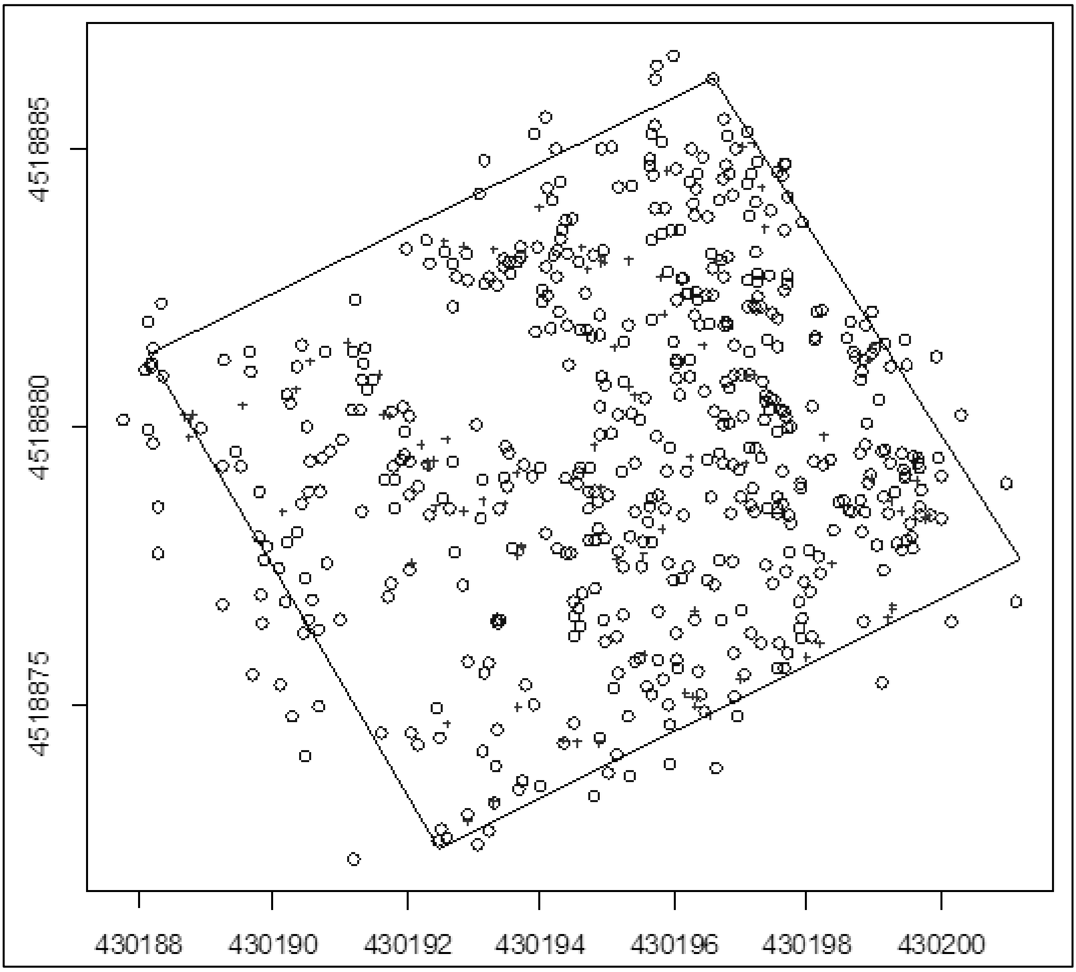

Figure S1. (G) Spatial distribution of adults (circles) and seedlings (crosses) in the Najarra population of *Silene ciliata*. Axes show UTM coordinates in meters.

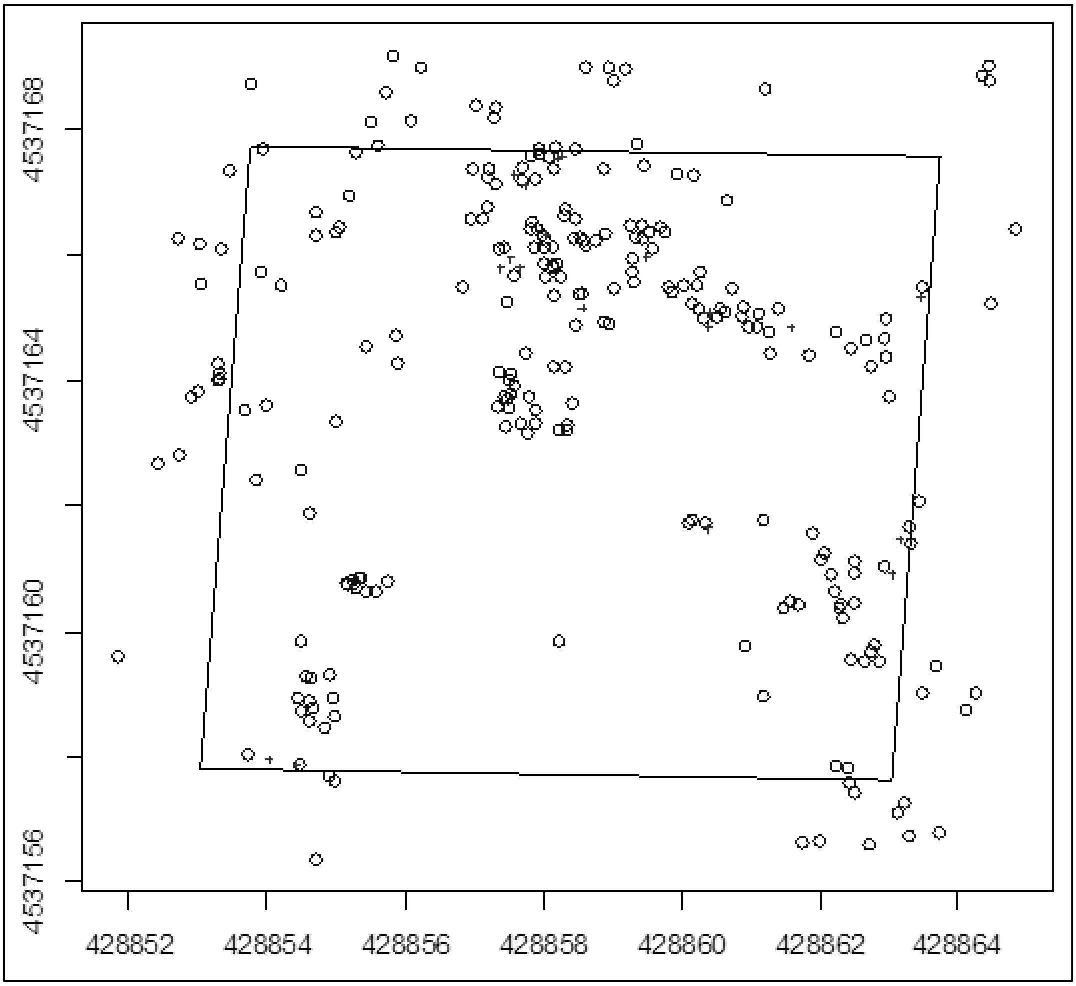

Figure S1. (H) Spatial distribution of adults (circles) and seedlings (crosses) in the Nevero population of *Silene ciliata*. Axes show UTM coordinates in meters.

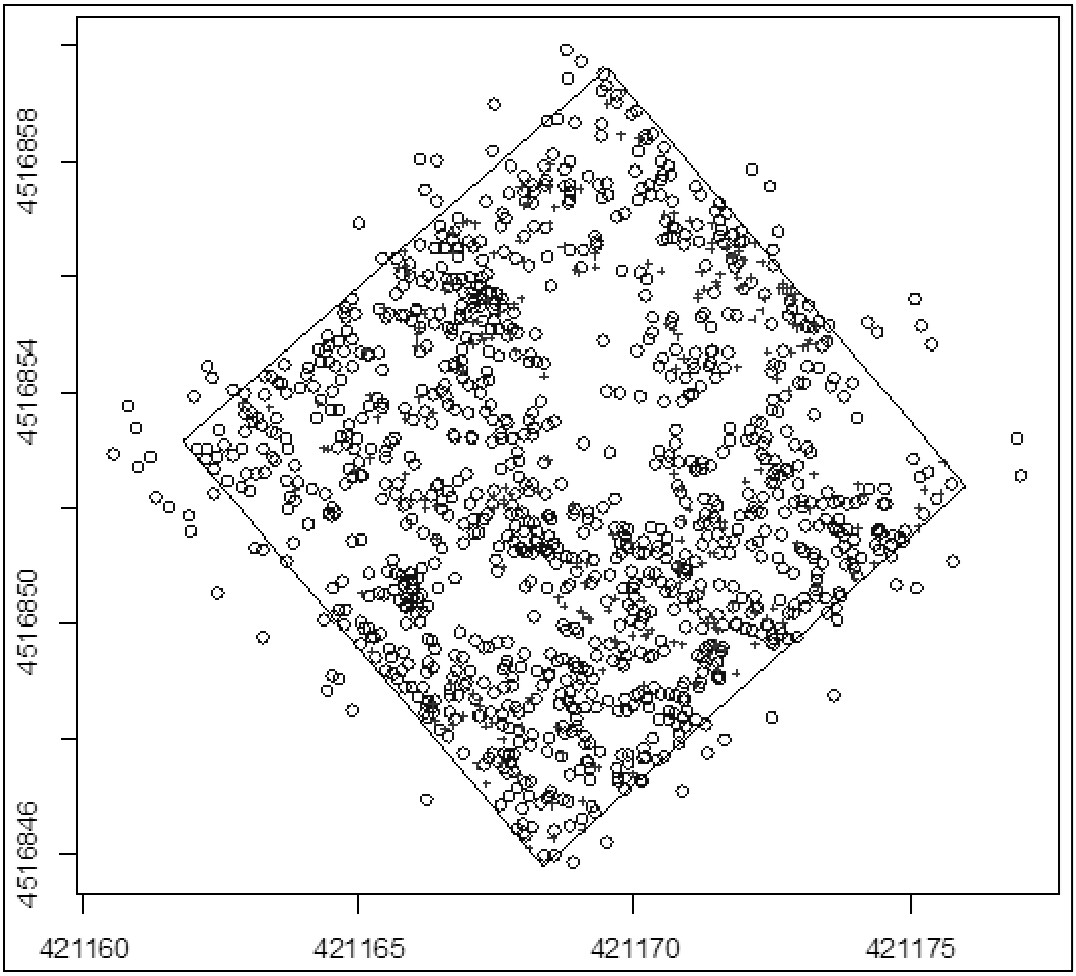

Figure S1. (I) Spatial distribution of adults (circles) and seedlings (crosses) in the Cabezas de Hierro population of *Silene ciliata*. Axes show UTM coordinates in meters.

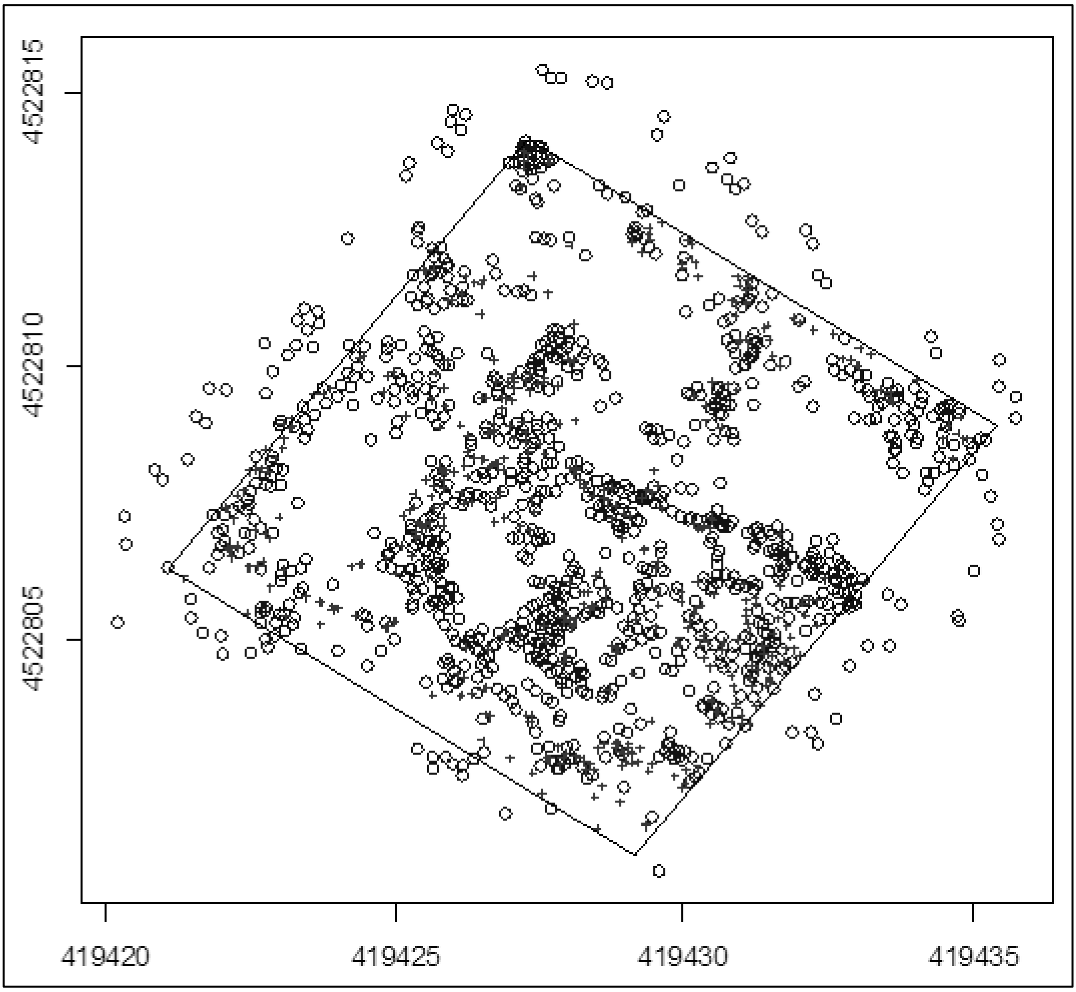

Figure S1. (J) Spatial distribution of adults (circles) and seedlings (crosses) in the Peñalara population of *Silene ciliata*. Axes show UTM coordinates in meters.

Table S1. Estimated parameters for the models fitted to the seedling recruitment data of *Silene ciliata (*cell size length 0.25 and 0.50 m)

		0.25 m					0.50 m				
populati	kernel	δ	u	β	θ	- log$L$	δ	u	β	θ	- log$L$
Peñalara	2Dt	0.33	3.20	0.010	0.511	1034.26	0.34	7.15	0.010	0.87	556.65
	Log-norm	0.34	0.66	0.010	0.511	1035.41	0.36	0.76	0.010	0.87	558.06
	WALD	0.34	0.68	0.010	0.510	1035.79	0.36	0.42	0.010	0.86	559.07
	Exp-pow	0.32	1.21	0.010	0.509	1034.49	0.34	1.54	0.010	0.87	556.60
Cabezas de Hierro	2Dt	0.42	172.46	0.003	0.523	708.02	0.41	5.16	0.003	0.91	420.42
	Log-norm	0.42	0.50	0.003	0.531	706.53	0.43	0.62	0.003	0.91	420.47
	WALD	0.42	1.49	0.003	0.531	706.48	0.43	1.09	0.003	0.91	420.69
	Exp-pow	0.41	2789.58	0.003	0.524	705.73	0.40	1.70	0.003	0.90	420.51
Nevero	2Dt	0.25	10.44	0.001	142.159	82.84	0.44	172.60	0.001	0.72	60.14
	log-norm	0.25	0.49	0.001	142.154	82.12	0.67	0.12	0.001	1.30	58.69
	WALD	0.25	0.89	0.001	142.150	82.07	0.67	47.81	0.001	1.48	58.69
	Exp-pow	0.25	2.10	0.001	142.053	82.85	0.56	398.50	0.001	0.96	59.05
Najarra	2Dt	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*Nc*
	Log-norm	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*Nc*
	WALD	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*Nc*
	Exp-pow	0.72	0.47	0.002	0.230	337.85	0.99	0.33	0.002	0.50	225.73
Laguna	2Dt	0.25	172.44	0.001	0.003	23.23	0.25	1.76	0.001	0.02	19.30
	Log-norm	0.28	0.36	0.001	0.003	23.24	0.24	0.76	0.001	0.02	19.29
	WALD	0.27	1.89	0.001	0.003	23.22	0.24	0.33	0.001	0.02	19.24
	Exp-pow	0.29	5.87	0.001	0.004	23.11	0.20	0.58	0.001	0.02	19.30

δ, mean dispersal distance (m)*;* u shape parameter; β, fecundity parameter (seedlings/cm)*;* θ, negative binomial parameter; – logL, log-likelihood; *nc* denotes models that did not converge.

Table S2. Estimated parameters for the four models fitted to the seedling recruitment data of *Armeria caespitosa.* (cell size length 0.25 and 0.50 m).

		0.25 m					0.50 m				
population	kernel	δ	u	β	θ	- log*L*	δ	u	β	θ	- log*L*
Cabezas de Hierro	2Dt	0.40	1.91	0.024	0.261	415.97	0.40	7.20	0.024	0.80	268.95
	Log-norm	0.34	0.84	0.024	0.259	415.96	0.43	0.61	0.024	0.80	268.62
	WALD	0.34	0.40	0.024	0.256	416.10	0.43	0.96	0.024	0.80	268.74
	Exp-pow	0.31	0.78	0.024	0.257	416.41	0.39	1.85	0.024	0.80	269.01
Najarra	2Dt	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*
	Log-norm	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*
	WALD	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*
	Exp-pow	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*
Loma Cabezas	2Dt	0.65	2.88	0.014	0.196	185.10	0.69	2.28	0.014	0.65	123.64
	Log-norm	0.82	1.14	0.014	0.225	184.52	0.92	1.21	0.014	0.76	123.30
	WALD	0.73	0.40	0.014	0.219	184.84	0.67	1.26	0.014	0.60	124.43
	Exp-pow	0.65	0.58	0.014	0.224	183.92	0.67	0.52	0.014	0.77	122.65
Collado de las Vacas	2Dt	0.41	1.75	0.011	0.463	151.63	0.43	1.85	0.010	0.75	99.30
	Log-norm	0.32	0.98	0.011	0.458	152.05	0.34	0.77	0.010	0.84	99.19
	WALD	0.33	0.18	0.011	0.424	152.79	0.36	0.41	0.010	0.85	98.63
	Exp-pow	0.31	0.54	0.011	0.455	152.64	0.37	0.66	0.010	0.68	100.90
Sierra de los Porrones	2Dt	0.25	1.84	0.022	0.387	75.93	0.26	3.58	0.024	0.46	54.49
	Log-norm	0.22	0.88	0.021	0.396	76.01	0.28	0.50	0.023	0.57	53.51
	WALD	0.23	0.20	0.021	0.417	75.79	0.28	0.99	0.023	0.57	53.59
	Exp-pow	0.20	0.43	0.024	0.350	76.60	0.27	1.32	0.023	0.45	55.04

δ, mean dispersal distance (m); u shape parameter; β, fecundity parameter (seedlings/cm); θ, negative binomial parameter; – logL, log-likelihood; *nc* denotes models that did not converge.

CAPÍTULO 2/CHAPTER 2

Variation in fine-scale genetic structure of *Silene ciliata* (Caryophyllaceae) across populations with different spatial aggregation patterns.

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Manuscrito en preparación.

ABSTRACT

The patterns and intensity of fine-scale spatial genetic structure (FSGS) can vary among populations within species depending on the interplay between different demographic and environmental factors. Theoretical models predict that FSGS will increase especially for local density variation due to static habitat heterogeneities, but few empirical studies have examined the differences in FSGS among populations with different degrees of spatial heterogeneity. In this study we used spatial autocorrelation methods to assessing the spatial demographic and genetic structure of five demographically stable but spatially heterogeneous populations of high-mountain specialist Silene ciliata Poiret (Caryophyllaceae). We found significant FSGS in three of the five stable populations of *S. ciliata* studied, and therefore, revealed among-population variation in the intensity of FSGS. There was a significant positive association between the spatial demographic and genetic structure. Population genetic diversity and inbreeding did not significantly vary as a function of the intensity of FSGS. Changes in environmental conditions due to variation in suitable habitat availability and microhabitat heterogeneity appeared to partially drive fine-scale aggregation of adults, and therefore, could be a main factor affecting FSGS. Contrary to findings in other species, the population with the highest plant density was also the population with the highest intensity of SGS, probably due to reduced pollen-dispersal distances. This result stresses the importance to combine FSGS analyses with explicit characterization of local spatial distribution of individuals and habitat to better understand the mechanisms generating intraspecific variation in FSGS across landscapes.

Keywords: environmental variation, heterogeneity, landscape, spatial ecology, spatial point pattern analysis.

INTRODUCTION

The generation of spatial genetic structure (SGS) within plant populations (*i.e.*, fine-scale SGS) results from the complex interaction between limited gene flow through seed and pollen dispersal, genetic drift, and local selection (Epperson & Li 1997; Vekemans & Hardy 2004). Under restricted seed dispersal within a continuous plant population, local inbreeding and family structures build up across generations in absence of homogenizing selection, leading to a genetic isolation-by-distance process (IBD, Wright 1943), which is enhanced if pollen dispersal is also restricted (Crawford 1984). Most natural plant populations exhibit IBD patterns because mating occurs preferentially between neighbouring individuals and because the majority of seed dispersal events occur over short distances (Hamrick & Trapnel 2011). The non-random spatial distribution of genotypes influences various evolutionary processes, such as biparental inbreeding, kin competition, effective population size and local adaptation, consequently affecting individual and population fitness (Linhart & Grant 1996; Escudero *et al.* 2003; Leblois *et al.* 2006). Moreover, studies of fine-scale SGS (FSGS) can provide indirect information on the scale of dispersal within populations (revised in Hamrick and Trapnell 2011), allow comparisons of the effects of different dispersal vectors or landscape features on historical gene flow (Vekemans and Hardy 2004; Hardy *et al.* 2006, Born *et al.* 2008, Gonzales *et al.* 2010), and provide knowledge on the level of within-populations inbreeding and of genetic drift (Wang *et al.* 2011).

The equilibrium pattern of IBD within populations is conveniently measured by the speed of decrease of the probability of gene identity with spatial distance, which is determined by the product between the rate of effective gene dispersal, σ_e, and effective population density, D_e (the ‘neighborhood size’ N_b, sensu Wright 1943; Rousset 2000). Both σ_e and D_e can vary among species and among populations within species, and therefore SGS patterns should exhibit variation at both hierarchical levels. Most empirical studies comparing plant FSGS patterns have focused on the influence of interspecific life-history variation, and particularly on testing whether species with different life form, mating system and seed and pollen dispersal mechanisms show different patterns of IBD (Vekemans & Hardy 2004, Hardy *et al.* 2006, Hamrick & Trapnell 2011). The extent of intraspecific (among-population) variation in FSGS and its ecological and demographic determinants have been much less studied, although historic events such as founder effects or past disturbances, intraspecific competition and microenvironmental selection have been invoked (Tero 2005, Chung *et al.* 2007; Matesanz *et al.* 2011).

