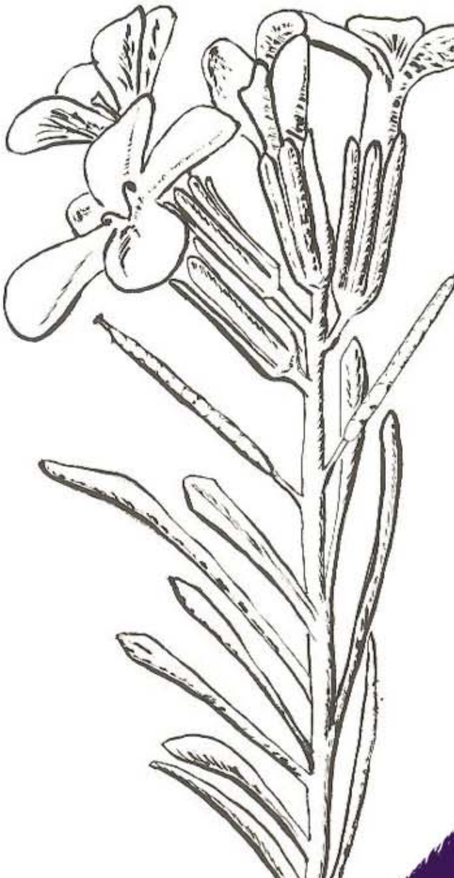
BIOLOGÍA DE LA CONSERVACIÓN
DE
Pseudomisopates rivas-martinezii
Y
Erysimum penyalarense,
DOS TÁXONES AMENAZADOS DE LA CORDILLERA CARPETANA



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CERTIFICAN:

Que los trabajos de investigación desarrollados en la memoria de tesis doctoral: **"Biología de la conservación de *Pseudomisopates rivas-martinezii* y *Erysimum penyalarense*, dos táxones amenazados de la Cordillera Carpetana"**, han sido realizados bajo su supervisión y son aptos para ser presentados por la Licenciada María Elena Amat de León Arce ante el tribunal que en su día se consigne, para aspirar al Grado de Doctor en Biología por la Universidad Rey Juan Carlos.

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ÁREA DE
BIODIVERSIDAD
Y CONSERVACIÓN

Biología de la conservación de *Pseudomisopates rivas-martinezii* y *Erysimum penyalarense*, dos táxones amenazados de la Cordillera Carpetana

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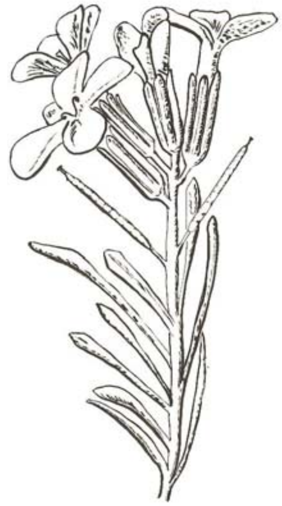
“There are more things in heaven and earth, Horatio,

Than are dreamt of in your philosophy.”

Hamlet

Introducción



1. Antecedentes e hipótesis general

1. Antecedentes e hipótesis general

A pesar del reconocido valor de la biodiversidad, muchas comunidades y especies están en regresión y en peligro de extinción debido a la pérdida de hábitat, la sobreexplotación, la contaminación o la introducción de especies invasoras. De hecho, numerosas especies se han extinguido ya (Frankham *et al*. 2004; Primack 2000; Sutherland 2000). La magnitud del problema es enorme, puesto que, aunque en el registro geológico fósil se han documentado extinciones masivas previas y los fenómenos equilibrados de extinción y especiación son actualmente considerados como parte de la historia de la vida (Willis & McElwainn 2002), nunca se había producido un patrón de extinción como el que se observa en la actualidad y producido por una sola especie (*Homo sapiens*). Es por ello que esta ha venido ya a denominarse “la sexta extinción” (Leakey & Lewin 1996). Además, esta situación es particularmente llamativa para las especies vegetales: la *Unión Internacional para la Conservación de la Naturaleza* (IUCN) ha clasificado recientemente el 53% de los musgos, el 23% de las gimnospermas, el 26% de las monocotiledóneas y el 54% de las dicotiledóneas como amenazadas en diferentes grados. Por su parte, los hábitats y especies de plantas de España no son una excepción a la situación mundial (Bañares *et al.* 2004; Rivas-Martínez & Penas 2005).

Centrándonos en los ecosistemas montanos, hay que destacar la gran riqueza de especies animales y vegetales (Körner & Spehn 2002; Körner 2004; Molau 2004), motivada por el aislamiento geográfico de muchas montañas, así como la gran variedad de tipos de hábitat derivados de la gran variabilidad ambiental. De hecho, 16 de los 25 centros de biodiversidad del mundo se encuentran en montañas, incluido el de la cuenca del Mediterráneo (Myers *et al.* 2000), que cuenta con un elevado número de endemismos (Thompson 2005). El número de especies vegetales endémicas aumenta con la altitud en la mayoría de los ecosistemas estudiados (Kessler 2000). De la misma manera, las zonas montanas de la Cordillera Carpetana o Sistema Central Ibérico no sólo contienen una gran cantidad de especies, sino que encierran también un alto número de endemismos. Aunque, en su mayoría, se trata de endemismos ibéricos, destacan también algunos pocos que le son exclusivos (Vargas & García 2008), como son *Erysimum penyalarense* (Rivas-Martínez *et al*. 1990) y *Pseudomisopates rivas-martinezii* (Sánchez-Mata 1988).