The patterns and intensity of FSGS can vary among populations within species depending on the interplay between different demographic and environmental factors (Hamrick & Trapnell 2011). If environmental, phenotypic or dispersal vector variation results in stable among-population differences in seed and/or pollen dispersal range, gene dispersal rates and thus equilibrium SGS patterns would then also differ across populations (with SGS decreasing for higher gene dispersal rates), everything else being equal. Differences in population density may generate intra-specific variation in FSGS as well, because effective population density directly affects N_b, with an increasing probability of genetic identity among neighbouring plants with decreasing density (Doligez *et al.* 1998, Vekemans & Hardy 2004; Hamrick & Trapnell 2011), potentially resulting in weak or absent FSGS in high density populations (Vekemans & Hardy 2004, Hardy *et al.* 2006, Chung *et al.* 2007).

Several recent empirical studies have revealed stronger FSGS in fragmented versus continuous habitats in conspecific plant populations (*e.g.,* Valbuena-Carabaña 2007, Gonzalez *et al.* 2010, Wang *et al.* 2011; but see Born *et al.* 2008). Although some of these studies failed to detect an association between FSGS and within-fragment density, the observed trend is consistent with theoretical predictions, because fragmentation will tend to reduce effective population density over larger scales, and may also reduce the gene dispersal rate, thereby increasing local inbreeding and genetic drift over generations. On the other hand, for a given global population density, numerical simulation studies showed that the spatial aggregation of adult individuals generally increases the magnitude of SGS in comparison to random or regular individual distributions (Doligez 1998; Leblois *et al.* 2004). Both species' life history traits and extrinsic biotic and abiotic factors can generate local aggregation patterns (Getzin *et al.* 2008; Chung *et al.* 2011). Theoretical models predict that SGS will increase especially for local density variation due to static habitat heterogeneities (Robledo-Arnuncio & Rousset 2010), while it may remain unaffected or even decrease (relative to random distribution of individuals) if aggregation is due to density-dependent regulation in homogeneous habitats (Barton *et al.* 2002; Robledo-Arnuncio & Rousset 2010).

Few empirical studies have examined the differences in FSGS among populations with different densities and degrees of spatial heterogeneity (Hamrick & Trapnell 2011), and fewer still combine FSGS analyses with explicit analysis of the local spatial distribution of individuals and habitat heterogeneities (*e.g.*, Chung *et al.* 2007, 2011; Jacquemyne *et al.* 2009). Further comparative studies of FSGS across populations with different demographic and habitat characteristics are needed to better understand the mechanisms generating intraspecific variation in FSGS across landscapes (*e.g.*, Gonzalez *et al.* 2010, Born *et al.* 2008, Wang *et al.* 2011).

In this study we assessed the spatial demographic and genetic structure of five demographically stable but spatially heterogeneous populations of high-mountain specialist *Silene ciliata* Poiret (Caryophyllaceae). The low effective seed dispersal distance of this species, together with short-distance pollen transport and the possibility of selfing (García-Fernández *et al.* 2012b, Lara-Romero *et al.* 2014) are likely to yield a marked within-population spatial genetic structure in equilibrium populations of *S. ciliata*. Furthermore, previous studies have shown strong variation in population structure (*e.g.*, density and spatial distribution of individuals) and physical environment (*e.g.*, microhabitat fragmentation and water availability) among populations (Giménez-Benavides *et al.* 2007, 2008, Lara-Romero *et al.* 2014). Therefore, the populations of *Silene ciliata* constitute suitable systems to study demographic and environmental determinants of intraspecific variation in FSGS. We hypothesized that among-population variation in population structure and physical environment would have affected the equilibrium between gene flow and genetic drift, modifying the intensity of SGS. We specifically expected more pronounced SGS in populations with lower plant density and stronger individual spatial aggregation. We addressed four main questions: (1) Is within-population genetic diversity spatially structured in the study species? (2) Does fine-scale SGS vary among populations? (3) If so, is this variation associated with differences in spatial population structure (*i.e.*, density and spatial distribution of individuals) and/or differences in suitable habitat availability? (4) Are population genetic diversity and inbreeding related to the intensity of within-population SGS?

METHODS

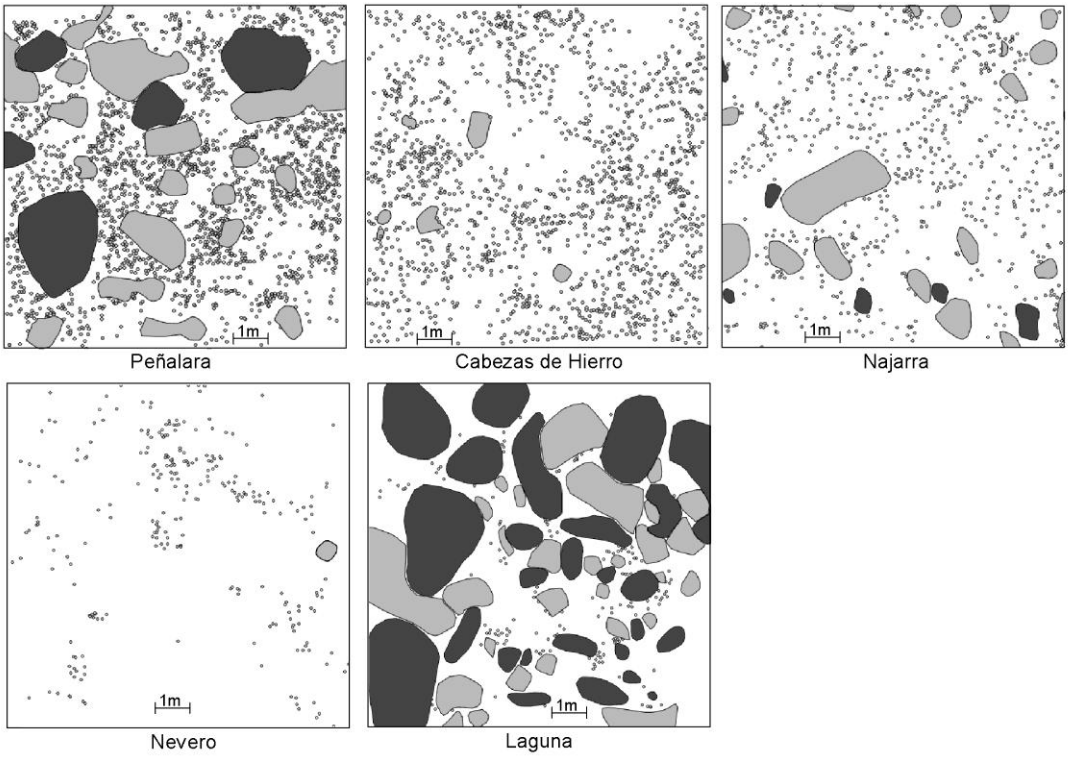
Study region and species

Silene ciliata Poiret (Caryophyllaceae) is a chamaephytic perennial cushion plant that occurs in the Mediterranean mountain ranges of southern Europe. It is self-compatible, although autogamy is restricted by pronounced protandry (Garcia-Fernandez *et al.*. 2012a). It blooms in late summer, with a peak in early August (Giménez-Benavides *et al.*. 2007). Flowering stems reach 15 cm in height and have 1–5 flowers. *S. ciliata* is pollinated at night by *Hadena consparcatoides Schawerda* (Lepidoptera: Noctuidae) but can also be pollinated by diurnal insects (Giménez-Benavides *et al.*., 2007). Fruit capsules, dehiscing by teeth, contain up to 100 seeds that are dispersed in August-September. Because its seeds lack specialized structures, seed dispersal is essentially barochorous.

The study was carried out in Sierra de Guadarrama, a mountain range located in central Spain, where *S. ciliata* reaches one of its southernmost distribution limits, with populations considered as relicts because of their isolation from populations in other mountain systems further north. It grows locally at altitudes from 1900 to 2430 m in dry cryophilic pastures dominated by *Festuca curvifolia* Lag ex Lange and other perennial plants that are interspersed in a shrub matrix characterized by *Cytisus oromediterraneus* Rivas Mart. & al. and *Juniperus communis subsp. alpina* (Suter) Čelak. Mean annual precipitation, measured at the Navacerrada Pass (40° 46′N, 4° 19′W; 1860 m a.s.l.), is 1330 mm with a pronounced dry season (< 10% of total annual rainfall) from May to October. Mean annual temperature is 6.3ºC, with mean monthly temperatures ranging from -1ºC in January to 16ºC in July (www.aemet.es). *S. ciliata* presents variable ploidy levels in natural populations; however, all individuals from the studied Guadarrama populations were diploid (2n = 24, García-Fernández *et al.*., 2012b).

Study plots

In August and September 2010, we established one 10x10m plot in each of five *S. ciliata* populations distributed along the Sierra de Guadarrama (Figure 1, Table 1). Additional information about the selected populations can be found in Lara-Romero *et al*. 2014. In each study plot, we mapped every adult *S. ciliata* individual using two high-resolution Differential Global Navigation Satellite System (DGNSS) receivers (Viva GS15, Leica, Switzerland) with an absolute accuracy of 5 cm for *x* and *y* coordinates. We also mapped the spatial position and contour of every neighboring shrub of *Cytisus oromediterraneus* and *Juniperus communis*, as well as the rocky outcrops and rocks greater than 50 cm in diameter which form unsuitable microhabitats for *S. ciliata.* Based on the spatial position of each adult plant, shrub, and rock, we constructed a map for each plot (Figure 1) using Quantum GIS, version 1.8 (http://www.qgis.org). Using this information, we estimated adult density (*D*), the availability of suitable habitat (*i.e.*, proportion of dry cryophilic pasture), and characterized habitat patches in each study plot by means of the shape index (*SI*) of the suitable habitat. *SI* measures the complexity of habitat boundary by calculating a normalized ratio of patch perimeter to area in which the complexity of patch shape is compared to a standard shape (circle or square) of the same size. It is not size dependent and equals one when the patch is a perfect circle/square while it becomes greater than one as the patch becomes more irregular (Berry 2007). *SI* is widely used in landscape ecology as a measure of landscape complexity and fragmentation (Berry 2007). The five populations spanned a gradient in adult density (*D*) and had different degrees of habitat heterogeneity (Table 1).


Peñalara

Cabezas de Hierro

Najarra

Nevero

Laguna

Figure 1. Spatial distribution of adult plants (circles), rocks (grey) and shrubs (dark grey) in each study plot of *S. ciliata*.

Table 1. Description of *S. ciliata* sampling sites.

Population	*UTM* coordinates (m)		*D*	D_e	Suitable habitat (%)	*SI*
Peñalara	419427.30	4522814.15	12.6	3.33	71.3	1.91
Cabezas de Hierro	421169.50	4516859.61	12.8	3.29	96.8	1.14
Najarra	430196.59	4518886.29	6.09	1.54	86.2	1.42
Nevero	428863.74	4537167.57	1.98	0.54	99	1.11
Laguna	419931.16	4521082.09	1.52	0.39	48.8	2.40

UTM, x,y geographical coordinates; *D*, census plant density (individuals/m2); D_e, effective plant density; *SI*, Shape index.

DNA extraction and microsatellite genotyping.

We collected leaf material of 96 adult plants per plot for genetic analysis. In order to increase sample size of shorter inter-individual distances for autocorrelation analysis, plants were selected using a clustered random sampling design (*sensu* Storfer *et al.* 2007). Namely we randomly selected 24 sample locations in each plot, where we collected leaf material of four individuals located in close proximity to each other. Portions of leaves with no sign of parasites, fungal infection or drought injuries were collected, and subsequently cleaned and dried in silica gel.

DNA was extracted using the DNeasy Plant minikit (QIAGEN, Barcelona, Spain) with 10–20 mg of dried *S. ciliata* tissue. Ten microsatellite loci previously used in genetic studies of *S. ciliata* were selected for genotyping: *Sci*1224, *Sci*1208, *Sci*0106, *Sci*1443 EST-2HTS, EST-8HTS, EST-37HTS, EST-X4-3, EST-G34D06 and EST-G47A02 (García-Fernández *et al.*, 2012b) Specifications of PCR reactions and amplification are detailed in García Fernández *et al.* (2012b). Samples were run on an automated DNA sequencer (ABI PRISM 3730, Applied Biosystems, California, USA) in Parque Científico de Madrid (Madrid, Spain). Fragment sizes were assigned to alleles using GeneMarker version 1.85 (SoftGenetics, State College, Pennsylvania, USA)

We evaluated the genotyping accuracy by the re-amplification and re-scoring of 10% of the samples. Furthermore, we estimated the occurrence of null alleles, large-alleles dropout and stuttering using the software MICRO-CHECKER (Van Oosterhout *et al.*. 2004). After checking, we discarded locus *Sci*1443 and EST-SSR X4-3 because of inconsistent allelic scoring and the possible presence of null alleles, respectively. No consistent linkage disequilibrium was found between pairwise comparisons across loci and populations (Log-likelihood ratio G statistic based on 5000 permutations performed in GENEPOP v. 4.1, Rousset, 2008).

Spatial distribution of reproductive plants within-populations.

We employed spatial point pattern analyses to characterize the spatial structure of adult plants at each of the five study populations. Specifically, we used the nonacumulative O-ring statistic (Wiegand & Moloney 2004) and the recently developed K2-index (Schiffers *et al.* 2008).

The nonacumulative O-ring O(*r*) is related to the derivative of the *K*-function and interpreted as the expected number of points (*i.e,* plants) in rings with radius *r* at a distance *m* from an arbitrary point (Wiegand and Moloney 2004). We employed *O(r)* to quantify the overall strength of spatial demographic structure within the study plot by quantifying the spatial variation in the intensity of the point pattern λ (*i.e.,* the mean number of plants per unit area) in relation to the average λ of the study plot, and also to detect heterogeneities in the point pattern. These heterogeneities are found when the function departs from the expected value for a random pattern at scales where plant-plant interactions should become unimportant, in a process known as “virtual aggregation” (Wiegand and Moloney 2004). We set the threshold where plant-plant interactions are not relevant at 1.4 m, which is over twice the mean effective seed dispersal distance detected in any of the populations (Lara-Romero *et al.* 2014). The K2-index (Schiffers *et al.* 2008) circumvents the “virtual aggregation” by relating λ at a given scale to λ at the next higher scale, allowing to determine up to which distance associations among individuals occur (*i.e.,* the strength of near-neighbour structure), complementing the information supplied by O(*r*) (Schiffers *et al.* 2008). The K2-index is related to the derivative of the pair correlation function $g(r)$ (Schiffers *et al.* 2008) and shows the expected differences in the number of points between a range of consecutive distances (Schiffers *et al.* 2008). O(r) and K2-index share the ring-based approach with spatial genetic autocorrelation methods, making the results of our spatial demographic and genetic analyses more directly comparable (Chung *et al.* 2007, 2011).

We calculated O(r) and K2-index using a ring width (r) of 0.1 m around each plant with a 0.1 m distance interval (d), and plotted as a function of distance to the plant (m). We used Ripley´s isotropic correction (Baddeley and Turner 2005) to correct for edge effects. The association between O(r) and K2-index and m was tested by computing O(r) and K2-index values for 25 distance classes and comparing these values with those expected under the null hypothesis of spatial randomness. To avoid bias in the estimates, distance classes were defined between m=0.10 m and a quarter of the plot width (2.5 m, Baddeley and Turner 2005). The confidence envelopes associated to the null hypothesis of spatial randomness was assessed by performing 999 random permutations of individual locations. We adjusted the statistical significance of the envelopes by the number of distance intervals used (α = 0.002). Values of the O(r) above and below the envelope indicates significant (α = 0.002) spatial clustering and repulsion compared to a CSR pattern, respectively. Distance classes where K2-index is different from zero indicate changing intensities, and therefore, the upper limit of distance ranges at which plants are not randomly distributed. Hence, significant positive and negative values of K2 are expected in the range of scales at which the pattern is regular and aggregated, respectively (Schiffers *et al.* 2008). Finally, to test for differences in the strength of spatial demographic structure among populations we also calculated for each population the slope of the nonaccumulative O-ring statistic ($b_{O(r)}$) and its associated 95% confidence intervals (95% CI) using least-squares regression theory (Zar 1999). All statistical analyses were performed in the open source software R *v* 3.0.2 using the package ‘spatstats’ (Baddely & Turner 2005).

Population genetic analyses.

We used GENEPOP v. 4.1 (Rousset, 2008) to calculate standard population genetic measures, including total number of alleles (A), observed (H_O) and expected (H_E) heterozygosity and inbreeding coefficients (F_{IS}). We tested for statistical differences in genetic diversity estimates (A, H_E and F_{IS}) across populations using the non-parametric Friedman rank sum test (Hollander & Wolf 1973) implemented in the R package "stats" (R Core Team, 2013). Deviations from Hardy Weinberg (HW) equilibrium within each population were tested using the Fisher's exact test implemented in GENEPOP. Genetic differentiation between populations (F_{ST}) was calculated using FSTAT v. 2.9.3 (Goudet 1995). The significance of F_{ST} values was evaluated based on 10 000 random permutations of multilocus genotypes among populations (Excoffier *et al.* 1992).

Characterization of within-population SGS

Bayesian clustering analysis

Within-population spatial discontinuities in genetic variation can arise as a consequence of biotic and abiotic landscape features that could act as a barrier to within-population gene flow (Storfer *et al.* 2007). To identify whether such genetic discontinuities occurred in each plot (*e.g.*, due to the spatial distribution of shrub and rock microhabitats) we used a Bayesian clustering method implemented in the R package 'GENELAND' version 4.0.3 (Guillot *et al.* 2005a) This approach uses Markov chain Monte Carlo (MCMC) procedures to estimate the number of genetic clusters, which is treated as an unknown parameter (Guillot *et al.* 2005a,b, 2008). Five independent runs of the spatial D-model were performed with 100 000 MCMC iterations, of which every hundredth one was saved. The spatial D-model approximates the domain of each population by the union of a few polygonal domains. This model corresponds with the spatial patterns expected under limited gene flow induced by the presence of physical barriers (Guillot *et al.* 2005a,b, 2008). We assumed linkage equilibrium between alleles across loci.

Spatial Genetic Autocorrelation Analysis

We used the spatial autocorrelation approach implemented in SPAGeDI version 1.4 (Hardy & Vekemans 2002) to assess SGS resulting from IBD. The approach, described by Vekemans and Hardy (2004), is based on genetic kinship coefficients between pairs of individuals *i* and *j* (F_{ij}, Loiselle *et al.*. 1995). To test the overall pattern of SGS in each population, F_{ij} were regressed on the spatial distance between individuals, d_{ij}, and its natural logarithm, ln(d_{ij}), giving the regression slopes (b_d and b_{Ld}) and the coefficients of determination (R^2_d and R^2_{Ld}) for each type of regression. Standard errors for F_{ij}, b_d and b_{Ld} were estimated by jackknifing over loci. We also obtained average pairwise F_{ij} over a set of distance intervals (*d*), and plotted them as a function of the distance. In all populations, mean F_{ij} was calculated at d = 0.5 m distance intervals up to a maximum distance of 10 m (above this threshold the number of pairwise comparisons was low in some populations). For each distance interval, computed values were compared to a 95% confidence interval around the null hypothesis of absence of SGS (*i.e.,* F_{ij} and d_{ij} or ln(d_{ij}) are uncorrelated). The 95% CI was constructed by performing 9999 random permutations of the spatial position of individuals.

To compare the intensity of SGS among populations, we calculated for each population the statistic *Sp*, which properly reflects the rate of decrease of F_{ij} with distance (Vekemans & Hardy 2004). We used the formula $Sp = –b_{dl}/(1–F_1)$, where F_1 is the mean F_{ij} at the smallest distance interval *d*. Mean *Sp* and standard error (SE) were calculated for each population over all loci. To test whether the statistic *Sp* differs among populations, the approximate 95% CIs were obtained as ±1.96 times the SE estimates obtained across loci. Additionally, to test for statistic association between the spatial demographic and genetic structure, we conducted correlation analysis between b_{Ld} and $b_{O(r)}$ and between *Sp* and the spatial scale up to K2-index significantly departure from SCR process. We also tested for correlational association between *Sp* and the availability of suitable habitat and their shape index (*SI*).

We also employed the software SPAGeDI to estimate Wright´s neighbourhood size (*Nb*, Wright 1943) and the effective gene dispersal rate (σ_e). In two-dimensional populations, Nb is related to σ_e and the effective population density (D_e) in the form $Nb = 4\pi D_e\sigma^2_e$ (Rousset 2000). Under Wright´s IBD model, Nb can be estimated as $Nb = –(1–F_1)/b_r$ where b_r is the restricted regression slope (b_{ld}) of F_{ij} on $\ln(d_{ij})$ in the range $\sigma_e < d < 20\ \sigma_e$ (Vekemans and Hardy, 2004). When D_e is known, σ_e can be estimated from Nb over the restricted range of distances by an iterative procedure implemented in SPAGeDI. Conveniently, D_e can be approximated as $D * N_e/N$ where N_e/N is the ratio of the effective over census population sizes. As suggested by Vekemans & Hardy 2004, we assumed that density was constant over time and computed N_e/N from $N_e/N \equiv 4/[2(1–F_I) + (1 + F_I)V)]$ (Kimura & Crow 1963), where V corresponds to the variance of the life time reproductive success among individuals. We used the demographic data reported by Giménez-Benavides *et al.* (2011) for the same study area to calculate V applying the equation $V = p^2X^2_MV_L + p^2X_LV_M + X_LX_MV_p$ (Barrowclough & Rockwee 1993), where L, M and p denotes reproductive life span, individual fecundity within a breeding season and probability of survival to breeding status of each propagule unit, respectively; with mean X and variance V. Following this procedure, the ratio of effective to census population sizes (N_e/N) was estimated at 0.264, 0.257, 0.252, 0.257 and 0.248, in Peñalara, Cabezas de Hierro, Najarra, Nevero and Laguna, respectively. These N_e/N ratios yielded effective densities ranging from 0.39 to 3.33 ind/m^2 (Table 1). We used these estimates of D_e to compute σ_e and Nb applying the iterative procedure implemented in SPAGeDI.

Approximate 95% confidence intervals for estimates of Nb were computed as $(F_1–1)/(b_r + 2SE_b)$ *and* $(F_1 – 1)/(b_r - 2SE_b)$, respectively, where SE_b is the standard error of b_r estimated by jackknifing over loci. When $b_r > 2SE$, the upper bound was reported as infinite (Fenster *et al.* 2003, Hardy *et al.* 2006). The 95% CI of σ_e was obtained using an analogous procedure.

Additionally, we used F_{IS} and F_{ij} to obtain indirect estimates of the selfing rate adjusted for biparental inbreeding, $\hat{s} = 2(F_{IS} – F_X)/(1 + F_{IS}–2F_X)$, where F_X is the coefficient of biparental inbreeding (Fenster *et al.* 2003). Following Chung *et al.* (2007), we used the intercept of the regression of pairwise F_{ij} on the natural logarithm of distance (b_{Ld}) as estimator of F_X. This is a conservative estimate of selfing rate because $\hat{s}$ assumes that outcrossing is restricted to nearest neighbor mating, where biparental inbreeding is highest (Chung *et al.* 2007).

RESULTS

Spatial distribution of reproductive plants within-populations.

The O-ring statistic O(*r*) showed a significant pattern of individual spatial aggregation at small scales that decreased strongly with distance and the magnitude of which differed largely among populations (Figure 2A). Peñalara showed the greatest neighbourhood intensity at small scales in relation to the intensity of the whole study area (slope of the nonaccumulative O-ring statistic: $b_{O(r)}$ = –4.41 (–5.70, –3.12). Nevero and Laguna exhibited an intermediate pattern: $b_{O(r)}$ = –2.19, 95% CI = (–2.72, –1.66) for Nevero; $b_{O(r)}$ = - 1.94 (–2.78, –1.10) for Laguna, while Cabezas de Hierro and Najarra showed the flattest functions, $b_{O(r)}$ = -1.43 (–1.88, –0.98) and $b_{O(r)}$ = –1.12 (–1.45, –0.79), respectively. Heterogeneities in the intensity of the point pattern were indicated by the departure of *g(r)* from CSR at large scales (*i.e.*, above 1.4 m) in all cases except in Cabezas de Hierro (Figure 2A).

Significant aggregation patterns at small scales were supported by K2-index estimates (Figure 2B), which were negative (indicating an aggregated pattern) up to 0.7 m in Laguna and 0.6 m in Nevero, and between 0.2 and 0.5 m in Peñalara. Najarra only showed significant aggregation between 0.1 and 0.2 m and Cabezas de Hierro did not show significant negative deviation from the null-hypothesis of CSR. The populations with higher plant density (Peñalara and Cabezas de Hierro) also showed a regular pattern at 0.1 m.

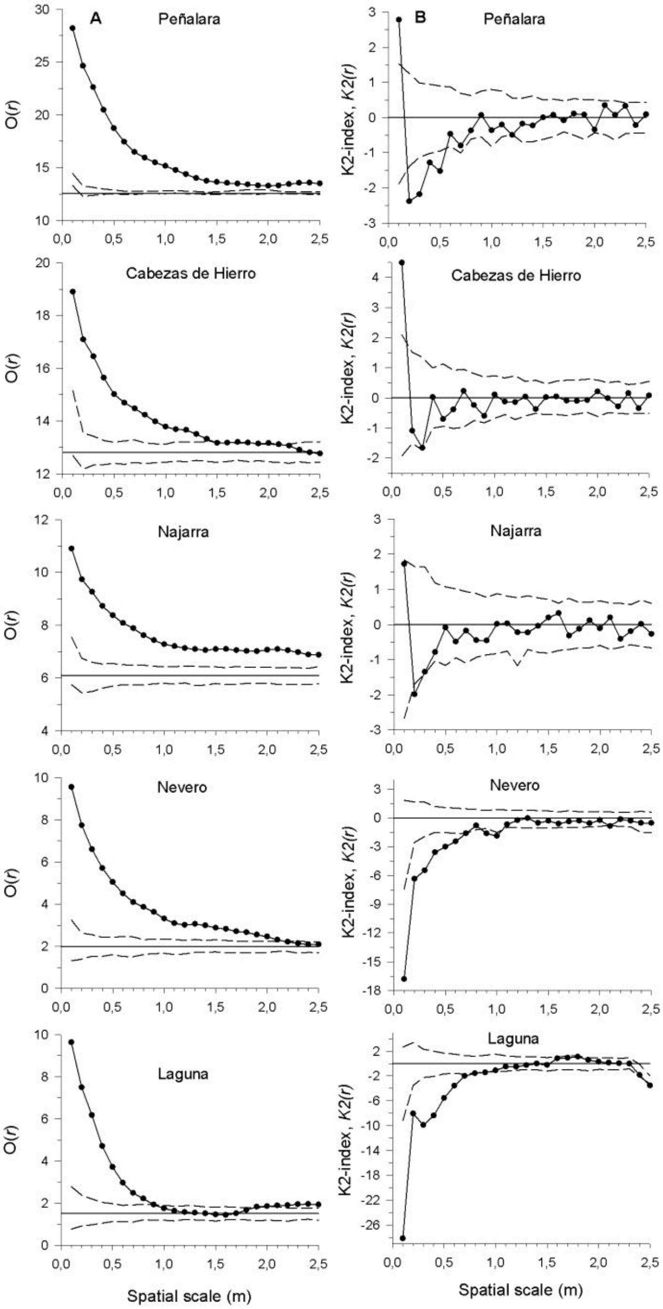


Fig 2. Spatial structure of individuals within *Silene ciliata* study plots using the O-ring statistic, $O_{(r)}$ (Panel A) and the K2-index, $K2_{(r)}$ (Panel B) for a ring of radius r with 0.1 m lags. The solid line indicates average intensity of the point pattern λ. Dashed lines indicate 95% confidence envelopes of random spatial structure after bonferroni adjustment. Note that y-axes show different scales for clarity.

Population genetic analyses

We scored a total of 144 different alleles across eight loci in 474 individuals, with a mean (± SE) number of alleles per locus of 10 (0.8). Detailed genetic diversity estimates for each marker and population are provided as Supplementary Data (Table S1). All five populations showed similar levels of genetic variation (Table 2). The average number of alleles ranged from 9 (± 1.9) in Najarra to 12 (± 2) in Nevero. Observed heterozygosities varied from 0.344 (± 0.090) in Laguna to 0.392 (± 0.079) in Cabezas de Hierro, whereas expected heterozygosities ranged from 0.504 (± 0.097) in Peñalara to 0.548 (± 0.098) in Nevero. Inbreeding coefficients (F_{IS}) varied between 0.235 (Peñalara) and 0.349 (Laguna). All populations showed significant departures from H-W equilibrium towards heterozygote deficiency, according to global tests across loci. The Friedman test did not detect significant differences among populations for any of the genetic diversity parameters. Indirect estimates of selfing rate $\hat{s}$ were 0.40, 0.45 0.48, 0.45 and 0.52 in Peñalara, Cabezas de Hierro, Najarra, Nevero and Laguna, respectively.

Population differentiation (F_{ST}) was low but significant ($F_{ST} = 0.052$, $p < 0.001$). Pairwise F_{ST} values were significantly different from zero for all population pairs (Supplementary data, Table S2), ranging between 0.030 (Peñalara - Nevero) and 0.082 (Najarra - Laguna).

Characterization of within-population SGS

All five independent runs of GENELAND gave one genetic group in each of the five populations, indicating that there were no genetic discontinuities or spatially structured gene pools in any of the studied populations.

No significant relationship was found between genetic kinship (F_{ij}) and geographic distance between individuals in Cabezas de Hierro and Najarra (b_L *and* b_{Ld} were not statistically different from 0; Figure 2, Table S3). By contrast, the slopes of linear regressions between kinship coefficients (F_{ij}) and both geographic distance and the logarithm of geographic distance were significantly negative in Peñalara, Nevero and Laguna (Figure 2, Table S3), as expected under IBD. In all three populations, the decrease of F_{ij} with distance better fitted a logarithmic relationship than a linear relationship ($R^2_{dL} > R^2_{d}$) (Table S3). The three populations with significant IBD showed significantly positive F_{ij} values in the first distance class ($d \leq 0.5$ m), rapidly declining and becoming non-significant in the second or third distance classes (Figure 3, Table S3). The *Sp* statistic confirmed the differences in the intensity of SGS among populations (Table S3). Peñalara showed the strongest SGS: *sp* = 0.0161, 95% CI = (0.0127, 0.0221); followed by Laguna and Nevero, *sp* =0.0117 (0.029, 0.0205) and *sp* = 0.0096 (0.0033, 0.0159), respectively. The lowest *Sp* values were found in Najarra and Cabezas de Hierro: *sp*= 0.0064 (0.0015, 0.0113) and *sp*= 0.0009 (0, 0.0054), respectively. We found a strong association between b_{Ld} and $b_{O(r)}$ (Pearson's product-moment correlation: $r = 0.90$, $p = 0.039$, $n = 5$) and a good but marginally significant correlation between *Sp* and K2-index ($r = 0.81$, $p = 0.096$, $n = 5$). There was not significant association between *Sp* and the availability of suitable habitat ($r = - 0.69$; $p = 0.198$, $n = 5$) and *SI* ($r = 0.65$; $p = 0.230$, $n = 5$).

Table 2. Genetic diversity estimates for *S. ciliata* populations.

Population	*N*	*A* (SE)	H_O (SE)	H_E (SE)	F_{IS}
Peñalara	96	10 (1.6)	0.384 (0.092)	0.504 (0.097)	0.235*** (5)
Cabezas de Hierro	94	10 (2.1)	0.392 (0.079)	0.548 (0.091)	0.285*** (6)
Najarra	95	9 (1.9)	0.360 (0.074)	0.524 (0.095)	0.316*** (4)
Nevero	96	12 (2)	0.391 (0.091)	0.548 (0.092)	0.283*** (5)
Laguna	93	11 (1.8)	0.344 (0.090)	0.531 (0.084)	0.349*** (6)

N, sample size; *A*, average number of alelles per locus; H_O and H_E, expected and observed heterozygosities; F_{IS}, average inbreeding coefficient; Number in parentheses in F_{IS} column are the number of loci that deviate significantly from H-W equilibrium after bonferroni correction; SE, standard error; *** P<0.001. There were no significant differences among populations for any of the genetic diversity estimators.

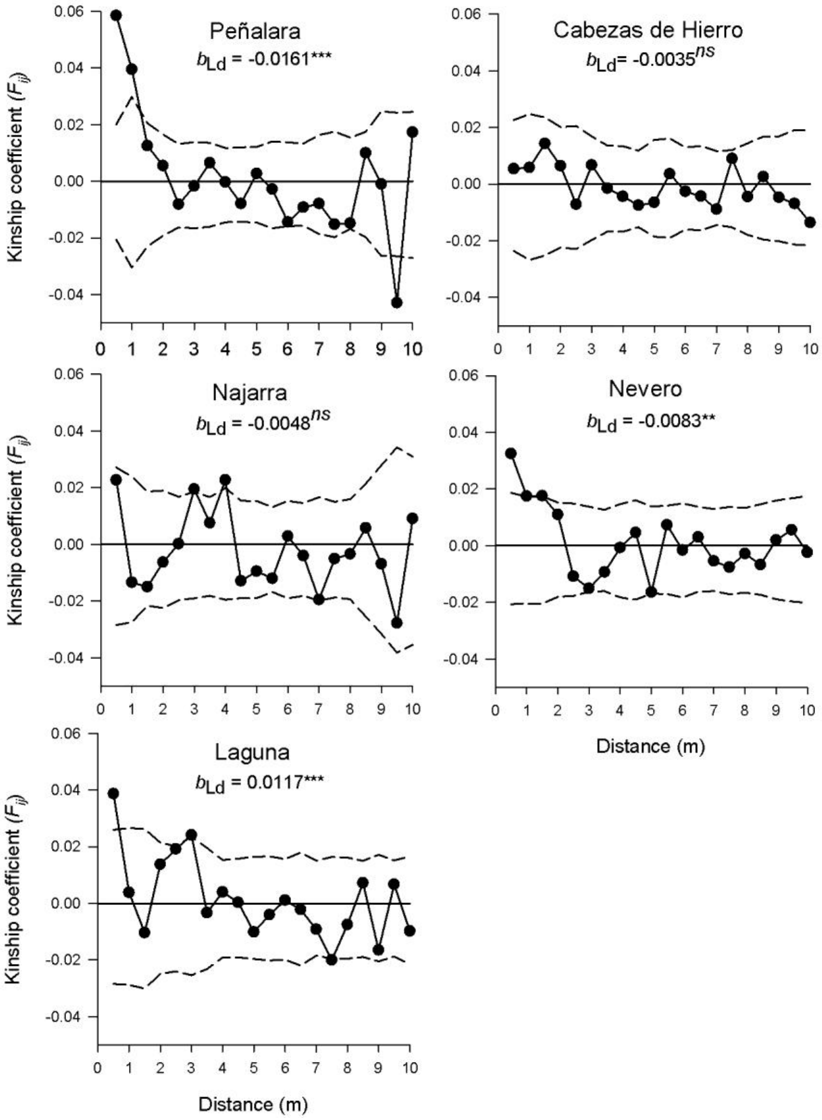


Figure 3. Average kinship coefficient, F_{ij}, plotted against geographical distance between individuals for five populations of *S. ciliata*. Dashed lines represent 95% confidence envelopes constructed under the null hypothesis that no autocorrelation exists from 9999 permutations. b_{Ld}, denote slopes of the regression of kinship coefficient values on the logarithm of the spatial distance, ln(*dij*).

We assessed Wright´s neighbourhood size (*Nb*) and the extent of gene dispersal (σ_e) for the three populations in which we detected SGS consistent with IBD. Estimates of the neighbourhood size were *Nb* = 158 individuals (95% CI: 121, 195) for Peñalara, *Nb* = 103 individuals (91, ∞) for Laguna and *Nb* = 106 individuals (81, ∞) for Nevero. *Nb* values in Peñalara were about one-tenth of the total number of individuals within the plot, while in Nevero and Laguna they were about 40 and 60% of total plot size, respectively. Using the independently estimated effective densities D_e (Table 1), we obtained a notably lower σ_e estimate for Peñalara (1.94 m, 95% CI: 1.74, 2.14) than for Nevero ($\sigma_e = 3.9$ m, 95% CI: 3.69, ∞) and Laguna ($\sigma_e = 4.65$ m, 95% CI: 4.17, ∞).

DISCUSSION

This study found significant FSGS in three of the five stable populations of *S. ciliata* studied, and therefore, revealed among-population variation in the intensity of fine-scale spatial genetic structure (FSGS). There was a significant association between spatial demographic and genetic structure. Populations with the weakest individual spatial aggregation did not show significant SGS, whereas, in the most spatially aggregated populations the slopes of linear regressions between the kinship coefficient (F_{ij}) and geographic distance were significantly negative, as expected under isolation by distance (Wright 1943). Population genetic diversity and inbreeding did not significantly vary as a function of the intensity of FSGS.

Effect of plant spatial distribution on FSGS

Assuming migration-drift equilibrium, observed differences in SGS among the study populations, as measured by *Sp*, should mainly result from differences in effective dispersal rate and/or effective population density Effective gene dispersal σ_e is properly defined as the asymptotic mean-square dispersal distance of gene lineages across generations (Rousset 2004). If the favourable habitat is not continuous, and especially if the discontinuities remains static across generations, some gene lineages will bounce between the edges of a favourable habitat patch over several generations until an inter-patch dispersal event occurs, and this landscape resistance to gene diffusion will result in σ_e values that may be substantially smaller than the mean-square parent-offspring distance

(σ) (Robledo-Arnuncio & Rousset 2010). As a result, SGS is expected to be stronger in spatially aggregated and heterogeneous than in continuous populations, even if parent-offspring dispersal distances remained unchanged. Although additional replicates are needed to get enough statistical power to detect possible significant associations, results are consistent with these theoretical predictions, and would suggest that a habitat-driven reduction in σ_e has increased SGS in Peñalara, Laguna and Nevero relative to the populations with more continuous distributions, even if average seed dispersal distances are largely invariant across populations (Lara-Romero *et al.* 2014).

Although empirical studies have often invoked environmental heterogeneity as a potential determinant of observed SGS variation (Valbuena-Carabaña 2007, Born *et al.* 2008, Gonzalez *et al.* 2010, Wang *et al.* 2011), its precise association with local individual aggregation and ensuing effects on effective gene dispersal rates have rarely been measured and discussed before. In the few studies that also quantified both spatial and genetic structure across plant populations, significant FSGS was found in spatially heterogeneous populations (Jacquemyne *et al.* 2009; Chung *et al.* 2011), while weak or absent FSGS was found in continuously distributed populations (Chung *et al.* 2007; Chung *et al.* 2008). However, the cited studies compared stable continuous populations versus expanding and senescing spatially heterogeneous populations, or seedlings versus adult plants within the same population. To the best of our knowledge, this is the first empirical study reporting some evidence about the possible relationship between plant aggregation and intra-specific SGS variation among stable plant populations.

Influence of habitat availability and global plant density on FSGS

Contrary to our expectations, the *Sp* statistic revealed that the population with the highest plant density (*i.e.*, Peñalara) also had the most pronounced FSGS. In this population, low suitable habitat availability (71.3%) together with high habitat heterogeneity (*SI* = 1.71) have probably forced greater spatial aggregation than in Cabezas de Hierro (suitable habitat = 96.8 %, *SI* = 1.14) and Najarra (suitable habitat = 86.2%, *SI* = 1.42), populations with high plant density (*D*) but weak spatial association between plants. As a result of the habitat-driven fine-scale aggregation of adults, the effective gene dispersal rate should be notably smaller in Peñalara, and therefore, may be the major factor explaining the observed differences among these three populations. There are, nonetheless, factors other than those measured in this study, that partially control habitat heterogeneity of the study species, such as interspecific competition. In particular, the fine-scale spatial aggregation of adults found in Nevero, with the habitat being 100% free of rocks and shrubs, may be explained by the presence of dominant patches of *Festuca curvifolia* (Sanchez-Pescador *et al.* 2014) with interspersed gaps where the rest of the plant species tend to occur. This may explain the strong spatial aggregation of *S. ciliata* individuals observed in Nevero population and the lack of correlation between *Sp* and the availability of suitable habitat and their shape index (*SI*).

FSGS in Peñalara was on the other hand non-significantly different than in Nevero and Laguna, in spite of having ten-fold larger population density, six-to-eight-fold larger effective density, and less-pronounced near-neighbour structure, which should in principle have resulted in larger neighbourhood size (weaker FSGS). σ_{e} was indeed considerably lower in Peñalara (σ_{e} = 1.94 m) than in Nevero and Laguna (σ_{e} = 3.9 m and 4.65 m, respectively). We hypothesize that this difference may have resulted from less extensive pollen dispersal in the highest density population. In plants, σ_e can be decomposed into effective seed and pollen gene dispersal rates: $\sigma_e^2 = \sigma_{e,s}^2 + \frac{1}{2}\sigma_{e,p}^2$ (Crawford 1984). Assuming as a rough approximation that $\sigma_{e,s}$ equals contemporary field estimates of mother-seedling axial square dispersal distance (σ_s) obtained through inverse modelling (σ_s = 0.27, 0.21 and 0.20 m for Peñalara, Nevero and Laguna, respectively; Lara-Romero *et al.* 2014), then, following Crawford (1984), the effective pollen gene dispersal rate $\sigma_{e,p}$ in Peñalara would be 1.35 m, less than half of those calculated for Nevero and Laguna (2.75 m and 3.28 m, respectively). This might be due to the greater pollinator mobility in low-density populations during foraging activities (Richards 1999; Born *et al.* 2008; Côrtes *et al.* 2013). Similar results were found for the congeneric species *Silene tatarica,* where *Sp* statistic was higher in denser populations as a consequence of restricted pollen dispersal (Tero *et al.* 2005).

Genetic diversity and inbreeding do not seem to be associated to variation in FSGS among populations

We found homogeneous values of genetic diversity (H_E) and coefficient of inbreeding (F_{IS}) across populations, which reveal the absence of relationship between population genetic diversity and inbreeding with FSGS. Concerning *Sp* and $F_{IS,}$ positive F_{IS} may be produced by biparental inbreeding and/or selfing (*i.e.*, self-pollination). Significant FSGS was only observed in three of the populations while F_{IS} was relatively high in all populations. Converseley, selfing rates ($\hat{s}$) ranged between 0.40 and 0.52 across populations, confirming previous evidence of the mixed mating system of this species with rather high levels of self-pollination (García-Fernández *et al.* 2012a,b). Thus, the occurrence of self-pollination in these populations (rather than biparental inbreeding induced by non-random spatial distribution of genotypes) may be the primary factor responsible for the high level of inbreeding observed in our study species.

Concerning *Sp* and H_E, García-Fernández *et al.*. (2012b) previously found substantial gene flow among populations in the same study site, which is supported by the low F_{ST} values obtained between our study populations. Furthermore, as previously discussed, low plant density may enhance gene dispersal by modifying pollinator behaviour and prevent genetic erosion in small populations (Born *et al.* 2008, Côrtes *et al.* 2013). On the other hand, predictions about the effect of IBD on the rate of decrease of allelic richness and heterozigosity are not unanimous, with different models predicting faster or lower rates of decrease (relative to random mating populations) depending on multiple factors such as population size, dispersal shape and range, habitat geometry and mutation (Leblois *et al.* 2006). All this suggests that FSGS can be concurrent with certain level of within and among-population gene dispersal and with maintained levels of genetic diversity within populations (Chung *et al.* 2007; Born *et al.* 2008; Wang *et al.* 2011). This may partially explain the lack of relationship between FSGS, plant density and genetic diversity found in this study.

Conclusions

Theoretical studies show that higher spatial heterogeneities in population density can increase the magnitude of FSGS in species with restricted dispersal (Doligez 1998, Barton *et al.* 2002; Robledo-Arnuncio & Rousset 2010). In the present study significant FSGS was only observed in populations with high or intermediate clustering, thereby supporting the importance of fine-scale aggregation of plants in intraspecific FSGS variation. Variation in suitable habitat availability, microhabitat fragmentation and intraspecific competition appeared to influence the fine-scale aggregation of adults, and therefore, could be a main factor affecting FSGS. Contrary to findings in other species (Vekemans and Hardy 2004, Hardy *et al.* 2006), the population with the highest plant density was also the population with the highest intensity of SGS, probably due to reduced pollen-dispersal distances. Although additional studies with even greater number of study plots are needed to gather statistical support for our findings, this study stresses the importance to combine FSGS analyses with explicit characterization of local spatial distribution of individuals and habitat to better understand the mechanisms generating intraspecific variation in FSGS across landscapes.

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SUPPORTING INFORMATION

Table S1. Genetic diversity indices for each microsatellite loci and population of *S. ciliata*. *Na*, number of alelles per locus; H_O and H_E, observed and expected heterozygosities; F_{IS}, average inbreeding coefficient.

Population	Locus	*Na*	H_O	H_E	F_{IS}
Peñalara	SSR-Sci1224	10	0.677	0.736	0.08
	SSR-Sci1208	9	0.740	0.768	0.046
	SSR-Sci0106	19	0.221	0.788	0.721*
	SSR-G34D06	9	0.656	0.706	0.071*
	EST-G47A02	5	0.083	0.130	0.359*
	EST-37HTS	12	0.323	0.437	0.262*
	EST-2HTS	4	0.146	0.166	0.186
	EST-8HTS	8	0.240	0.327	0.268*
C. de Hierro	SSR-Sci1224	8	0.620	0.663	0.065
	SSR-Sci1208	7	0.456	0.708	0.358*
	SSR-Sci0106	20	0.376	0.864	0.579*
	SSR-G34D06	10	0.733	0.780	0.06*
	EST-G47A02	4	0.128	0.196	0.351*
	EST-37HTS	12	0.278	0.474	0.416*
	EST-2HTS	4	0.096	0.170	0.439*
	EST-8HTS	17	0.462	0.552	0.163*
Nevero	SSR-Sci1224	11	0.740	0.814	0.091*
	SSR-Sci1208	6	0.521	0.649	0.198*
	SSR-Sci0106	20	0.289	0.810	0.644*
	SSR-G34D06	13	0.763	0.737	-0.036
	EST-G47A02	4	0.042	0.082	0.489*
	EST-37HTS	8	0.209	0.331	0.37*
	EST-2HTS	18	0.250	0.585	0.574*
	EST-8HTS	13	0.313	0.397	0.213*

Table S1 (cont.)

Population	Locus	*Na*	H_O	H_E	F_{IS}
Najarra	SSR-Sci1224	7	0.522	0.624	0.165*
	SSR-Sci1208	8	0.653	0.717	0.09*
	SSR-Sci0106	21	0.358	0.847	0.579*
	SSR-G34D06	9	0.484	0.712	0.322*
	EST-G47A02	3	0.053	0.102	0.484*
	EST-37HTS	10	0.233	0.572	0.593*
	EST-2HTS	5	0.116	0.150	0.23*
	EST-8HTS	9	0.463	0.487	0.049*
Laguna	SSR-Sci1224	9	0.602	0.656	0.082
	SSR-Sci1208	6	0.688	0.744	0.076
	SSR-Sci0106	20	0.250	0.703	0.646*
	SSR-G34D06	12	0.641	0.768	0.166*
	EST-G47A02	3	0.054	0.143	0.625*
	EST-37HTS	8	0.130	0.358	0.636*
	EST-2HTS	14	0.215	0.616	0.652*
	EST-8HTS	12	0.174	0.280	0.38*
Mean	-	10	0.374	0.531	0.299*

Table S2. Matrix of pairwise differentiation (F_{ST}) among populations. * F_{ST} values significantly different from zero after Bonferroni correction.

	Peñalara	Cabezas de Hierro	Nevero	Najarra	Laguna
Peñalara	-				
Cabezas de Hierro	0.033*	-			
Nevero	0.030*	0.037*	-		
Najarra	0.055*	0.045*	0.071*	-	
Laguna	0.043*	0.07*	0.054*	0.082*	-

Table S3. Estimates of within-population spatial genetic structure at five populations of *S. ciliata*.

Population	F_1 (SE)	b_d (R_d^2)	b_{Ld} (R_{Ld}^2)	*Sp* (SE)
Peñalara	0.059*** (0.012)	-0.0037*** (0.0045)	-0.0161*** (0.0086)	0.0161 (0.0042)
Cabezas de Hierro	0.006^{ns} (0.008)	-0.0011^{ns} (0.0004)	-0.0035^{ns} (0.0004)	0.0009 (0.0023)
Najarra	0.023^{ns} (0.019)	-0.0013^{ns} (0.0004)	-0.0048^{ns} (0.0005)	0.0064 (0.0025)
Nevero	0.038** (0.012)	-0.0013* (0.0008)	-0.0083** (0.0030)	0.0096 (0.0032)
Laguna	0.034*** (0.021)	-0.0025** (0.0022)	-0.0105*** (0.0032)	0.0117 (0.0045)

F_1, mean F_{ij} value between individuals separated at the minimum distance interval*;* b_d and b_{Ld}, denote slopes of the regression of kinship coefficient values on the spatial distance, *dij*, and its logarithm, ln(*dij*), respectively. The associated coefficient of determination R^2 is shown in brackets; *Sp*, strength of the genetic structure calculated for the minimum distance class in each population; SE, standard error. *ns* $P > 0.05$, * $P<0.05$, ** $P < 0.01$, *** $P<0.001$.

CAPÍTULO 3/CHAPTER 3

Genetic variation in flowering phenology and reproductive performance in a Mediterranean high-mountain specialist.

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ABSTRACT

Adaptive responses to past climate change may play an important role in the persistence of high-mountain plants, which are vulnerable to global warming. *Armeria caespitosa* is a high-mountain plant, endemic to the Iberian Central Range. Differences in abiotic environment along the elevational gradient impose the species to two opposing stress gradients concerning water stress and duration of the growth season. Furthermore, the species is found in two interspersed, contrasting microhabitats (rocky outcrops and dry cryophilic grasslands) that have different effects on plants depending of the altitude. As a result of this, the species shows great among-population variation in many reproductive and vegetative traits. We used a common garden approach to assess whether this phenotypic variation has a genetic basis or is the result of plastic responses shaped by heterogeneous environmental conditions. Plants from the high-elevation edge and dry cryophilic grasslands flowered earlier and produced more viable fruits but were smaller. These results confirm that among-population variation in flowering phenology and reproductive performance traits in *A. caespitosa* is partially genetically based. Results also showed that the stronger selection response in favor of early-flowering individuals in the low-edge populations did not correspond with greater proportion of early-flowering individuals. Genetic variability associated with flowering onset may be relevant in coping with the impacts of ongoing global warming.

Keywords: common garden, flowering phenology, reproductive performance, mountain plants, local adaptation, altitudinal gradients.

INTRODUCTION

High-mountain plant species are among the organisms thought to be especially vulnerable to global warming (Thuiller *et al.,* 2005; Nogues-Bravo *et al.,* 2007). This becomes a critical issue considering the exceptionally high warming rates projected for the 21st century (Nogues-Bravo *et al.,* 2007). The main response of high-mountain plants to this temperature increase seems to be upward range shifts tracking their current climatic niche (Gottfried, Pauli & Futschik, 2012; Pauli, Gottfried & Dullinger, 2012). However, this may not be feasible for some species because upslope habitat may not be available due to interspecific competition, unfavorable soil conditions or because other species already inhabit summit areas (Pauli *et al.,* 2003; Körner *et al.,* 2007; Larsen, Brehm & Navarrete, 2011). Furthermore, the seed dispersal capacity of some high-mountain species may not be large enough to allow them to track their climatic niche under rapid climate warming (Lara-Romero *et al.,* 2014). In these cases, plants can prevent local extinction by varying their phenotype by means of phenotypic plasticity and/or genetic change through an adaptive evolutionary process (Davis, Shaw & Etterson, 2005; Jump & Peñuelas, 2005; Miner *et al.*, 2005; Gienapp *et al.,* 2008; Chuine *et al.*, 2010; Lloret *et al.*, 2012).

Phenotypic plasticity allows for a more rapid and flexible adjustment to environmental change than adaptation by natural selection (Sultan, 2000; Miner *et al.*, 2005). However, in the long run, plastic response to extreme environmental changes has its limits (Bradshaw, 1965; Bradshaw & McNeilly, 1991; DeWitt *et al.*, 1998; Jump & Peñuelas, 2005; de Jong, 2005; Gienapp *et al.,* 2008). Adaptive evolution can greatly influence the patterns and rates of response to new climatic conditions (Jump & Peñuelas, 2005; Davis and Shaw, 2001; Davis *et al.,* 2005; Parmesan, 2006; Gienapp *et al.,* 2008) and complement other responses, including plasticity or migration (Davis *et al.,* 2005, Parmesan, 2006).

Environmental-change driven phenotypic responses in mountain plant populations has been reported in several studies (Inouye, Morales & Dodge, 2002; Dune *et al.,* 2003; Inouye, 2008; Kudo & Hiro, 2006; Giménez-Benavides *et al.,* 2011). Nevertheless knowledge on the evolutionary consequences of such phenotypic shifts is still scarce

(Gienapp, 2008; Chuine *et al.,* 2010). A change in a species' environment can impose new or intensified selection regimes on traits that may lead to genetically based evolutionary shifts (Hoffmann & Hercus, 2000; Davis *et al.,* 2005; Parmesan, 2006; Gienapp *et al.,* 2008; Munguía-Rosas *et al.,* 2011). However, a heritable trait under natural selection may be prevented from an adaptive response due to evolutionary constraints such as correlations among traits, lack of sufficient genetic variability, fluctuating selection or deleterious mutations (Linhart & Grant, 1996; García-Ramos and Kirkpatrick, 1997; Hoffmann & Hercus, 2000; Jump & Peñuelas, 2005; Parmesan, 2006; Gienap *et al.*, 2008). Actually, many phenotypic differences observed in natural populations may simply be the result of non-evolutionary variation (Miner *et al.,* 2005; Levin, 2009). Consequently, in order to determine a species' potential to adapt to ongoing climate warming, it is critical to know if phenotypic shifts along environmental gradients have a genetic basis.

Armeria caespitosa (Ortega) Boiss. *in* DC. (Plumbaginaceae) is a high-mountain specialist plant, endemic to the Iberian Central Range. Differences in abiotic environment along the elevational gradient impose the species to two opposing stress gradients concerning water stress and duration of the growth season. Hence, plants at higher altitudes experience more favourable conditions in terms of temperature and water availability but have less time to grow, flower and disperse (Gimenez-Benavides *et al.*, 2007; García-Camacho & Escudero 2009; Gimenez-Benavides *et al.,* 2011, Lara-Romero *et al.,* 2014). Furthermore, the species is found in two interspersed, contrasting microhabitats (rocky outcrops and dry cryophilic high-mountain grasslands) that have different effects on plants depending of the elevation. Overall, nutrient and water content in soil is likely to be higher in grassland than in rocks (Körner 2003). Nevertheless, rocky outcrops allow for higher number of orientations in less space than grasslands enabling plants to reach favorable exposure conditions depending on the altitude: orientations with low insolation in summer at lower altitudes and orientations with greater exposure to prevailing wind at higher altitudes. The latter allows earlier snowmelt at rocky outcrops than at grasslands (Palacios, de Andrés & Luengo, 2003), which may increase the length of growing season and reproductive success of the species (García-Camacho & Escudero

2009; Giménez-Benavides *et al.*, 2011). Earlier studies have shown that *A. caespitosa* is likely to withstand environmental conditions along these complex gradients varying some ecologically important traits along its distribution range. Hence, differences in reproductive performance and demographic vital rates have been found in populations at each microhabitat and edges of the species elevation range (García-Camacho & Escudero, 2009; García-Camacho, Iriondo & Escudero, 2010; García-Camacho *et al.*, 2012). This reproductive and demographic variation suggests that *A. caespitosa* is able to persist at the low edge of its elevation range through "demographic compensation" (García-Camacho *et al.*, 2012); a mechanism by which a decrease in certain vital rates (*i.e.*, life span and fecundity rates) imposed by stressful environmental conditions is buffered by compensatory changes in other demographic vital rates (*i.e.*, growth rate) due to phenotypic plasticity and/or local adaptation (Doak & Morris, 2010). This species seems to have the potential to respond to early snowmelt in warm years by means of shifts in reproductive phenology (García-Camacho & Escudero, 2009; Giménez-Benavides *et al.*, 2011). This ensures an adequate match between flowering period and optimal environmental conditions, which is essential for reproductive success since the growing season is usually very short due to water shortage derived from high temperatures and lack of precipitation as the summer progresses (Giménez-Benavides, Escudero & Iriondo, 2007; Giménez-Benavides *et al.*, 2011). Moreover, flowering onset in this species has been found to be under selection: plants that bloomed earlier flowered for longer periods and produced more fruits (Giménez-Benavides *et al.*, 2011). However, the stronger selection responses for earlier flowering at the lower elevations (Giménez-Benavides *et al.*, 2011) indicates that there is spatial variation across the different elevations and microhabitats. Nevertheless, it remains unknown whether the selective advantage of early-flowering individuals is great enough to cause the predominance of early-flowering phenotypes in nature (Giménez-Benavides *et al.*, 2011).

In this context, we aimed to assess whether the observed variation in a suite of reproductive and vegetative traits in natural populations has a genetic basis or is the result of plastic responses shaped by heterogeneous environmental conditions. We used a common garden approach to study the variation of such traits in four populations of *A.*

caespitosa. Common garden experiments allowed us to control the effect of environmental factors on the expression of the traits of interest, ensuring that any differences found among the considered populations would have a genetic origin (Turesson, 1922; Clausen, Keck & Hiesey, 1940; Kawecki & Ebert, 2004). We hypothesized that part of the phenotypic variability observed in natural environments has a genetic basis and therefore, we would detect significant differences among populations in traits related to demographic and reproductive performance and flowering phenology. Concerning flowering phenology, we asked whether the stronger selection responses for earlier flowering observed at lower elevations are promoting the predominance of early-flowering phenotypes (Giménez-Benavides *et al.*, 2011).

MATERIAL AND METHODS

Study species and source populations

Armeria caespitosa (Ortega) Boiss. *in* DC. is a high-mountain dwarf chamaephytic cushion plant, endemic to the mountains of central Spain (Sierra de Guadarrama, Ayllón and East Gredos). It grows at elevations from 1600 to 2430 m in two interspersed but contrasting microhabitats: crevasses and ledges of granite and gneiss outcrops (hereafter rock microhabitat), and in dry cryophilic *Festuca curvifolia* Lag. *ex* Lange fell fields (hereafter pasture microhabitat). *A. caespitosa* is self-incompatible and pollinated by bees, bumblebees and syrphid flies (García-Camacho, Mendez & Escudero, 2009). Its flowers are grouped in short-scaped flowerheads of 19 ± 8 (mean ± SD) flowers. Further information on the natural history of *A. caespitosa* is available in García-Camacho and Escudero (2009) and García-Camacho *et al.*, (2009, 2010, 2012).

The study site was located in the Sierra de Guadarrama mountain range (Madrid, Spain). Vegetation composition of these high-mountain systems is detailed in Escudero *et al.*, (2004). We replicated the design in García-Camacho and Escudero (2009) to allow the comparison of previous results on the phenology and reproductive output of the species in its natural environment. Hence, we selected four *A. caespitosa* populations at the high and low edges of the species distribution range (hereafter, high-edge and low-edge populations, respectively), two in the rock microhabitat and two in the pasture microhabitat (Table 1). We avoided isolated or small populations to minimize bias due to confounding factors. Thus, we selected genuine functional populations with more than 300 individuals under similar environmental conditions, such as orientation and shrub cover. These populations could not be replicated because they constitute the edge populations of *A. caespitosa* in the Sierra de Guadarrama. On the Western distribution edge in Sierra de Gredos, *A. caespitosa* hybridizes with a close congener, *Armeria bigerrensis* subsp. *bigerrensi*s, whereas on the Eastern distribution edge in Sierra de Ayllón *A. caespitosa* populations are small and scarce. More information on the selected populations can be found in García-Camacho & Escudero (2009).

Seed collection and cultivation of plants

In July 2006, we collected fruits from 100 healthy individuals haphazardly chosen at each site and microhabitat (fruits were collected from only 60 plants on the cliffs of Site 1 due to inaccessibility). Fruits were dissected in the laboratory and all the seeds collected per population were pooled. Plants were grown in the CULTIVE Facility situated at 690 m a.s.l. in Universidad Rey Juan Carlos, Móstoles, Spain (http://servicat.escet.urjc.es:8080). Mean annual precipitation and temperature measured at the experimental field of the CULTIVE Facility are 494 mm (13 – 51 mm) and 14.4 ºC (5.8 - 24.9ºC), respectively. In spring 2007, we sowed 200 seeds from each population in rectangular plastic pots (5 cm^3 volume approximately) with a commercial substrate enriched with NPK and distributed them haphazardly in a greenhouse bench. We allowed plants to develop without stress caused by temperature, nutrient or water limitations. In April 2010, we transplanted the plants that had grown into the reproductive stage (188 plants in total) in 1800 cm^3 rectangular plastic pots with a commercial substrate enriched with NPK. We randomly distributed the pots in the experimental field of the CULTIVE Facility to allow natural pollination. Once again, plants were cultivated without stress caused by temperature, nutrient or water limitations. Because maternal effects are usually only pronounced at the seedling stage and decrease over time (Mousseau & Fox, 1998), we only collected data after the plants had grown under homogeneous conditions for five years, minimizing bias in results due to maternal effects. Voucher specimens were deposited in the Universidad Rey Juan Carlos Germplasm bank (Supporting Information, Table S1).

Sampling methods

We waited until the flowering season of 2011 to monitor phenology and reproductive performance. At that time we also measured the maximum diameter of each plant both as an estimation of plant size at flowering time and growth rate (as all plants were sown at the same time). We counted the total number of flowers per plant every 7 days throughout the flowering period for a total of 15 censuses. All phenological variables were measured with reference to 15th of February. When fruits were ripe at the end of the flowering period, we collected and counted all the flowerheads from each plant. We dissected ten flowerheads per individual, counted the total number of fruits and viable fruits per flowerhead (fruits with one fully matured viable seed) and obtained the mean values for each variable. Following García-Camacho & Escudero (2009) and Nieto-Feliner, Izuzquiza & Lansac (1996), we considered fruits to be viable when they had enlarged and hardened enclosing calyces with wide intercostal spaces, patent hairs and wide-open scarious involucres, which were resistant to pressure from forceps.

We also calculated four individual-based phenological variables: flowering onset, duration, moment and synchrony. Flowering onset was defined as the number of days elapsed between the 15th February and the first flower on the plant. Duration was estimated as the number of days elapsed between the onset and end of blooming and moment as the number of days elapsed between the blooming of the first flower in all populations studied and the flowering peak. Finally, synchrony was calculated considering the following function (Albert, Escudero & Iriondo, 2001):

$$S_i = \frac{1}{n-1} \sum_{j=1}^{n} \frac{a_{ij}}{b_{ij}}$$

where n is the number of plants, a_{ij} is the number of days during which individuals *i* and *j* are in flower simultaneously, and b_{ij} is the number of days during which at least one of them is in flower.

Table 1. Ecogeographic characterization of *A. caespitosa* sampling sites.

Sampling site	Elevational range	Habitat	Geographic coordinates	Altitude (m)	Pm (mm)	Tm (ºC)
Porrones	Low	Rock	40° 45′N, 3° 56′W	1750	1133 (25 – 130)	8.2 (5.2 – 18.3)
Loma de Cabezas	Low	Pasture	40° 46’N, 3° 56′W	1950	1351 (30 – 153)	5.7 (-0.7 – 15.1)
Cabezas de Hierro	High	Rock	40° 48′N, 3° 55′W	2350	1594 (34 – 193)	4.2 (-2.8 – 13.7)
Cabezas de Hierro	High	Pasture	40º 47’N, 3º 56’W	2300	1594 (34 – 193)	4.2 (-2.8 – 13.7)

Pm: mean annual precipitation, *Tm:* mean annual temperature. Minimum and maximum monthly precipitation and temperatures are provided in brackets. Climatic data obtained from Gonzalo-Jiménez (2011).

Statistical analysis

We used General Linear Models (hereafter GLMs) to test for genetic differences in reproductive phenology. Hence, we fitted models for onset, duration, moment and synchrony. All these models included position in the elevational range (hereafter elevational range) and microhabitat as categorical predictors. As in García-Camacho & Escudero (2009), we considered range edge instead of elevation, because we were especially interested in how plants behave at the local low and high edges of their ranges. Similarly, rock and pasture were included as the two levels of microhabitat. We also included interaction effects between elevational range and microhabitat. In the case of the models fitted for duration, synchrony and moment, we included plant size and onset as predictor continuous variables.

We also used GLMs to assess differences in reproductive output. We fitted models for number of flowerheads, fruits per flowerhead and viable fruits per flowerhead. All these models included elevational range, microhabitat and their interaction effects as predictors. In the case of the model fitted for number of flowerheads, we included plant size and onset as continuous predictor variables, while in models fitted for fruits per flowerhead and viable fruits per flowerhead, the continuous predictor variables were plant size and the two phenological variables onset and synchrony. In addition, we fitted GLMs with elevational range, microhabitat and their interaction to model plant size. We did not include moment and duration in the models as predictor continuous variables because both phenological traits were significantly related to onset (see below).

We fitted all models applying the principle of parsimony by means of a stepwise model simplification process or deletion test (Crawley, 2007). We achieved this by fitting a maximal model (*i.e,* the model containing all the predictor variables mentioned above) and then removing sequentially non-significant interaction terms and explanatory variables from the maximal model to obtain a final model containing nothing but significant and non-redundant predictor variables (*i.e.,* minimal adequate model, Crawley, 2007). The continuous predictor variables were entered first in the models to minimize potential effects of plant size and/or phenological variables before calculating categorical predictor's effects. We assumed Poisson error and log link functions for all the response variables except fruits per flowerhead and viable fruits per flowerhead, for which we included a quasi-Poisson error and a log-link function.

To examine the effect of elevational range and microhabitat on the long-term survival of adult plants, we tested for differences in mean survival times using the log-rank X^2- test of homogeneity among groups of the Kaplan-Meier product-limit survival analysis (Kleinbaum & Klein, 2005). Data for the survival analysis were collected in May 2010, September 2010, May 2011 and September 2011. All statistical analyses were performed in the open source software package R *v* 3.0.2 (R Core Team, 2014).

RESULTS

Means, standard deviations and sample size of phenology and reproductive variables for each population are presented in Supporting Information, Table S2.

Models fitted for onset and duration found a significant effect of elevational range and microhabitat (Table 2a, b). Plants from the highest elevation and from the pasture microhabitat flowered earlier and had shorter flower duration, once the negative effect of flower onset on flower duration had been taken into account (Figure 1; Table 2a, b; Table S2). No significant elevational range x microhabitat interactions was found. The remaining phenological variables were not significantly affected by elevational range and microhabitat. However, onset significantly affected flowering moment and duration (Table 2b, c). Hence, plants that flowered earlier had a longer flowering duration and a shorter flowering moment. Plant size did not affect any of the studied phenological variables.

Elevational range and microhabitat had a significant effect on the number of flowerheads (Table 3). Plants from low-edge populations and the pasture microhabitat produced more flowerheads (Figure 2a, Table 3). There was also a significant elevational range x microhabitat interaction due to a greater variation between microhabitats at the low-edge populations (Figure 2a, Table 3). Plant size and onset also had a significant positive and negative effect on number of flowerheads, respectively (Table 3).

Table 2. Minimal adequate model for reproductive phenology of *Armeria caespitosa* plants grown in the common garden experiment.

	Solution for effects				**Deviance Change**	
Effect	**Parameter value**	**SE**	***z* value**	***Pr*(>|*z*|)**	χ^2	**Pr(>χ^2)**
a) Flowering onset						
Intercept	3.957	0.023	171.81	<0.001	-	-
Range	-	-	-	-	5.02	0.02
Low	0.062	0.026	2.41	<0.01		
Microhabitat	-	-	-	-	15.19	<0.001
Rock	0.099	0.025	3.04	<0.01	-	-
b) Flowering duration						
Intercept	4.712	0.056	83.94	<0.001	-	-
Onset	-0.021	0.001	-20.03	<0.001	67.60	<0.001
Range	-	-	-	-	6.31	0.01
Low	0.084	0.032	2.67	<0.01		
Microhabitat	-	-	-	-	5.60	0.02
Rock	0.075	0.031	2.38	0.02	-	-
c) Flowering moment						
Intercept	4.226	0.047	89.84	<0.001	-	-
Onset	0.002	0.0008	2.75	<0.01	7.61	<0.01

SE: Standard Error. Missing factors were removed during model simplification process based on a deletion test. Missing levels of factors (range: high; microhabitat: pasture) are included in the intercept.

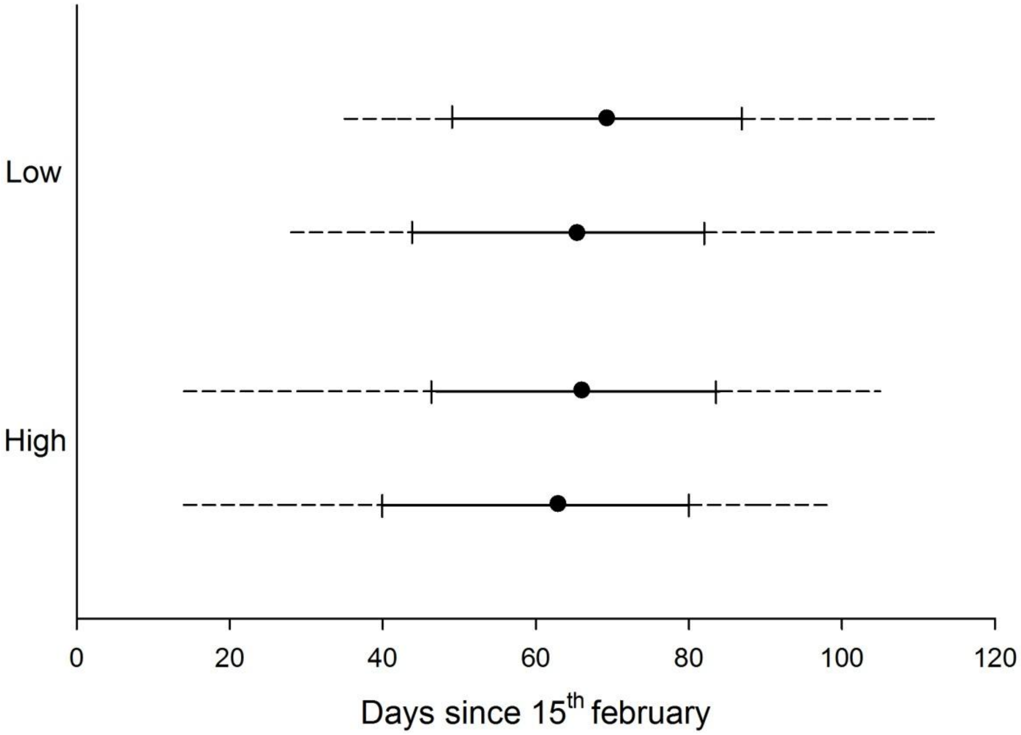

Figure 1. Variation in flowering phenology between microhabitats and between elevation edges. Horizontal bars indicate flowering onset, duration and end per elevation edge and microhabitat. Dashed lines represent the range of flowering duration in each study population; solid lines delimited by small vertical bars represent the range of mean individual flowering duration. Circles represent the mean flowering moment.

Table 3. Minimal adequate model for number of flowerheads of *Armeria caespitosa* plants grown in the common garden experiment.

	Solution for effects				**Deviance change**	
Effect	**Parameter value**	**SE**	***z* value**	***Pr*(>|z|)**	χ^2	***Pr*(>χ^2)**
Intercept	2.84	0.11	25.96	<0.001	-	-
Plant size	0.012	0.001	12.79	<0.001	67.41	<0.001
Onset	-0.007	0.001	-7.09	<0.001	38.35	<0.001
Range	-	-	-	-	67.41	<0.001
Low	0.575	0.036	15.94	<0.001	-	-
Microhabitat					57.94	<0.001
Rock	-0.099	0.046	-2.15	0.03	-	-
Range:Microhabitat	-	-	-	-	10.08	<0.01
Low:Rock	-0.177	0.056	-3.18	<0.01	-	-

SE: Standard Error. Missing factors were removed during model simplification process based on a deletion test. Missing levels of factors (range: high; microhabitat: pasture) are included in the intercept.

Number of flowerheads was positively related to total fruits per flowerhead, whereas non-significant effects of elevational range and microhabitat were found (Figure 2b, Table 4a). On the other hand, elevational range had a significant effect on the number of viable fruits per flowerhead, although a significant elevational range x microhabitat interaction was also found (Table 4b). Consequently, the number of viable fruits per flowerhead was greater at the high-edge populations in the pasture microhabitat than at the low-edge populations but was lower in the rock microhabitat (Figure 2c, Table4b). Plant size and the two phenological variables onset and synchrony were not significantly related with fruit performance of plants grown in the common garden experiment.

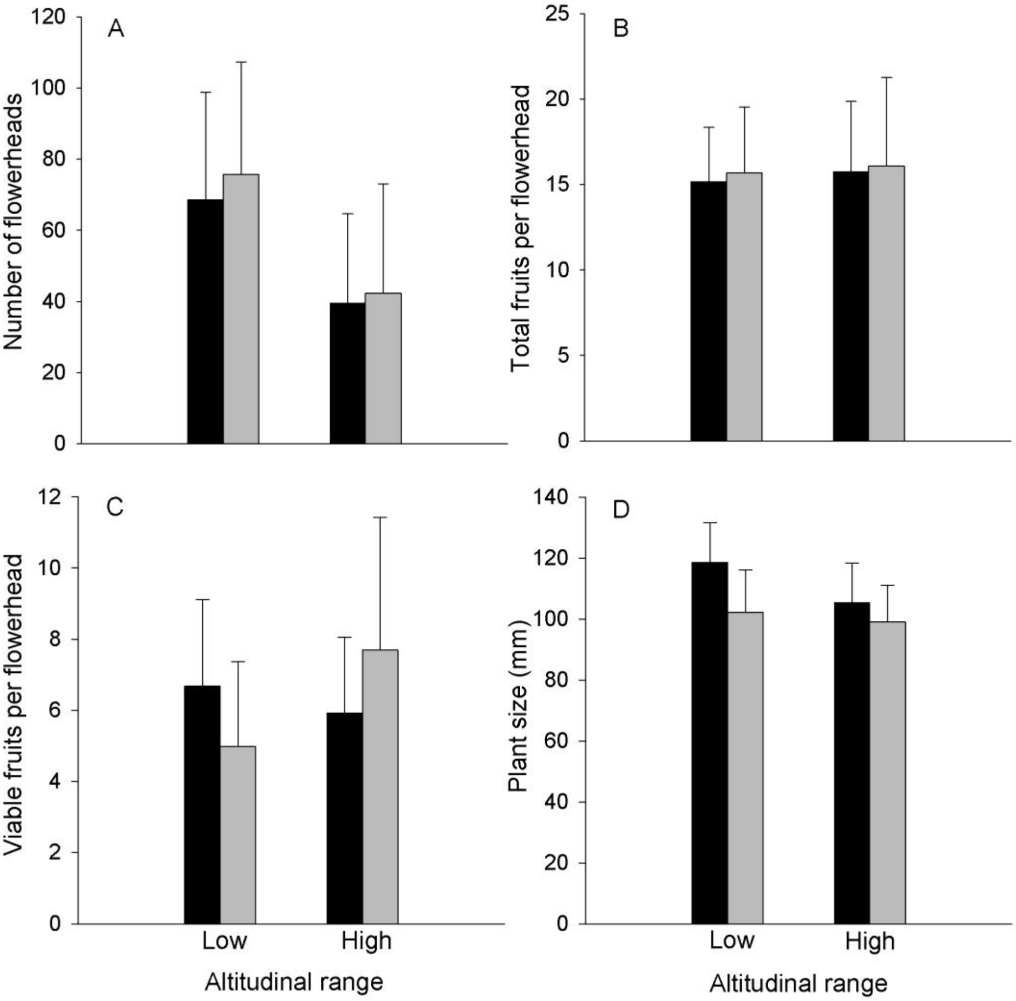

Figure 2. Mean values and standard deviations for the components of reproductive success and plant size of *Armeria caespitosa* in the common garden experiment. Black bars: rock microhabitat; grey bars: pasture microhabitat.

Table 4. Minimal adequate model fitted for fruit performance of *Armeria caespitosa* plants grown in the common garden experiment.

	Solution for effects				Deviance change	
Effect	**Parameter value**	**SE**	***t* value**	**Pr(>|*t*|)**	χ^2	**Pr(>χ^2)**
a) Total fruits per flowerhead						
Intercept	2.661	0.05	9.80	<0.001	-	-
Flowerheads	0.002	0.0007	2.10	0.04	4.31	0.04
b) Viable fruits per flowerhead						
Intercept	2.040	0.076	26.74	<0.001	-	-
Range	-	-	-	-	5.32	0.02
Low	-0.432	0.110	-3.93	<0.001	-	-
Microhabitat	-	-	-	-	0.11	0.74
Rock	-0.262	0.121	-2.16	0.03	-	-
Range:Microhabitat	-	-	-	-	11.09	<0.001
Low:Rock	0.553	0.167	3.32	<0.01	--	--

SE: Standard Error. Missing factors were removed during model simplification process based on a deletion test. Missing levels of factors (range: high; microhabitat: pasture) are included on the intercept.

A significant effect of elevational range and microhabitat on plant size was found (Table 5). Plants were found to be smaller in the high-edge populations and in the pasture microhabitat (Figure 2d, Table 5). Kaplan-Meier product-limit survival analysis did not find significant differences in the shape of the survival curves across elevations or microhabitats (log-rank $\chi^2 = 5.6$, $df = 3$, $P = 0.13$).

Table 5. Minimal adequate model fitted for plant size of *Armeria caespitosa* plants grown in the common garden experiment.

	Solution for effects				**Deviance change**	
Effect	**Parameter value**	**SE**	***z* value**	***Pr*(>|*z*|)**	**χ^2**	***Pr*(>χ^2)**
Intercept	4.571	0.017	270.56	<0.001	-	-
Range	-	-	-	-	12.63	<0.001
Low	0.072	0.019	3.82	<0.001	-	-
Microhabitat	-	-	-	-	36.53	<0.001
Rock	0.112	0.019	6.06	<0.001	-	-

SE: Standard Error. Missing factors were removed during model simplification process based on a deletion test. Missing levels of factors (range: high; microhabitat: pasture) are included on the intercept.

DISCUSSION

This study shows that in *A. caespitosa*, among-population variation in ecologically relevant traits related to reproductive performance and flowering phenology is genetically based. The inherent genetic variation observed in flowering onset was not consistent with the selective pressures previously identified in natural populations.

Flowering onset

The common garden was established at lower elevation than the natural populations. As a result, plants flowered several weeks earlier in our common garden (early March) than in natural populations (early May: García-Camacho & Escudero, 2009). This is congruent with previous studies that have shown that *A. caespitosa* comes into bloom soon after snowmelt. These results further support the idea that this species is photoperiodically neutral (García-Camacho & Escudero, 2009; Giménez-Benavides *et al.*, 2011) and that a temperature threshold triggers flowering onset. In natural conditions, *A. caespitosa* flowers earlier at the low-elevation edge, because the critical threshold temperature is reached earlier. However, under common garden conditions, flower onset was earlier in plants from the high-elevation edge. This suggests that the threshold temperature for flowering is lower for individuals from the high-elevation edge.

The genetic variation in flowering onset detected in the common garden experiment between the low and high-elevation edges does not correspond with the selective pressures previously identified in natural populations, where the selection response for earlier flowering was found to be stronger in the low-elevation edge and in the rock microhabitat, but practically negligible in all the other studied populations (Giménez-Benavides *et al.*, 2011). The results reported by Giménez-Benavides *et al.* (2011) are based on selection differentials and gradients. These analyses reveal the strength and direction of the selection acting on each trait just during the selective episodes studied (Lande & Arnold 1983, Arnold & Wade 1984), while the genetically based variations found in a common garden experiment are the outcome of both recent and more ancient processes (Kawecki & Ebert 2004). Consequently, selection responses within a population and genetic differentiation between populations for flowering onset do not need to be causally connected. No selection pressures are expected when the populations have reached their optimal because phenotypes are already differentiated; whereas selection pressures may be strong and different when populations are yet undifferentiated but growing in different environments (Lande & Arnold 1983, Arnold & Wade 1984, Kawecki & Ebert 2004).

Flowering onset at lower temperatures at the high-elevation edge may be linked to the shorter effective growth period on the mountain summits in terms of temperature and the positive correlation between flowering duration and seed production found in natural populations (Giménez-Benavides et al 2011). In this context, earlier flowering at lower temperatures would increase seed production. On the contrary, the threshold temperature for flowering is reached at the low-elevation edge earlier in the year when sudden spring freezing events are more likely. These episodic spring freezing events may eliminate the advantage of flowering at an earlier date. Giménez-Benavides *et al.* (2011) found directional selection for early flowering genotypes at the lower-elevation edge for two consecutive years, one which was climatically benign and one which was extremely warm and dry. However, episodic late spring freezing events may stabilize selection at a higher threshold temperature when averaged throughout time.

Similarly, the earlier flowering onset found in the pasture microhabitat compared to the rock microhabitat may be related to earlier snowmelt in the latter due to its lower specific heat (Körner, 2003) and greater exposure to prevailing winds (Palacios, de Andrés & Luengo, 2003). In this way, plant populations in the pasture microhabitat would have shorter effective growth periods and need to lower the threshold temperature for flowering onset to maintain the length of the reproductive period and maximize seed production. As a result, the growing season is shorter at the high-elevation edge and in the pasture microhabitat (García-Camacho & Escudero 2009, Giménez-Benavides *et al.,* 2011, Escudero *et al.,* 2012), which may have led to selective pressures towards earlier flowering onset. This is supported by the lower flowering duration showed by plants from these populations in relation to plants from low-elevation edge and rock microhabitat under common garden conditions. In any case, substantial within-population variation in flowering onset was also detected in the common garden experiment (coefficients of variation 0.2 - 0.4). This suggests that relevant genetic variation in flowering onset also exists at the population level.

Flowerheads, viable fruits per flowerhead and plant size

Previous studies on the reproductive success of *A. caespitosa* in the same natural populations used for this study found no significant differences between populations in terms of number of flowerheads (García-Camacho & Escudero, 2008). In contrast, the number of viable fruits per flowerhead was higher at the higher-elevation edge and at the pasture microhabitat in the climatically benign year 2004, but no significant differences were found in the warmer and drier year of 2005 (García-Camacho & Escudero, 2009). Therefore, it is clear that differences in reproductive success greatly depend on environmental conditions. These previous studies also showed that higher-elevation edge and pasture microhabitat tend to harbor smaller plants than lower-elevation edge and rock microhabitat (García-Camacho & Escudero 2008). The sign of the differences found in the natural populations in benign conditions is in accordance with those found in the common garden experiment, suggesting that differences in these traits are also partially due to inherent genetic variation between populations. This variation may be related to the way plants allocate resources to reproduction versus further vegetative growth and survival (Cousens, Dytham & Law, 2008) and may explain the process of "demographic compensation" detected in this species (García-Camacho *et al.*, 2012). These findings agree with those of previous studies that have reported genetic differences in reproductive traits among high-mountain plant populations (e.g. Pluess & Stöcklin, 2005; Stöckin, Kuss & Pluess, 2009; Frei *et al.*, 2011). Once again, relevant variation in these traits was also found at the within-population level in the common garden experiment, suggesting substantial genetic variation within populations (coefficients of variation: 0.4 - 0.7 and 0.4 - 0.5 for number of flowerheads and number of viable fruits per flowerhead, respectively).

Genetic variation in the context of global warming

The inherited capacity to shift flowering onset may be of adaptive value for natural populations under ongoing global warming. In mountains, temperature and precipitation modifications due to climate change seem to primarily affect snow and snowmelt timing (Beninston *et al.*, 1997; Dye, 2002; Laternser & Schneebeli, 2003; García-Romero *et al.*, 2010) and, consequently, the onset and length of the growing season (Inouye *et al.*, 2002; Dune *et al.*, 2003; Inouye, 2008; Kudo & Hiro, 2006; Giménez-Benavides *et al.*, 2011). This effect is particularly critical in Mediterranean mountains because summer precipitation is scarce and soil moisture mainly depends on the supply of snowmelt water, which decreases progressively as the season advances (Giménez-Benavides *et al.*, 2007). In this context, the genetic variation found within *A. caespitosa* populations may allow them to advance their flowering period in response to warmer conditions, increasing flowering duration and reproductive performance (Giménez-Benavides *et al.*, 2011). This would represent an adaptive response that may enhance persistence under ongoing global warming (Linhart & Grant 1996; Davis, Shaw & Etterson, 2005; Gienapp *et al.*, 2008; Chuine *et al.*, 2010).

Limitations of the common garden experiment

It should be noted that the observed genetic differences among populations may not necessarily be adaptive. In theory, they could also be caused by neutral genetic drift resulting from historical processes irrelevant for the contemporary persistence of populations (Bradshaw, 1965; Linhart & Grant, 1996; Kawecki & Ebert, 2004; Gienapp *et al.,* 2008), or even by cryptic genetic variation (Rutherford, 2000; Ghalambor *et al.,* 2007). Reciprocal transplants or tests of population responses to specific experimental conditions are needed to explore the adaptive significance of the variation in phenotypes among populations (Kawecki & Ebert, 2004).

The environmental conditions experienced in the common garden differed from those in the natural populations because the experimental site was located at a lower elevation than the populations from the low and high-elevation edges. Therefore, the phenotypes expressed in this experiment may not be the same as those that would have been expressed in the natural populations. In any case, the climatic conditions in the common garden are expected to have been equally advantageous for all the studied populations, as they were grown without significant limitations due to temperature conditions or water and mineral nutrient availability.

Conclusions

This study has established the presence of genetic variation in a suite of female reproductive traits of *Armeria caespitosa*. Trait differences between plants of the populations studied found in the common garden experiment are only partially in accordance with those found in the natural populations, suggesting that differences in these traits are partially due to genetic variation between populations. Furthermore, the later flowering onset found in the low-edge populations did not correspond with the stronger selection response in favor of early-flowering individuals previously reported in the low-edge populations. The inherited capacity of this species to shift its flowering phenology coupled with the observed genetic variation within populations indicates that there is adaptive potential to respond to ongoing global warming. Future research should assess the adaptive significance of the documented variation in phenology and life history traits by means of reciprocal transplants in the field.

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SUPPORTING INFORMATION

Table S1. Voucher information for populations used in the study.

Collection Locality [a]	**Microhabitat**[b]	**Voucher specimen accesión no.**[c]	**Geographic coordinates**
Cuerda de los Porrones, Madrid	Rock	E0265	40° 45′N, 3° 56′W
Loma de Cabezas, Madrid	Pasture	E0268	40° 46’N, 3° 56′W
Cabezas de Hierro Menor, Madrid	Rock	E0270	40° 48′N, 3° 55′W
Cabezas de Hierro Menor, Madrid	Pasture	E0270	40º 47’N, 3º 56’W

[a] Locality and Spanish province.

[b] Microhabitat in which the specimen was collected: rock (rocky outcrops), pasture (dry cryophilic high-mountain grasslands).

[c] Vouchers deposited at Universidad Rey Juan Carlos, Departamento de Biología y Geología, Germplasm bank. (http://www.gbif.org/dataset/59bf2c83-1e3c-40c8-9437-39ce3d3d462c).

Table S2. Reproductive output, size, and phenological traits, of *Armeria caespitosa* in the common garden experiment. All the variables are expressed as mean ± sd.

	Low		**High**	
Variable	Rock (n=26)	Pasture (n= 37)	Rock (n= 22)	Pasture (n=26)
Size (mm)	118.58 ± 13.91	102.19± 14.26	105.36± 13.23	99.08± 12.15
Onset (days)	49± 10	44± 13	47± 15	40± 14
Duration (days)	38± 9	38± 12	37± 15	40± 14
Moment (days)	69± 3	65± 5	66± 4	63± 5
Synchrony	0.60± 0.10	0.60± 0.08	0.58± 0.10	0.58± 0.09
Flowerheads	68.42± 30.33	75.76± 31.6	39.5± 25.1	42.31± 30.76
Total fruits per flowerhead	15.16± 3.20	15.69± 3.84	15.76± 4.11	16.10± 5.18
Viable fruits per flowerhead	6.68± 2.43	4.99± 2.38	5.92± 2.14	7.69± 3.73

CAPÍTULO 4/CHAPTER 4

Complex and contrasting effects of shrub encroachment in high-mountain pollination networks.

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Manuscrito en preparación.

1. ABSTRACT

Mutualistic interactions are among the most important forces structuring ecological communities and can be strongly influenced by global change. In this study, we used network analysis tools to assess the effects of shrub encroachment on plant-pollinator interaction in the dry cryophilic grasslands of Sierra de Guadarrama mountain range (central Spain). In these mountains, the shrub belt is encroaching and replacing cryophilic pastures as a result of the interplay among some global change drivers. We constructed quantitative visitation networks to compare replicated shrub encroached and un-encroached plots. Results showed distinctive effects of shrub encroachment on plant and pollinator species because encroached pastures increased richness and visitation patterns of pollinators, but also increased pollinator-mediated competition among plant species, which can potentially result in reduced reproductive success. In spite of the central role of shrub species the structure of the neworks was robust to the effects of shrub encroachment, although it slightly increased network generalisation. This study advances our understanding of the role of shrub encroachment in high-mountain ecosystems and reveals that they are important in mediating not only plant–pollinator interactions but also plant–plant interactions. These findings point out the need of an integrative approach to study the effect of global change drivers on species interactions and their impact on the structure and functioning of ecological communities.

Key words: competition, specialization, generalization, global change, interactions, niche variation.

2. INTRODUCTION

Global environmental change drivers cause species extinction, modify species distributions and promote the emergence of novel communities, ultimately affecting the state of ecosystems and their associated services (Sala *et al.*, 2000; Didham *et al.*, 2007; Tylianakis *et al.*, 2008; Algar *et al.*, 2009). High-mountain ecosystems are particularly responsive to global environmental change drivers (Nogues-Bravo *et al.*, 2007; Wookey *et al.*, 2009; Pauli *et al.*, 2012). Indeed, some studies have identified significant shifts in plant species distributions and community composition in response to climate warming, and extensification of traditional land management (Tasser & Tappeiner, 2002; Pauli *et al.*, 2012; Brandt *et al.*, 2013). In many cases, these alterations have been related explicitly to an increase in density, cover and biomass of indigenous native woody plants, to the detriment of herbaceous flowering plants (Sanz-Elorza *et al.*, 2003; Wookey *et al.*, 2009; Brandt *et al.*, 2013). Numerous studies document the effect of shrub encroachment on the abiotic environment (*e.g.,* soil erosion and nutrient cycling), plant-plant interactions and vegetation composition (Wookey *et al.*, 2009; Eldridge *et al.*, 2011). By contrast, we lack empirical data showing how shrub encroachment modifies plant-animal interactions (*e.g.*, herbivory, parasitism, pollination: Tylianakis *et al.*, 2008; Ferreira *et al.*, 2013). This is indeed a relevant issue that needs to be addressed as species interactions are among the most important forces structuring ecological communities (Bascompte & Jordano, 2007; Gilman *et al.*, 2010; Tylianakis *et al.*, 2010; Svenning *et al.*, 2014).

Harsh environmental conditions of high-mountain ecosystems limit pollinator richness and pollination services for plant reproduction (Inouye & Pyke, 1988; Kearns & Inouye, 1994; Dupont *et al.*, 2003; but see Bingham & Orthner, 1998). In this context of limiting resources, shrub encroachment may increase the functional complementarity among plant species in the community when plant species differ in their supply of nutritional resources (from the perspective of pollinators) (Ebeling *et al.*, 2008; Mitchell *et al.*, 2009; Blüthgen & Klein, 2011). According to biodiversity → (functional) niche hypothesis (Blüthgen & Klein, 2011), higher diversity of functionally complementary plants contributes to better sustain pollinators' requirements. This implies a positive feedback of biodiversity at both trophic levels in which more flowering plant species would tend to harbour higher diversity of pollinator species at encroached areas (Ebeling *et al.*, 2008; Blüthgen & Klein, 2011). In addition to enhancing nutritional resources, new shrub species would stabilize flowering abundance along the season. Both effects may lead to more stable and diverse populations of pollinators in encroached areas comparing to pasture communities (MacArthur, 1955; Bolnick *et al.*, 2007; Ebeling *et al.*, 2011). This stability would narrow the specific niche's breadths according to the "Niche Variation hypothesis" (Van Valen, 1965). Consequently, in the encroached pastures the overall niche breadth of the pollinator's community would increase by narrowing the specific niche breadths of each component (Vázquez & Stevens, 2004; Bolnick *et al.*, 2007; Ebeling *et al.*, 2011).

The response of pollinators to shrub encroachment may affect indirect plant-plant interactions between shrubs and co-flowering forb species mediated by shared pollinators. Empirical evidence shows that changes in plant communities sometimes lead to increase plant-plant competitive interactions, whereas other studies document enhanced facilitation between flowering plants. For instance, pollinators may shift their preference from forbs to shrubs in encroached areas, thus leading to decreased visitation to forb plants that may reduce their seed set (Revised in Morales & Traveset, 2009). The strength of the plant-plant competition for pollinators depends on their flowering phenology and phenotypic similarity in floral resources (Jordano *et al.*, 2003; Morales & Traveset, 2009; Olesen *et al.*, 2011). Shrub species tend to have less accessible flowers, and therefore, competition for pollinators between pasture and shrub flowering plants may take place only in a subset of plant visitors that can have access to shrub flowers, such as bumblebees and bees (Fenster *et al.*, 2004; Fontaine *et al.*, 2006). On the other hand, higher pollinator diversity in encroached pastures may increase visitation rates to forb species increasing their reproductive output (Johnson *et al.*, 2003; Ghazoul, 2006).

The above-described community-wide patterns in plant–pollinator and plant-plant interactions can be explored through the application of a species interaction network approach (Jordano, 1987). Interaction networks summarize the diversity of interactions among species in a community and they are widely used to explore the effect of changes in species composition and interactions on ecological communities (*e.g.*, Lopezaraiza-

Mikel *et al.*, 2007; Aizen *et al.*, 2008; Vilá *et al.*, 2009; Kaiser-Bunbury *et al.*, 2011). Topological properties of these networks inform on community organization and they are instrumental to predict community dynamics in response to key changes (Jordano *et al.*, 2003; Proulx *et al.*, 2005; Bascompte & Jordano, 2007; Blüthgen, 2010).

In this study, we used network analysis tools to assess the effects of shrub encroachment on high-mountain plant-pollinator interactions at the community level. The study was carried out in orophyllous cryophilic pastures of Sierra de Guadarrama, a mountain range located in central Spain. In these mountains, the shrub belt is encroaching and replacing the cryophilic pastures because livestock grazing has declined and snow cover has reduced, presumably due to increased winter temperatures (Sanz-Elorza *et al.*, 2003; Garcia-Romero *et al.*, 2010). Two dominant leguminous species of the shrub belt have showy floral displays and co-flower with the pasture community (Castroviejo *et al.*, 1990; Escudero *et al.*, 2004). Hence, it is expected that shrub species affect the structure and dynamic of the pollination networks in encroached areas compared to pastures with no shrubs. Specifically, we asked:

(1) Does shrub encroachment change pollinator diversity and visitation patterns in cryophilic pastures?

(2) Do indirect plant-plant interactions between shrubs and forbs mediated by pollinators arise in encroached pasture areas? If so, are they facilitative or competitive?

(3) Does shrub encroachment affect the topology of the plant–pollinator network?

3. MATERIAL AND METHODS

3.1 Study system

The present study was carried out in the orophyllous cryophilic pastures of Sierra de Guadarrama, a mountain range located in the Central System in Spain (40º 50’N, 3º 57’W; Figure 1). Dry cryophilic pastures occur in the higher summits above the tree line between 2000 and 2430 m.a.s.l, characterized by a Mediterranean-Alpine-type climate (Escudero *et al.*, 2004). The dominant vegetation is an extremely short pasture (rarely exceeding 3 cm height) dominated by graminoid *Festuca curvifolia* Lag. ex Lange with several creeping chamaephytes and caespitosous grasses (hereafter forb species). Timberline is located between 1900 and 2100 m and is dominated by stunted pines (*Pinus sylvestris*), which appear interspersed in a shrub matrix characterized by *Cytisus oromediterraneus* (Fabaceae) Rivas Mart. *et al.*,., *Adenocarpus hispanicus* (Fabaceae) (Lam. DC.) and *Juniperus communis subsp. alpina* (Cupressaceae) (Suter) Čelak. The first two shrubs (hereafter shrub species) co-flower with forb species. These species produce showy resource-rich flowers with higher reward levels for pollinators compared to the small and resource-poor flowers produced by forb species (Castroviejo *et al.*, 1990).

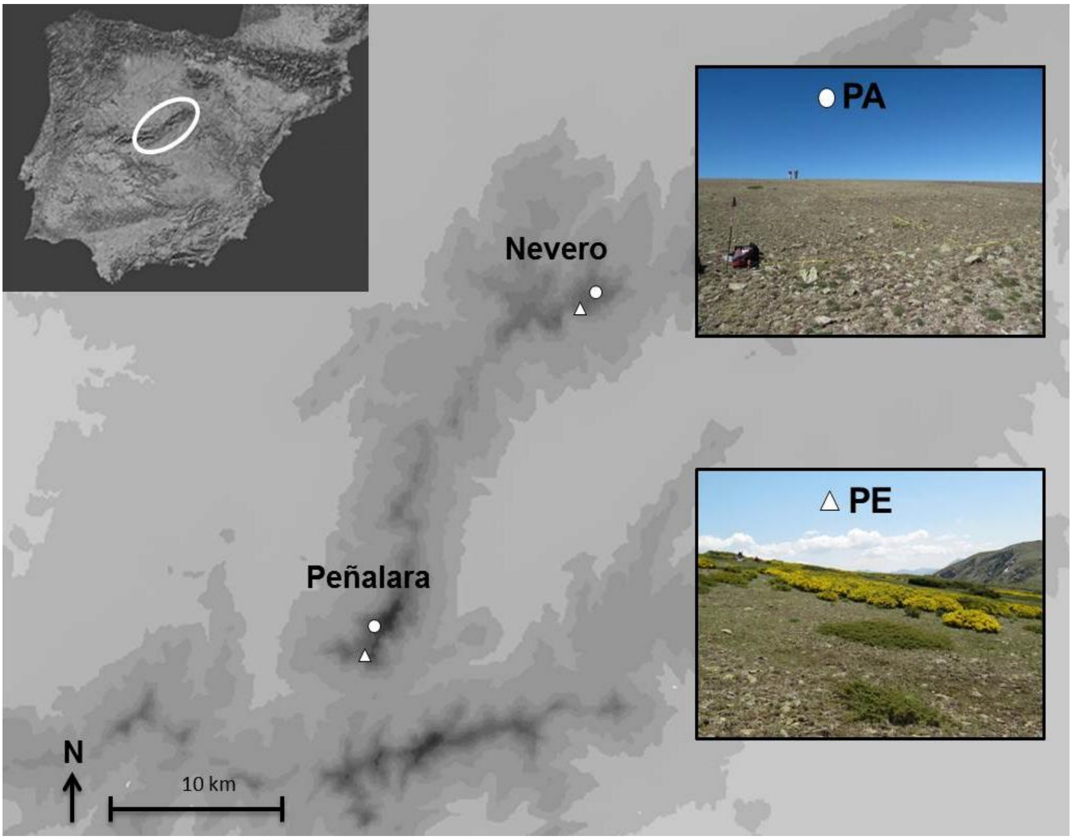

Figure 1. Location of the sampling sites in Sierra de Guadarrama (Spain). Darker-shaded areas indicate higher altitude. Circles denote pasture habitat (PA) whereas triangles denote encroached pastures (PE). Upper left map indicates the location of Sierra de Guadarrama in the Iberian Peninsula. The photographs on the right side show PA and PE sites in Nevero to illustrate the differences between habitat types. The sampling plots were located at 2264 m (Peñalara-PA), 2214 m (Peñalara-PE), 2202 m (Nevero-PA) and 2126 m (Nevero-PE) above sea level.

3.2 Sampling design and field survey

3.2.1 Study sites

We replicated the study in two localities separated by a distance of about 17 kilometres (Peñalara and Nevero peaks, Figure 1). In each locality, we selected two sampling sites; a pasture site dominated by forb species where shrub species are absent (hereafter, PA) and one located in an encroached pasture (hereafter, EP) with ~25% of their area covered by shrub species. We selected EP sites with moderate level of shrub encroachment to avoid comparing communities in which forb species have already been displaced. Sampling sites were 700 and 900 m apart from each other in Peñalara and Nevero, respectively. Previous studies show that this distance allows spatially independent pollinator communities (Aizen *et al.*, 2008; Kaiser-Bunbury *et al.*, 2009; Padrón *et al.*, 2009). Differences in altitude of sampling sites within localities and between habitat types (*i.e.,* EP *vs.* PA) were less than 100 m (Figure 1). All sampling sites had an NW orientation and similar plant communities (74% of forb species occurred in both habitat types, Table S1).

3.2.2 Monitoring of plant-pollinator interactions

We collected data throughout the flowering period (13 June to 28 July 2011) for a total of 10 observation days for each sampling site at Nevero and 11 census days for each sampling site at Peñalara. We added a census day to Peñalara in order to balance the number of hours surveyed in both sites (approximately 160 hours per site). Weather determined the number of census days, as warm, dry and light wind conditions are unusual in high-mountain environments (Dupont *et al.*, 2003). Observations were made on sunny days without wind, from 10:00 to 18:00 h when the environmental conditions at these altitudes allow pollination activity (Dupont *et al.*, 2003). To collect visitation data, we established two 60 x 100m sampling plots in each locality (one at PA and another at EP) where we set 10 linear transects across the width of the plots (60m long and 5m wide). We made successive walks through the line transects and we noted the identity and number of contacts between plant and insect species. Using this methodology the complete area of each sampling plot was observed. Transect methods are very effective for monitoring plant-pollinator interactions in environments where time available for observations is limited (Gibson *et al.,* 2011), as in the case of high-mountain environments. We defined a plan-pollinator interaction as the contact of an insect with the reproductive organs of a flower for more than 1 second (Gibson *et al.,* 2011). The two sampling sites in each locality were simultaneously surveyed by two work teams. Insects were captured and determined to the lowest taxonomic category possible with the help of experts (see acknowledgements). Voucher specimens were deposited at Rey Juan Carlos University. We grouped the pollinators into functional groups to facilitate the detection of general patterns. We define 'functional group' as a set of pollinators that tend to interact with flowers in a similar way (Fenster *et al.,* 2004). Following the methodology employed in Gómez *et al.,* (2008) we used criteria of similarity in size, proboscis length, foraging behaviour and feeding habits rather than taxonomic relationship. We established eleven functional groups (Appendix 1, Table S1). Plant species were determined in the field. The number of flowering plants of each species (hereafter flowering plant abundance) was recorded weekly in 10 transects (60m long and 2m wide) per sampling site.

3.3 Data analysis

3.3.1 Effect of shrub encroachment on species assemblages and visitation patterns

We used linear mixed models (LMMs) to test whether shrub encroachment affected the number of flowering plants, number of plant-pollinator interactions, total number of visits and the number of visits per flowering plant. All models included habitat type (PA and EP) as a fixed factor, and locality and census day nested within locality as random factors to control for potential temporal and spatial non-independence (Zuur *et al.*, 2009). In the case of the models fitted for the number of interactions, total number of visits and the number of visits per flowering plant we included also flowering plant abundance as a continuous predictor variable because it affects visitation patterns (Vázquez, 2005; Blüthgen *et al.*, 2006).

From the pollinators' perspective we checked whether the total number of pollinator species differed between habitat types by using rarefaction curves. Rarefaction methods allow for comparison of datasets standardizing by sampling effort (Gotelli &

Colwell, 2001). We used ECOSIM ver 7.71 (Gotelli & Entsminger, 2004) to compute sample-based rarefaction curves and their 95% confidence interval curves for each habitat type pooling data from all census days and sampling sites. Curves were rescaled by the number of observations (*i.e.,* number of visits) to allow for species richness comparison (Gotelli & Entsminger, 2004). Then, we applied LMM to test whether shrub encroachment affected visitation patterns across functional groups. All models included habitat type, functional group, and their interaction terms as fixed factors, and locality and census day nested within locality as random factors, in addition to flowering plant abundance as continuous predictor.

From the plant species point of view, we tested whether shrub encroachment affected the number of interactions, total number of visits, and the number of visits per flowering plant by applying LMMs. We compared whether visitation patterns differed among plant species according to their life form (fixed factor: shrub or forb) in EP. Note that both life forms only co-occur at EP sites. Once we assessed the effect of the life form in shaping pollinators' preference, we estimated the effect of habitat type (fixed factor) by comparing visitation patterns among forb species that are found at PA and EP sampling sites. The model also included life form and the interaction term with habitat type as fixed factors. All models fitted for plant species included locality and census day nested in locality as random factors and flowering plant abundance as continuous predictor.

We assumed Gaussian error for all the LMM models. Response variables were ln-transformed to reach normality. Residuals did not meet the homocedasticity requirement, and therefore, we modelled the variance of the residuals as σ^2 multiplied with the power of the absolute value of the flowering plant abundance (*i.e.,* "power of the covariate" variance structure, Zuur *et al.,* 2009). We performed all statistical analyses in the open source software R *v* 3.0.3 using the package "lmer" (Pinheiro *et al.,* 2014).

3.3.2 Topological network metrics

We built quantitative bipartite networks for full-season data for each habitat type (Jordano, 1987). We used visitation frequencies as a surrogate for interaction strength (Vázquez *et al.*, 2005; Vázquez *et al.*, 2007). We defined visitation frequency between a pollinator *i* and a plant species *j* as the total number of visits of *i* to *j* multiplied by relative specific flowering plant abundance of *j*, *i.e.* the total number of visits was weighted by flowering plant abundance. We calculated nine network-level topological metrics:

1) number of plant species (*P*);

2) number of pollinator species (*A*);

3) total number of interactions (*I*);

4) total number of visits (*V*);

5) Connectance ($C=I/AP$), i.e. the fraction of realised interactions in the network (Jordano, 1987);

6, 7) Weighted linkage for plants (lw_p) and pollinators (lw_a) (also called weighted generality and vulnerability) as the effective mean number of partners per plant (lw_p), and per pollinator (lw_a) (Bersier *et al.*, 2002). Both indices are, therefore, a measure of the niche breadth;

8) Index of network specialization (*H'2*), that quantifies the degree of niche divergence of elements within an entire bipartite network (Blüthgen *et al.*, 2006; Blüthgen *et al.*, 2008). It ranges from 0 (low specialisation, high niche overlap) to 1 (high specialisation, low niche overlap); and

9) Interaction strength asymmetry (*AS*), that measures whether dependence (*i.e.*, the proportion of realised interactions in each species) between plants and pollinators is reciprocal or asymmetric (Bascompte *et al.*, 2006). It ranges from -1 (higher dependence in the lower trophic level, i.e., plants) to 1 (higher dependence in the higher trophic level, i.e, pollinators).

We also estimated three species-level metrics for each plant (*p*) and pollinator (*a*) species:

1) Linkage level (*L;* the number of links or partners that each species had in the network);

2) Index of species specialization (*d'*), expresses levels of specialization of each species based on its discrimination from random selection of partners (Blüthgen *et al.*, 2006; Dormann *et al.*, 2008). It ranges from 0 (no specialisation, or species that interact with their partners proportionally to their availability) to 1 (perfect specialist, or species that disproportionately interact with rare partners); and

3) Species strength (*S*) is the sum of dependencies of the partners relying on each species (Bascompte *et al.*, 2006). It quantifies the importance of each species from the perspective of their partners. This metric is a quantitative extension of the linkage level (Bascompte *et al.*, 2006).

All these metrics were calculated using the R package 'bipartite' *v.* 2.0.4 (Dormann *et al.*, 2008). We used unpaired two-sample *t*-test to check for differences on species-level metrics between habitats, trophic level (*i.e.,* plant and pollinators) and functional group of pollinators and one-sample *t*-tests to test for differences between plant life forms within EP. We compared the observed lw_p, lw_a, *H'2, AS, L, d', S* values with values from a random network, in which interactions of plant species and pollinators were randomized (10,000 permutations) with the null model proposed by Vazquez *et al.*, (2007). The model randomized the total number of interactions recorded in the observed interaction matrix assigning interspecific interactions according to species-specific probabilities proportional to species' relative abundances, but maintaining the connectance of the observed matrices (Vázquez *et al.*, 2007).

We finally estimated the modularity (*M*) of the networks which measures the extent to which species interactions are organized into modules (Olesen *et al.*, 2007). Network modules are groups of species more strongly linked to each other than to species in other modules (Olesen *et al.*, 2007; Tylianakis *et al.*, 2010). We used the software NETCARTO to estimate *M* of the networks employing the algorithm *SA* (Guimera & Amaral 2005). We assessed the significance level of *M* running an *SA*-analysis of 100 random networks with the same species degree distribution as the empirical one (Guimera & Amaral, 2005; Olesen *et al.*, 2007).

3 Results

Effect of shrub encroachment on species assemblages and visitation patterns

Overall, the number of flowering plants, number of interactions and the total number of visits recorded per census was higher in encroached pastures (EP) than in pastures (PA), while the number of visits per flowering plant was lower (Fig. 2). Flowering plant abundance only influenced the number of visits per plant ($F_{1, 19}$ 74.57, $p <$ 0.001; β = -0.0012; t = -8.64, p < 0.001), and was more stable along the flowering season in EP (weekly mean ± SD: 0.9 plant/m^2 ± 0.5) than in PA (0.6 plant/m^2± 0.7).

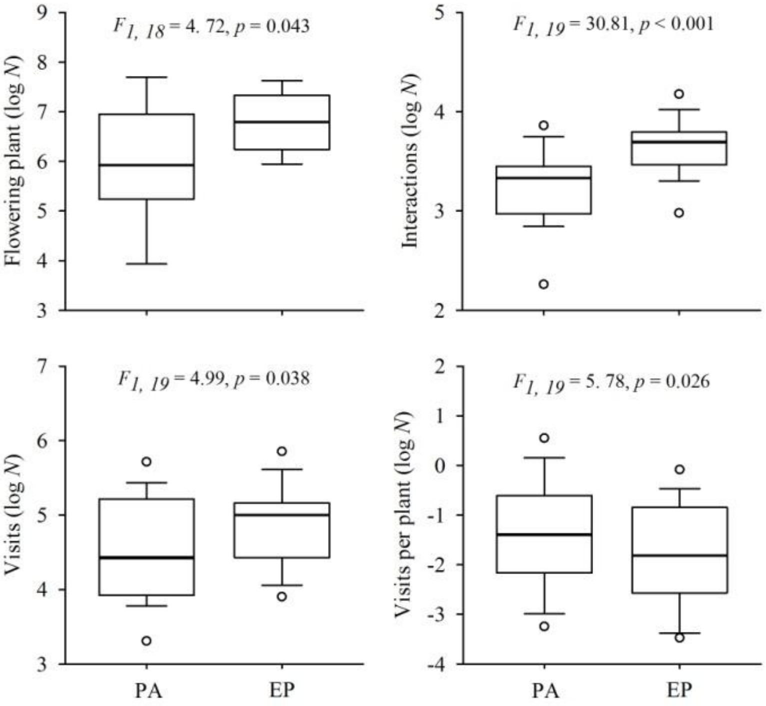

Figure 2. Number of flowering plants, number of interactions, total number of visits and number of visits per flowering plant measured in pastures (PA) and encroached pastures (EP). In boxplots, thick lines represent the median, boxes the lower and upper quartile, and whiskers above and below the box indicate the 90th and 10th percentiles. LMM results for testing the effects of shrub encroachment are displayed above each graph.

Pollinator richness was higher in EP than in PA in all functional groups (Table S1). As a result, sample-based rarefied estimates of pollinator richness were significantly higher in EP than in PA (Curves rescaled to 2000 visits: 108 species, 95% CI: 102-113; 91 species, 95% CI: 87–94, respectively). The main functional groups of pollinators did not vary between habitat types: butterflies were the predominant pollinators [28 species (23.5%) at EP, 17 species (18.1%) at PA]; followed by hoverflies [13 species at EP (10.9%), 14 species at PA (14.9%)]; bees [13 species (10.9%) at EP, 11 species (11.7%) at PA]; and beetles [12 species (10.1%) at EP, 11 species (11.7%) at PA]. The greater number of species in EP compared to PA resulted in an increased number of interactions and visits recorded for each functional group (Table 1, Table S2: $\beta = 0.42$, $t = 2.39$, $p = 0.02$ for number of interactions; $\beta = 0.78$, $t = 2.45$, $p = 0.01$ for number of visits). There was also a significant effect of the functional group on the visitation patterns observed (Table 1) due to the higher number of interactions and visits by butterflies, hoverflies, small bees and beetles in both habitat types (Table S2). Furthermore, the interaction habitat type x functional group was not significant (Table 1), suggesting that insects from all eleven functional groups responded to the shrub encroachment in a similar manner. Flowering plant abundance did not affect visitation patterns (Table 1).

Table 1. LMM results for testing the effects of habitat type, functional group and flowering plants abundance on the number of interactions and total number of visits recorded for each functional group of pollinators.

	Num. *Df*	Den. *Df*	Number of interactions		Number of visits	
			F value	*Pr*(>|*F*|)	*F* value	*Pr*(>|*F*|)
Intercept	1	419	1109.51	**<0.001**	773.74	**<0.001**
Habitat type	1	419	20.75	**<0.001**	14.16	**<0.001**
Functional group	10	419	34.96	**<0.001**	25.40	**<0.001**
Plants abundance	1	419	0.07	0.78	0.10	0.76
Habitat type: F. group	10	419	1.42	0.17	1.34	0.21

Num. *Df*, numerator degrees of freedom of fixed effects; Den. *Df*, denominator degrees of freedom for fixed effects. Parameter estimates fitted for fixed effects and standard deviations fitted for random effects are provided as Supplementary data (Table S2).

Life form (*i.e.,* shrub and forb species) affected the number of interactions with pollinators and the number of visits per flowering plant observed in EP (Table 2a). Hence, shrub species had more interactions with pollinators (Table S3: $\beta = 0.53$, $t = 2.43$, $p = 0.02$) and received more visits per flowering plant (Table S3: $\beta = 0.81$, $t = 2.35$, $p = 0.02$) than forb species. Models fitted for forb species that occurred at both habitat types showed that the number of interactions with pollinators and total number of visits recorded for forb species did not differ between PA and EP (Table 2b). There was a significant effect of plant species on these two variables (Table 2b). Thus, *Jasione crispa, Jurinea humilis* and *Senecio pyrenaicus* had greater number of interactions and visits in comparison with the rest of the forb plants (Table S4). The interaction habitat type x plant species was not significant (Table 2b). In contrast, the number of visits received per flowering plant was significantly lower in EP (Table 2b, Table S4: $\beta = - 1.61$, $t = - 3.04$, $p <0.01$). There was also a significant habitat type x plant species interaction for this variable (Table 2b). Although all forb species received less visits per flowering plant in encroached pastures, two of them showed greater negative effect of shrub encroachment, namely *Biscutella intermedia* ($\beta = - 2.30$, $t = - 2.39$, $p = 0.02$) and *Solidago virgaurea* ($\beta = -1.63$, $t = -1.98$, $p = 0.04$) (Table S4). Flowering plant abundance had a significant effect on all models fitted for plant species (Table 2a,b), which suggests that visitation patterns to plant species in both habitat types were partially explained by this variable. Hence, plant species with greater flowering plant abundance had more interactions with pollinators (Table S3, S4), and therefore, received more visits (Tables S3, S4). However, flowering plant abundance was inversely related to the number of visits per flowering plant (Tables S3, S4).

Table 2. LMM results for testing the effects of habitat type, plant species, life form and flowering plant abundance on number of interactions, total number of visits and number of visits per flowering plant recorded for each plant species.

	Num. *Df*	Den. *Df*	Number of interactions		Number of visits		Visits per flowering plant	
			F value	*Pr*(>|*F*|)	*F* value	*Pr*(>|*F*|)	*F* value	*Pr*(>|*F*|)
a) Species that occurred at encroached pastures								
Intercept	1	115	356.87	**<0.001**	423.69	**<0.001**	140.69	**<0.001**
Life form	1	115	3.91	**0.05**	1.12	0.29	12.26	**<0.001**
Plants abundance	1	115	10.88	**<0.01**	19.46	**<0.001**	53.28	< **0.001**
b) Species that occurred at both habitat types								
Intercept	1	155	580.23	**<0.001**	645.22	**<0.001**	359.16	**<0.001**
Habitat type	1	155	0.019	0.89	0.12	0.73	25.40	**<0.001**
Plant species	10	155	4.64	**<0.001**	4.70	**<0.001**	22.00	**<0.001**
Plants abundance	1	155	26.98	**<0.001**	37.84	**<0.001**	47.71	**<0.001**
H. Type:P. Species	10	155	0.52	0.87	0.45	0.92	2.36	**0.01**

Num. *Df*, numerator degrees of freedom of fixed effects; Den. *Df*, denominator degrees of freedom for fixed effects. Parameter estimates fitted for fixed effects and standard deviations fitted for random effects are provided as Supplementary Data (Table S3, S4).

Topological network metrics

Topological network metrics confirmed that the total number interactions and visits recorded were higher in EP than in PA (Table 3). Four functional groups of pollinators (butterflies, hoverflies, small bees and beetles) accounted for the majority of interactions and visits in both habitat types. They summed up to 63% of interactions and 74% of visits in PA and up to 60% of interactions and 76% of visits in EP. The forb species *Jasione crispa*, *Jurinea humilis* and *Senecio pyrenaicus* were involved in 40% of all links and received 59 % of all visits at PA, while these species together with the shrub species *Cytisus oromediterraneus and Adenocarpus hispanicus* were involved in 54% of all links and had 62% of all visits at EP. Nevertheless, most plant species were highly connected, with 10 species (85%) at PA and 12 species (80%) at EP that had at least 10 interactions and 37 visits or more. For further details, the number of interactions and total number of visits recorded for each plant species are shown in Table S1. Regarding network-level metrics, connectance (*C*) values were similar between habitat types, while the index of network specialization (*H'2*) was slightly lower in EP than in PA (Table 3). Weighted linkage for plants (lw_p) was four times lower than for pollinators (lw_a) showing that plants interact with a high number of pollinator species. Regarding habitat types, lw_p and lw_a in EP increased by 25% and 17%, respectively, compared to PA (Table 3). The negative interaction strength asymmetry ($AS = -0.40$) in PA indicated stronger dependence in the higher trophic level, i.e., in the pollinators. In contrast, EP had low asymmetry values ($AS = 0.08$), implying reciprocal dependence between trophic levels, *i.e.*, plants and pollinators (Table 3). The plant-pollinator networks of both habitat types were not significantly modular: $M = 0.34$ (mean ± SD, of 100 randomizations: $M = 0.35 \pm 0.007$; $p > 0.05$) for PA and $M = 0.32$ (0.33 ± 0.006; $p > 0.05$) for EP.

Means of all species-level metrics, namely number of links (*L*), species specialization (*d'*) and species strength (*S*) estimated for both plant (*p*) and pollinators (*a*) were similar between habitat types (*t*-test: all $p > 0.05$; Table 3). This pattern was constant for all pollinator functional groups (*t*-test: all $p > 0.05$), but not for plant life forms: linkage ($L = 31.5 \pm 7.5$) and strength ($S = 11.08 \pm 5.7$) of shrub species were higher than those of forb species in EP ($L = 20.86 \pm 4.0$, $t = 2.66$, $p < 0.02$; $S = 6.53 \pm 2.03$, $t = 2.23$, *p*

< 0.02). Furthermore, mean L and mean S were significantly higher in plants than in pollinators at both PA and EP (*t- test:* $p < 0.001$), but non-significant differences were found for mean d' (*t- test:* $p > 0.05$).

The observed lw_p, lw_a, $H'2$, AS, L, d', S values were significantly different than expected by chance as they did not fall within the 95% confidence intervals obtained from 10,000 random networks.

Table 3. Topological network metrics for a bipartite plant-pollinator network obtained for pastures (PA) and encroached pastures (EP).

Metrics	PA	EP
Network-level metrics		
Number of plants (P)	15	17
Number of animals (A)	94	119
Number of interactions (I)	268	367
Number of visits (V)	2423	3116
Connectance (C)	0.19	0.18
Weighted plant linkage (lw_p)	8.76	11.57
Weighted pollinator linkage (lw_a)	2.36	2.86
Network specialization $(H'2)$	0.49	0.42
Interaction strength asymmetry (AS)	-0.4	0.08
Species level metrics (Mean ± *SE*)		
Plant linkage (L_p)	17.87 ± 3.07	22.12 ± 3.68
Pollinator linkage (L_a)	2.82 ± 0.27	3.13 ± 0.26
Plant species specialization (d'_p)	0.33 ± 0.04	0.31 ± 0.04
Pollinator species specialization (d'_a)	0.25 ± 0.02	0.24 ± 0.02
Plant species strength (S_p)	6.33 ± 1.70	7.06 ± 1.94
Pollinator species strength (S_a)	0.16 ± 0.04	0.14 ± 0.03

SE, standard error. Non-significant differences between PA and EP were found in all tested species level metrics ($p > 0.05$)

4 DISCUSSION

Overall, the number of flowering plants, number of interactions and the total number of visits recorded was higher in encroached pastures (EP) than in pastures (PA), while the number of visits per flowering plant was lower. Shrubs had more interactions with pollinators and received more visits per flowering plant than forb species, while the number of visits received per flowering plant of the forb species was significantly lower in EP. From the perspective of pollinators, shrub encroachment increased richness and visitation patterns of all functional groups. The topology of the plant-pollinator network remained relatively invariant between habitats types with a slightly increase of network generalization in EP. Species-level metrics did not significantly change between habitat types, although linkage and strength of shrub species were higher than those of forb species in EP.

Effects of shrub encroachment on pollinator diversity and visitation patterns

The increase in the number of pollinator species (*i.e.,* species richness) in all functional groups is congruent with our expected results based on the "biodiversity → (functional) niche hypothesis" that predicts higher pollinator's biodiversity in rich-plant communities (Ebeling *et al.*, 2008; Blüthgen & Klein, 2011). This implies a positive feedback between species richness of both trophic levels: more flowering plant species in EP derives into higher pollinator species richness. The greater pollinator species richness in EP may be responsible for the increased number of interactions and visits of all functional groups, which imply an increase on total niche breadth of the pollinator community. This overall increase can be achieved by each species specializing on a narrow range of plants or, alternatively, by each pollinator using a wide set of available plants (Vázquez & Stevens, 2004; Bolnick *et al.*, 2007; Mitchell *et al.*, 2009).

Generalization is a prevalent feature in plant-pollinator networks (Ollerton, 1996; Waser *et al.*, 1996). As a matter of fact, networks in pastures showed similar levels of generalization as previously reported metrics in plant-pollinator networks (Blüthgen & Klein, 2011; mean ± SE: $H'2$= 0.55 ± 0.04 vs. $H'2$=0.49, t = 1.62, p = 0.12). Based on the "Niche Variation hypothesis" we expected increased specialization in EP. Yet, network metrics showed that shrub encroachment did not change pollinator niche breadth (lw_a, d'_a); rather, the index of network specialization (*H'2)* decreased suggesting an increased generalisation (higher niche overlap). Generalization is considered to be a beneficial strategy in most plant-pollinator networks (Waser *et al.*, 1996; Johnson & Steiner, 2000; Tur *et al.*, 2013; Svenning *et al.*, 2014). This is because pollination systems with intermediate functional niche complementarity (*sensu* Blüthgen *et al.*, 2011, moderate generalization and/or specialization) may be more resilient against detrimental effects of disturbance, population fluctuations and extinctions of associated species (Yachi & Loreau, 1999; Valone & Barber, 2008; but see Vázquez & Simberloff, 2002).

Plant-plant interactions mediated by shared pollinators

Generalization can improve the stability of a community, but also enhance interspecific competition when the ecosystem functions represent a limited resource (Blüthgen & Klein, 2011), as may be the case in our study system. Shrub encroachment increased plant linkage (*i.e.,* L_p and lw_p) while reducing the number of visits per flowering plant of forb species without affecting their specialization (d'_p). These findings suggest the existence of pollinator-mediated competition among plant species as a result of pollinator sharing, which can potentially result in reduced reproductive success of some plant species (Morales & Traveset, 2009).

Competition for pollinators between plants can be mediated by two processes: a) the presence of other preferred species hampers the visit of native species (*i.e.,* explotation barriers: Santamaría & Rodríguez-Gironés, 2007); b) lack of flower constancy (*i.e.*, pollinators switch between flowers of different species during their foraging activities increasing heterospecific pollen deposition) (Lopezaraiza-Mikel *et al.*, 2007; Morales & Traveset, 2008; Morales & Traveset, 2009). Previous studies have shown that generalists have the largest potential to compete with other plant species through these two types of processes (Lopezaraiza-Mikel *et al.*, 2007; Morales & Traveset, 2008; Morales & Traveset, 2009; Seifan *et al.*, 2014). In our plant-pollinator network, shrub species acted as super-generalist plants, as supported by their low degree of specialization (d') but high linkage (L_p) and pollinator visitation. As a result, the two processes controlling pollinator-mediated competition among plant species may be clearly increased by the integration of shrub species into the network. Further research is needed to evaluate the effective outcome of this integration (increased stability *vs.* increased competition). For instance, the evaluation of the presence of shrub pollen grains in the pollen transport network (Lopezaraiza-Mikel *et al.*, 2007) and the joint estimation of the "quantity" (*i.e.,* visit number) and "quality" (*i.e.,* visit efficiency) component of the interaction and the subsequent effect on plant fitness (Fenster *et al.*, 2004; Mitchell *et al.*, 2009; Seifan *et al.*, 2014) would provide very valuable information.

Effects of shrub encroachment on the topology of plant –pollinator network

In spite of the central role of shrub species, the structure of the networks was very robust to the effects of shrub encroachment (*e.g.,* connectance *C*, modulariry *M* and *H'2*). This result is congruent with previous findings in the literature where strong shifts on network properties were found only in highly invaded habitats, when invader species have already displaced native ones (*e.g.,* Aizen *et al.*, 2008).

The robustness of our study network to external changes supports previous empirical and theoretical works that show that the topology of mutualistic networks is robust to dynamic changes (Jordano *et al.*, 2003; Bascompte & Jordano, 2007). Some authors have argued an explanation based on the role of forbidden links and linkage constraints caused by phenological uncoupling, morphological mismatchings, trait complementarity and/or physiological–biochemical constraints (Jordano *et al.*, 2003; Bascompte *et al.*, 2006; Santamaría & Rodríguez-Gironés, 2007; Olesen *et al.*, 2008; Olesen *et al.*, 2011). Among these constraints, phenological uncoupling has been identified as a main factor driving linkage constraints in generalized systems of pollination (Jordano *et al.*, 2003; Olesen *et al.*, 2011). More recently, it also has been suggested as the principal mechanism that generates complementary among ecologically generalized species in plant-pollinator mutualism (Blüthgen & Klein, 2011). Phenological uncoupling is widespread in mountain systems, since flowering periods are often markedly short, and seasonality pronounced, while many pollinators are active for several months (Totland, 1994; Gimenez-Benavides *et al.*, 2011). Consequently, phenological uncoupling may be a primary factor controlling network topology and functional niche complementarity in our study system (Morente-López, Lara-Romero & Iriondo, unpublished results).

The relative invariance of network structure implies that plant-pollinator interaction networks might be less prone to detrimental effects of disturbance than previously thought (Petanidou *et al.*, 2008; Hegland *et al.*, 2009; Vilá *et al.*, 2009), as may be the case of the plant-pollinator network studied here. However, multiple drivers of global change may act simultaneously on multiple processes creating challenges in predicting potential disruption across entire networks of species interactions (Tylianakis *et al.*, 2008; Svenning *et al.*, 2014). For instance, some studies have shown that climate warming may affect phenophases and distributions of pollinator species, thereby generating temporal and spatial mismatches between plants and pollinators (Memmott *et al.*, 2007; Hegland *et al.*, 2009). The predicted result of these disruptions is the extinction of pollinators, plants and their interactions (Memmott *et al.*, 2007) that would deprive alpine populations of their ecological services. Despite this, mismatches in pollination interactions and the subsequent effects in the availability of mutualistic partners are still rarely explored in high-mountain communities, thus requiring additional research (Hegland *et al.*, 2009).

Conclusions

This study reveals distinctive effects of shrub encroachment on plant and pollinator species because it increased richness and visitation patterns of pollinators, but also increased pollinator-mediated competition among plant species, which can potentially result in reduced reproductive success for key native forb species. In spite of the central rol of shrub species the topological structure of the network appeared to be robust to the integration of shrub species. These findings advances our understanding of the role of shrub encroachment in high-mountain ecosystems and point out the need of an integrative approach to study the effect of global change drivers on species interactions and associated ecosystem services.

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8. APPENDIX 1.

Functional group of pollinators defined following the criteria of similarity in size, proboscis length, foraging behaviour and feeding habits.

(i) Bumblebees, pollen-and nectar-collecting members of the bee genus Bombus;

(ii) Bees, mostly pollen- and nectar-collecting females measuring 7 mm in body length or larger;

(iii) Small bees, mostly pollen- and/or nectar-collecting females smaller than 7 mm;

(iv) Wasps, including aculeate wasps, large parasitic wasps collecting only nectar;

(v) Bee flies, long-tongued nectar-collecting Bombyliidae;

(vi) hoverflies, nectar- and pollen-collecting Syrphidae and short-tongued Bombyliidae;

(vii) Flies, including nectar- and pollen- feeding species mainly belonging to the families tachinidae and muscidae;

(viii) Small flies, including feeding nectar- and/or pollen-feeding species smaller than 5 mm;

(ix) Beetles, including species collecting nectar and/or pollen;

(x) Butterflies (including diurnal moths), all nectar collectors;

(xi) Others, including nectar-collecting ants, small parasitic wasps, Microlepidoptera (micromoths), bugs and other occasional flower visitors.

9. SUPPORTING INFORMATION

Table S1. List of species studied in each habitat type. Acronim, scientific name, family, functional group and number of interactions and total number of visits recorded are indicated for each species.

Acronim	Scientific name	Family	F. Group	Encroached Pasture		Pasture	
				Interactions	Visits	Interactions	Visits
adehis	Adenocarpus hispanicus (Lam.) DC.	Fabaceae	Plant	24	255	n.p.	n.p.
armcae	Armeria caespitosa (Gómez Ortega) Boiss.	Plumbaginaceae	Plant	16	33	21	100
bisint	Biscutella intermedia (Boiss. & Reut.) Malag.	Cruciferae	Plant	4	12	8	36
cytoro	Cytisus oromediterraneus Rivas Mart. & al.	Fabaceae	Plant	39	205	n.p.	n.p.
eupwil	Euphrasia willkommii Freyn.	Scrophulariaceae	Plant	2	3	4	23
gagnev	Gagea nevaensis (Boiss.) O. Bolòs	Liliaceae	Plant	n.p.	n.p.	5	8
genlut	Gentiana lutea L.	Gentianaceae	Plant	9	37	n.p.	n.p.
hiease	Pilosella vahlii (Froel.) F.W. Sch. & Sch. Bip.	Compositae	Plant	10	31	n.p.	n.p.
hiebla	Pilosella argylocoma (Fr.) F.W. Sch. & Sch. Bip.	Compositae	Plant	6	14	n.p.	n.p.
hiesp	Lentodon sp. L.	Compositae	Plant	23	139	24	303
jascri	Jasione crispa (Pourr.) Samp.	Campanulaceae	Plant	44	570	31	414
jurhum	Jurinea humilis (Desf.) DC.	Compositae	Plant	47	723	40	691
leualp	Leucanthemopsis alpina (L.) Heywood	Compositae	Plant	28	176	28	189
ransp	Ranunculus sp. L.	Ranunculaceae	Plant	n.p.	n.p.	4	7
sedbre	Sedum brevifolium DC.	Crassulaceae	Plant	24	187	17	155
sedcan	Sedum candollei Raym.	Crassulaceae	Plant	n.p.	n.p.	18	58
senpyr	Senecio pyrenaicus L.	Compositae	Plant	48	377	36	316
silcil	Silene ciliata Pourr.	Caryophyllaceae	Plant	8	88	4	19
solvir	Solidago virgaurea L.	Compositae	Plant	22	155	18	47

Table S1 (Cont).

Acronim	Scientific name	Family	F. Group	Encroached Pasture		Pasture	
				Interactions	Visits	Interactions	Visits
thypra	Thymus praecox Opiz.	Labiatae	Plant	23	108	17	57
bombus3	Bombus sp. (Latreille, 1802)	Apidae	Bombus	1	2	2	8
bommax	Bombus maxillosus (Klug, 1817)	Apidae	Bombus	3	8	4	167
bomter	Bombus terrestris (Linnaeus, 1758)	Apidae	Bombus	10	168	n.p.	n.p.
andfla	Andrena flavipes (Panzer 1799)	Andrenidae	Bees	6	10	1	4
andlab	Andrena labialis (Kirby, 1802)	Andrenidae	Bees	n.p.	n.p.	1	1
andnit	Andrena nitida (Müller 1776)	Andrenidae	Bees	1	6	1	2
andova	Andrena ovatula (Kirby 1802)	Andrenidae	Bees	5	54	1	14
andpil	Andrena pilipes (Fabricius, 1781)	Andrenidae	Bees	2	5	n.p.	n.p.
andsue	Andrena suerinensis (Friese 1884)	Andrenidae	Bees	3	7	n.p.	n.p.
antret	Anthophora retusa (Linnaeus, 1758)	Anthophoridae	Bees	n.p.	n.p.	1	1
apimel	Apis melifera (Linnaeus, 1758)	Apidae	Bees	5	17	1	4
dasmor	Dasypoda morotei (Quilis, 1928)	Melittidae	Bees	n.p.	n.p.	2	3
halcon	Halictus confusus (Smith, 1853)	Halictidae	Bees	9	56	6	17
halqua	Halictus quadricinctus (Fabricius, 1776)	Halictidae	Bees	3	8	2	8
halrub	Halictus rubicundus (Christ, 1791)	Halictidae	Bees	3	5	n.p.	n.p.
halsca	Halictus scabiosae (Rossi 1790)	Halictidae	Bees	1	1	2	7
haltet	Halictus tetrazonius (Klug, 1817)	Halictidae	Bees	1	1	n.p.	n.p.
hopmit	Hoplitis mitis (Nylander, 1852)	Megachilidae	Bees	4	18	3	3
hylpra	Hylaeus praenotatus (Förster, 1871)	Colletidae	Bees	1	1	n.p.	n.p.
cercuc	Ceratina cucurbitina (Rossi, 1792)	Anthophoridae	Small Bees	2	2	n.p.	n.p.
lasalb	Lasioglossum albipes (Fabricius, 1781)	Halictidae	Small Bees	4	4	1	1
lascos	Lasioglossum costulatum (Kriechbaumer, 1873)	Halictidae	Small Bees	4	10	3	5

Table S1 (Cont).

Acronim	Scientific name	Family	F. Group	Encroached Pasture		Pasture	
				Interactions	Visits	Interactions	Visits
lasdus	Lasioglossum dusmeti (Blüghten, 1924)	Halictidae	Small Bees	1	1	n.p.	n.p.
lasiog	Lasioglossum leucopus y cupromicans (Kirby 1802 & Pérez, 1903)	Halictidae	Small Bees	14	378	14	339
lasleu	Lasioglossum leucozonium (Schrank, 1781)	Halictidae	Small Bees	n.p.	n.p.	1	6
lassub	Lasioglossum subfasciatum (Imhoff, 1832)	Halictidae	Small Bees	4	60	1	3
lasxan	Lasioglossum xanthopus (Kirby, 1802)	Halictidae	Small Bees	1	1	n.p.	n.p.
sphsca	Sphecodes scabricollis (Wesmael, 1865)	Halictidae	Small Bees	4	12	3	4
panban	Panurgus banksianus (Panzer 1806)	Panurginae	Small Bees	8	22	2	13
pancal	Panurgus calcaratus (Panzer 1807)	Panurginae	Small Bees	4	15	2	17
bracon	Braconidae (Benoit, 1949)	Braconidae	Wasps	n.p.	n.p.	1	1
cerare	Cerceris Arenaria (Linnaeus, 1758)	Crabronidae	Wasps	4	46	5	24
cerchy	Cerceris rhybyensis (Linnaeus, 1771)	Crabronidae	Wasps	1	1	n.p.	n.p.
ichneu	Ichneumonidae	Ichneumonidae	Wasps	1	1	n.p.	n.p.
odyner	Odynerus sp. (Latreille, 1802)	Eumenidae	Wasps	3	3	n.p.	n.p.
ophyon	Ophyon (Fabricius, 1798)	Ichneumonidae	Wasps	2	2	1	1
philan	Philantus sp. (Fabricius, 1790)	Sphecidae	Wasps	2	8	n.p.	n.p.
podalo	Podalonia tydei y Podalonia sp. (Le Guillou, 1842 & Fernald, 1927)	Sphecidae	Wasps	2	17	n.p.	n.p.
sphex	Sphex sp. (Linnaeus, 1758)	Sphecidae	Wasps	6	43	8	78
tenmes	Tenthredo mesomela (Linnaeus, 1758)	Sphecidae	Wasps	1	1	n.p.	n.p.
chfes	Chrysotoxum festivum (Linnaeus, 1758)	Syrphidae	Hoverflies	3	7	2	3
chinter	Chrysotoxum intermedium (Linnaeus, 1758)	Syrphidae	Hoverflies	2	4	n.p.	n.p.
chsp	Chrysotoxum sp. (Linnaeus, 1758)	Syrphidae	Hoverflies	n.p.	n.p.	3	4

Table S1 (Cont).

Acronim	Scientific name	Family	F. Group	Encroached Pasture		Pasture	
				Interactions	Visits	Interactions	Visits
didint	Didea intermedia (Loew, 1854)	Syrphidae	Hoverflies	2	4	1	2
erissi	Eristalis similis (Fallen, 1817)	Syrphidae	Hoverflies	11	248	9	217
eriste	Eristalis tenax (Linnaeus, 1758)	Syrphidae	Hoverflies	9	185	9	174
eupco	Eupeodes corollae (Fabricius, 1794)	Syrphidae	Hoverflies	12	32	7	57
mercry	Merodon crypticus (Marcos-García, Vujic & Mengual, 2007)	Syrphidae	Hoverflies	1	1	1	1
parag	Paragus sp. (Latreille, 1804)	Syrphidae	Hoverflies	1	1	n.p.	n.p.
scaeva	Scaeva albomaculata/pyrastri (Macquart, 1842 & Linnaeus, 1758)	Syrphidae	Hoverflies	11	104	10	119
sersil	Sericomyia silentis (Harris, 1776)	Syrphidae	Hoverflies	n.p.	n.p.	1	1
sphscr	Sphaeroforia scripta (Linnaeus, 1757)	Syrphidae	Hoverflies	10	46	3	4
sphsp	Spaherophoria sp. (Linnaeus, 1758)	Syrphidae	Hoverflies	9	18	6	11
syrrib	Syrphus ribesii (Linnaeus, 1758)	Syrphidae	Hoverflies	1	3	1	3
syrtor	Syrphus torvus (Osten Sacken, 1875)	Syrphidae	Hoverflies	n.p.	n.p.	1	1
volele	Volucella elegans (Loew, 1862)	Syrphidae	Hoverflies	n.p.	n.p.	1	2
volpel	Volucella pellucens (Loew, 1862)	Syrphidae	Hoverflies	1	2	n.p.	n.p.
convir	Conophorus virescens (Fabricius, 1787)	Bombyliidae	Bee Flies	1	18	2	63
hemham	Hemipenthes hamifera (Loew, 1854)	Bombyliidae	Bee Flies	5	31	1	12
hemmor	Hemipenthes morio (Loew, 1854)	Bombyliidae	Bee Flies	1	2	1	1
phthi	Phthiria sp. (Evenhuis, 1985)	Bombyliidae	Bee Flies	6	13	6	14
sysgra	Systoechus gradatus (Wiedemann in Meigen, 1820)	Bombyliidae	Bee Flies	5	37	4	29
vilhot	Villa hottentotta (Linnaeus, 1758)	Bombyliidae	Bee Flies	3	23	5	11
calli	Calliphoridae	Calliphoridae	Bigflies	n.p.	n.p.	1	1

Table S1 (Cont).

Acronim	Scientific name	Family	F. Group	Encroached Pasture		Pasture	
				Interactions	Visits	Interactions	Visits
dilmin	Dilophus minor (Strobl, 1900)	Bibionidae	Bigflies	n.p.	n.p.	1	1
dysma	Dysmachus sp. (Loew, 1860)	Asilidae	Bigflies	1	3	1	1
musci	Muscidae	Muscidae	Bigflies	3	5	2	4
neocor	Neomyia cornicina (Fabricius, 1781)	Muscidae	Bigflies	1	1	n.p.	n.p.
tachi1	Tachinidae1	Tachinidae	Bigflies	2	2	n.p.	n.p.
tachi2	Tachinidae2	Tachinidae	Bigflies	4	6	2	4
tachi3	Tachinidae3	Tachinidae	Bigflies	3	9	n.p.	n.p.
tachi4	Tachinidae4	Tachinidae	Bigflies	1	2	n.p.	n.p.
tephri	Tephritidae	Tachinidae	Bigflies	1	1	n.p.	n.p.
antho1	Anthomyiidae1	Anthomyiidae	Smallflies	1	1	n.p.	n.p.
antho2	Anthomyiidae2	Anthomyiidae	Smallflies	1	1	1	2
antho3	Anthomyiidae3	Anthomyiidae	Smallflies	n.p.	n.p.	1	9
chloro	Chloropidae	Chloropidae	Smallflies	3	4	n.p.	n.p.
dypt1	Megaselia & Meoneura (Róndani, 1856)	Phoridae &Carnidae	Smallflies	5	9	6	13
myotes	Myopa testacea (Linneaus 1767)	Conopidae	Smallflies	1	2	n.p.	n.p.
phecin	Pherbellia cinerella (Fallén, 1820)	Sciomyzidae	Smallflies	3	7	1	1
salspho	Saltella sphondylii (Schrank, 1803)	Sepsidae	Smallflies	7	52	6	52
scaster	Scathophaga stercoraria (Linnaeus, 1758)	Scathophagidae	Smallflies	1	3	n.p.	n.p.
sepneo	Sepsis neocynipsea (Melander & Spuler, 1917)	Sepsidae	Smallflies	1	5	1	1
adsger	Adscita geryon (Hübner 1813)	Zygaenidae	Butterflies	n.p.	n.p.	1	2
aglurt	Aglais urticae (Linneo, 1758)	Nymphalidae	Butterflies	5	74	6	39
argagl	Argynis aglaja (Linnaeus, 1758)	Nymphalidae	Butterflies	1	5	1	2

Table S1 (Cont).

Acronim	Scientific name	Family	F. Group	Encroached Pasture		Pasture	
				Interactions	Visits	Interactions	Visits
argsp	Argyniss sp. (Rafinesque 1815)	Nymphalidae	Butterflies	1	1	1	2
colalf	Colias alfacariensis (Berger, 1948)	Pieridae	Butterflies	1	2	n.p.	n.p.
colcro	Colias croccea (Geoffroy, 1785)	Pieridae	Butterflies	4	30	5	20
crambi	Crambidae (Meyrick, 1890)	Crambidae	Butterflies	n.p.	n.p.	1	2
eremeo	Erebia meolans (Prunner, 1798)	Nymphalidae	Butterflies	5	48	1	1
eretri	Erebia triaria (Prunner, 1799)	Nymphalidae	Butterflies	1	2	n.p.	n.p.
helvir	Heliothis viriplaca (Ochsenheimer, 1816)	Noctuidae	Butterflies	2	6	n.p.	n.p.
hipalc	Hipparchia alcyone (Denis & Schiffermüller, 1775)	Nymphalidae	Butterflies	n.p.	n.p.	n.p.	n.p.
hipsem	Hipparchia semele (Linnaeus, 1758)	Nymphalidae	Butterflies	2	3	n.p.	n.p.
inaio	Inachis Io (Linnaeus, 1759)	Nymphalidae	Butterflies	1	1	1	1
isslat	Issoria lathonia (Linnaeus, 1758)	Nymphalidae	Butterflies	11	551	9	159
lamboe	Lampides boeticus (Linnaeus, 1767)	Lycaenidae	Butterflies	1	4	2	2
lycalc	Lycaena alciphron (Rottemburg, 1775)	Lycaenidae	Butterflies	2	2	1	2
lycphl	Lycaena phlaeas (Linnaeus, 1761)	Lycaenidae	Butterflies	3	3	n.p.	n.p.
macste	Macroglossum stellatarum (Linnaeus, 1758)	Sphingidae	Butterflies	5	25	5	54
manjur	Maniola jurtina (Linnaeus, 1758)	Nymphalidae	Butterflies	1	1	n.p.	n.p.
meldid	Melitaea didyma (Esper, 1779)	Nymphalidae	Butterflies	2	2	n.p.	n.p.
panpan	Pandoriana pandora (Denis & Schiffermüller, 1775)	Nymphalidae	Butterflies	3	32	4	207
parapo	Parnassius apolo (Linnaeus, 1758)	Papilionidae	Butterflies	3	8	n.p.	n.p.
piebra	Pieris brassicae (Linnaeus, 1758)	Pieridae	Butterflies	2	3	2	2
pienap	Pieris napi (Linnaeus, 1758)	Pieridae	Butterflies	1	2	n.p.	n.p.

Table S1 (Cont).

Acronim	Scientific name	Family	F. Group	Encroached Pasture		Pasture	
				Interactions	Visits	Interactions	Visits
pierap	Pieris rapae Linnaeus, 1758)	Pieridae	Butterflies	1	1	n.p.	n.p.
pondap	Pontia daplidice (Linnaeus, 1758)	Pieridae	Butterflies	1	1	n.p.	n.p.
pyrali1	Pyralidae1	Pyralidae	Butterflies	1	2	n.p.	n.p.
pyrali2	Pyralidae2	Pyralidae	Butterflies	2	6	3	10
synant	Synanthedon sp. (Hübner 1819)	Sesiidae	Butterflies	2	2	1	1
vanata	Vanessa atalanta (Linnaeus, 1758)	Nymphalidae	Butterflies	1	1	3	47
vancar	Vanessa cardui (Linnaeus, 1758)	Nymphalidae	Butterflies	2	46	n.p.	n.p.
acmcoa	Acmaeoderella coarctata coarctata (Lucas, 1846)	Buprestidae	Beetles	3	11	4	6
chryso	Chrysomelidae (Latreille, 1802)	Chrysomelidae	Beetles	1	1	n.p.	n.p.
cocsep	Coccinella septepunctata (Linnaeus, 1758)	Coccinellidae	Beetles	4	15	6	26
curcul1	Curculionidae1	Curculionidae	Beetles	1	3	3	7
curcul2	Curculionidae2	Curculionidae	Beetles	n.p.	n.p.	1	1
enicop	Enicopus sp. (Scopoli, 1763)	Dasytidae	Beetles	7	108	10	162
enipil	Enicopus pilosus (Scopoli, 1763)	Dasytidae	Beetles	n.p.	n.p.	1	1
helper	Heliopathes perrouidi (Mulsan & Rey, 1854)	Tenebrionidae	Beetles	1	1	2	4
lablon	Labidostomis longimana (Linnaeus, 1760)	Chrysomelidae	Beetles	n.p.	n.p.	n.p.	n.p.
lytves	Lytta vesicatoria (Linnaeus, 1758)	Meloidae	Beetles	2	30	n.p.	n.p.
mylvar	Mylabris variabilis (Pallas, 1781)	Meloidae	Beetles	7	105	2	3
nitidu	Nitidulidae (Latreille, 1802)	Nitidulidae	Beetles	3	10	5	14
phalac1	Phalacridae1	Phalacridae	Beetles	2	18	2	7
phalac2	Phalacridae2	Phalacridae	Beetles	3	11	2	2
phyvul	Phyllodecta vulgatissima (Linnaeus, 1758)	Chrysomelidae	Beetles	1	2	n.p.	n.p.

Table S1 (Cont).

Acronim	Scientific name	Family	F. Group	Encroached Pasture		Pasture	
				Interactions	Visits	Interactions	Visits
camlat	Camptopus lateralis (German, 1817)	Alydidae	Others	1	1	n.p.	n.p.
forfus	Formica fusca (Linnaeus 1758)	Formicidae	Others	2	4	2	3
lygaei2	Lygaeidae (Schilling, 1829)	Lygeidae	Others	n.p.	n.p.	2	5
lygeid	Lygeidae (Schilling, 1829)	Lygeidae	Others	n.p.	n.p.	1	1
micbla	Microlepidoptero 1	-	Others	1	2	1	2
micbri	Microlepidoptero 2	-	Others	1	1	n.p.	n.p.
miccam	Microlepidoptero 3	-	Others	1	1	1	1
micman	Microlepidoptero 4	-	Others	n.p.	n.p.	2	2
micpar	Microlepidoptero 5	-	Others	6	32	10	65
micral	Microlepidoptero 6	-	Others	2	5	n.p.	n.p.
mirida	Miridae (Hahn, 1831)	Miridae	Others	n.p.	n.p.	1	1
scioco	Sciocoris sp. (Fallén, 1829)	Pentatomidae	Others	n.p.	n.p.	1	2
spisax	Spilostethus saxatilis (Scopoli, 1763)	Lygeidae	Others	1	1	n.p.	n.p.
tetimp	Tetramorium impurum (Förster 1850)	Formicidae	Others	n.p.	n.p.	2	4

Table S2. Parameter estimates and standard deviation fitted for fixed and random effects by LMM performed for testing the effects of habitat type, functional group and flowering plants abundance on number of interactions and total number of visits recorded for each functional group.

		Number of interactions			Number of visits		
		Parameter Value	*t* value	*Pr*(>|*t*|)	Parameter Value	*t* value	*Pr*(>|*t*|)
Fixed effects							
Intercept		0.40	3.05	**<0.001**	0.85	3.62	**<0.001**
Habitat type	Encroached	0.42	2.39	**0.02**	0.78	2.45	**0.01**
Functional Group	Big flies	0.04	0.22	0.83	-0.59	-1.87	0.06
	Bees	0.34	1.93	0.06	0.10	0.31	0.76
	Beetles	1.04	5.86	**<0.01**	1.20	3.78	**<0.001**
	Bee flies	0.08	0.44	0.66	0.04	0.11	0.91
	Butterflies	1.20	6.78	**<0.001**	1.50	4.75	**<0.001**
	Others	0.47	1.68	0.10	0.36	1.15	0.25
	Smallbees	0.99	5.60	**<0.001**	1.53	4.83	**<0.001**
	Smallflies	0.49	2.76	0.01	0.37	1.15	0.25
	Hoverflies	1.37	7.72	**<0.001**	1.87	5.90	**<0.001**
	Wasps	0.28	1.61	0.11	0.31	0.97	0.33
Plant abundance		0.0001	0.27	0.78	0.0001	0.31	0.75
H. Type:F. Group	Encroached:Flies	-0.19	-0.76	0.45	-0.20	-0.44	0.66
	Encroached:Bees	0.15	0.61	0.54	0.03	0.08	0.94
	Encroached:Beetles	-0.25	-1.02	0.31	-0.31	-0.70	0.48
	Encroached:Bee flies	-0.26	-1.06	0.29	-0.54	-1.20	0.23
	Encroached:Butterflies	-0.01	-0.05	0.96	-0.10	-0.21	0.83
	Encroached:Others	-0.62	-2.50	**0.01**	-1.21	-2.69	0.01
	Encroached:Small bees	-0.15	-0.59	0.56	-0.49	-1.08	0.28
	Encroached:Small flies	-0.34	-1.39	0.17	-0.71	-1.59	0.11
	Encroached:Hoverflies	-0.03	-0.13	0.90	-0.47	-1.04	0.30
	Encroached:Wasps	-0.28	-1.15	0.25	-0.70	-1.56	0.12
Random effects							
				SD			SD
Intercept				0.18			0.31
Study site				0.18			0.31
Residual				0.64			0.96

SD, standard deviation. Significant effects ($p < 0.05$) are indicated in bold. Missing levels of factors (Habitat type: Pasture; Functional group: Bumblebees) are included on the intercept. Bumblebees was the functional group with the lowest number of interactions and visits measured, which facilitates the comparison among functional groups.

Table S3. Parameter estimates and standard deviation fitted for fixed and random effects by LMM performed to test the effects of life form and flowering plant abundance on number of interactions, total number of visits and number of visits received per flowering plant.

		Number of interactions			Number of visits			Visits per flowering plant		
		Parameter Value	*t* value	*Pr*(>|*t*|)	Parameter Value	*t* value	*Pr*(>|*t*|)	Parameter Value	*t* value	*Pr*(>|*t*|)
Fixed effects										
Intercept		1.21	12.88	**<0.001**	1.94	14.02	**<0.001**	-0.98	-6.69	**<0.001**
Life form	Shrub	0.53	2.43	**0.02**	0.54	1.68	0.09	0.81	2.35	**0.02**
Plant abundance		0.0009	3.30	**<0.01**	0.0019	4.41	<**0.001**	-0.0029	-7.29	**<0.001**
Random effects										
				SD			SD			SD
Intercept				0.00004			0.00004			0.00014
Study site				0.00002			0.00001			0.00001
Residual				0.93977			1.37602			1.62155

SD, standard deviation. Significant effects ($p < 0.05$) are indicated in bold. Missing levels of factors (Life form: Herb) are included on the intercept.

Table S4. Parameter estimates and standard deviation fitted for fixed and random effects by LMM performed to test the effects of habitat type, plant species and flowering plant abundance number of interactions, total number of visits and number of visits received per flowering plant.

		Number of interactions			Number of visits			Visits per flowering plant		
		Parameter Value	*t* value	*Pr*(>|*t*|)	Parameter Value	*t* value	*Pr*(>|*t*|)	Parameter Value	*t* value	*Pr*(>|*t*|)
Fixed effects										
Intercept		0.33	0.86	0.39	1.15	2.01	**0.04**	-2.07	-4.36	**<0.001**
Habitat type	Encroached	0.14	0.30	0.77	0.15	0.21	0.83	-1.61	-3.04	**<0.01**
Plant species	Armcae	0.76	1.52	0.13	0.20	0.27	0.78	0.48	0.78	0.44
	Bisint	0.49	0.89	0.37	0.42	0.52	0.61	2.72	3.54	**<0.001**
	Pilvah	0.60	1.29	0.20	0.85	1.23	0.22	1.10	1.91	0.06
	Jascri	1.00	2.14	**0.03**	1.34	1.93	0.06	0.83	1.45	0.15
	Jurhum	1.78	3.89	**<0.001**	2.24	3.32	**<0.001**	2.15	3.82	**<0.01**
	Leualp	0.78	1.68	0.09	0.71	1.03	0.30	1.40	2.38	**0.02**
	Sedbre	0.83	1.80	0.07	1.01	1.47	0.14	0.11	0.19	0.85
	Senpyr	1.57	3.36	**<0.001**	1.83	2.66	**0.01**	2.19	3.84	**<0.01**
	Solvir	1.02	1.95	0.05	0.46	0.59	0.55	2.88	3.98	**<0.001**
	Thypra	0.95	1.77	0.08	0.91	1.15	0.25	2.81	3.95	**<0.001**
Plant abundance		0.0009	5.29	**<0.001**	0.0015	6.01	**<0.001**	-0.0014	-6.52	**<0.001**
H. Type:P. species	Encroached:armcae	0.16	0.25	0.80	-0.08	-0.08	0.93	-1.01	-1.27	0.21
	Encroached:bisint	-0.69	-0.95	0.35	-1.00	-0.93	0.35	-2.30	-2.39	**0.02**
	Encroached:pilvah	0.20	0.33	0.75	-0.02	-0.02	0.98	0.13	0.18	0.86
	Encroached:jascri	-0.18	-0.31	0.76	-0.24	-0.27	0.79	0.56	0.78	0.44
	Encroached:jurhum	-0.52	-0.90	0.37	-0.44	-0.52	0.61	-0.23	-0.32	0.75
	Encroached:leualp	0.13	0.22	0.83	0.12	0.14	0.89	-0.28	-0.38	0.70
	Encroached:sedbre	-0.44	-0.75	0.46	-0.78	-0.88	0.38	-0.15	-0.22	0.83
	Encroached:senpyr	-0.20	-0.34	0.74	-0.44	-0.49	0.63	0.72	0.98	0.33
	Encroached:solvir	-0.05	-0.08	0.94	0.51	0.53	0.60	-1.63	-1.98	**0.04**
	Encroached:thypra	-0.04	-0.06	0.95	-0.04	-0.04	0.97	0.19	0.21	0.83
Random effects										
				SD			SD			SD
Intercept				0.00005			0.00008			0.15597
Study site				0.01324			0.35383			0.00191
Residual				0.98578			1.43571			1.10141

SD, standard deviation. Significant effects ($p < 0.05$) are indicated in bold. Missing levels of factors (Habitat type: Pasture; Plant species: Silcil) are included on the intercept. Table S1 contains acronyms used for plant species. Silcil was the plant species with the lowest number of interactions and visits measured, which facilitates the comparison among plant species.

CONCLUSIONS

CONCLUSIONS

1) No single kernel function provided the best fit across all populations, although estimated mean dispersal distances were short in all cases (range: 0.23 – 0.71 m). In addition to intrinsic limitations of primary seed dispersal, effective seed dispersal in these two species may be further restricted by post-dispersal factors related to summer drought. The limited dispersal range of the two studied species raises uncertainty on their ability to track their climatic niche through migration under ongoing rapid global warming.

2) The altitudinal gradient appears to influence effective seed dispersal patterns in *S. ciliata* and *A. caespitosa* through adult plant fecundity and seedling density, but not through the effective seed dispersal range. There were significant differences in the fecundity parameter among populations in both species and in the mean dispersal distance among populations of *A. caespitosa*, while differences in the mean dispersal distance among *S. ciliata* populations were only marginally significant. These results call for a case-by-case analysis in a wider range of plant taxa and environments to assess the prevalence and magnitude of intraespecific dispersal variation.

3) Significant FSGS has been found in three of the five stable populations of *S. ciliata* studied, which reveals among-population variation in the intensity of FSGS.

4) There was a significant positive association between the spatial demographic and genetic structure of the five populations of *S. ciliata* studied. Changes in environmental conditions due to variation in suitable habitat availability and microhabitat heterogeneity appeared to partially drive fine-scale aggregation of adults, and therefore, could be a main factor affecting FSGS.

5) Population genetic diversity and inbreeding did not significantly vary as a function of the intensity of FSGS. This finding reveals the absence of relationship between both genetic indices and FSGS. This suggest that population genetic diversity and inbreeding are not related with fine-scale environmental variation.

6) The common garden approach confirmed that among-population variation in flowering onset, number of flowerghead and number of viable fruits in *A. caespitosa* is partially genetically based.

7) The stronger selection response in favor of early-flowering individuals in the low edge of *A. caespitosa* elevational range did not correspond with greater proportion of early-flowering individuals. Nevertheless, the observed genetic variability within populations in flowering onset indicates that there is adaptive potential to respond to ongoing global warming.

8) Shrub encroachment increased richness and visitation patterns of pollinators, but also increased pollinator-mediated competition among plant species, which can potentially result in reduced reproductive success.

9) Shrub species acted as super-generalist plants, as supported by their low degree of specialization but high linkage and pollinator visitation. In spite of the central role of shrub species the structure of the networks remained relatively unchanged. This result is congruent with previous findings in the literature suggesting that plant-pollinator mutualism may be robust to the effects of global change.

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