Desgraciadamente, los ecosistemas alpinos están sometidos en la actualidad a severos procesos de degradación ambiental, que tienen como resultado la extinción de buena parte de las especies que los integran. La gravedad de estas amenazas reside principalmente en el bajo nivel de resiliencia de estos ecosistemas ante las perturbaciones (Spiegelberger 2006) y a su vulnerabilidad frente al cambio climático (Pauli *et al.* 2007). En este sentido se ha documentado la regresión de los pastizales alpinos mediterráneos de la Sierra de Guadarrama, debido al avance de piornales y enebrales (Sanz-Elorza *et al.* 2003). No obstante, resultan escasos aún los datos rigurosos sobre aspectos básicos de la biología de las especies altimontanas que nos ayuden a predecir su respuesta frente a tales amenazas.

No debemos perder de vista que, en última instancia, la viabilidad de las poblaciones reside en su dinámica demográfica, de forma que la extinción de las poblaciones se producirá cuando la tasa de mortalidad supere a la de natalidad. Esto implica determinar en qué medida los factores que amenazan a una población se traducen en un declive poblacional. La literatura reciente aporta una revisión de las amenazas más comunes para la supervivencia de los organismos vegetales (Tabla 1), que se pueden clasificar en tres categorías: (1) amenazas impuestas por las condiciones ambientales cambiantes (estocasticidad ambiental), (2) amenazas resultantes de la perturbación de las interacciones interespecíficas básicas (perturbación de interacciones bióticas), y (3) amenazas que afecten a la variabilidad genética (desequilibrios genéticos) (Oostermeijer 2003). La primera categoría tiene a menudo como resultado la destrucción de los individuos y su hábitat; mientras que las dos siguientes, asociadas a un pequeño tamaño poblacional o al aislamiento, suelen ser consecuencia de la primera categoría. Es por ello, que podríamos distinguir causas primarias y causas secundarias (Oostermeijer 2003).

Estocasticidad ambiental Cambio climático Destrucción del hábitat Alteración del suelo: erosión, urbanización, deforestación, aclaramiento de tierras, etc. Cambios en usos del territorio o abandono Eutrofización y fertilización Dinámica del fuego (supresión) Prácticas agrícolas, pesticidas y herbicidas Prácticas ganaderas Deposición de nitrógeno Actividades turísticas Establecimiento de áreas protegidas Sobreexplotación	**CAUSAS PRIMARIAS**
Perturbación de interacciones bióticas Introducción de especies invasoras Declive demográfico Reducción en la abundancia polinizadores Reducción en la abundancia de dispersores Herbivoría (ramoneo, pisoteo) Sucesión de la vegetación, competencia interespecífica **Desequilibrios genéticos** Endogamia Deriva y erosión genética Hibridación Acumulación de mutaciones Pérdida de alelos S	**CAUSAS SECUNDARIAS**

Tabla 1. Factores que condicionan el estado de amenaza de las plantas, extraídos de la literatura de la conservación y clasificadas según su origen (adaptado de Oostermeijer 2003).

Para afrontar dichas causas de amenaza la biología de la conservación abarca la conservación de todos los componentes de la biodiversidad (genes, poblaciones, especies, comunidades y ecosistemas) que hayan sido perturbados directa o indirectamente por actividades humanas u otros agentes (Soulé 1985). Esta ciencia surgida en los años 70 es una ciencia multidisciplinar que se desarrolla en respuesta a la actual crisis de biodiversidad. Se trata, por tanto, de una disciplina de crisis que aúna ciencias naturales como la ecología, la biología de poblaciones, la genética o la evolución, con otras ciencias sociales como la economía, la sociología o la antropología (Primack 1993). Su finalidad es la de proveer los fundamentos y herramientas para conservar la biodiversidad (Soulé 1985).

Son muchos los criterios e índices que se utilizan para establecer el valor de la biodiversidad (Heywood & Iriondo 2003). No obstante, ante la inherente escasez de recursos económicos destinados a la conservación, la prioridad y el valor asignable a las distintas especies no puede considerarse equivalente. Algunos autores argumentan que las especies con mayor divergencia -bien sea genética, taxonómica o ecológica- contribuyen en mayor medida a la biodiversidad total que aquellas especies que están más relacionadas entre sí. En este sentido la sistemática molecular ofrece la posibilidad, por un lado, de medir la diferenciación genética entre especies y, por otro, de asignar la pertenencia a distintos grupos o clados (Vane-Wright *et al.* 1991; Faith 1992). Muchos trabajos de investigación de especies amenazadas se centran en el estado actual de las especies descuidando la importancia de su historia evolutiva. Es por ello que el planteamiento de la presente memoria doctoral considera el conocimiento que, del origen y evolución de las especies, proporcionan la taxonomía y las filogenias moleculares. Tomando dicho conocimiento como punto de partida para profundizar en la perspectiva que de su situación de amenaza y dinámica actual ofrece la biología de poblaciones.

Si descendemos al nivel de población, hay que destacar que las poblaciones de especies no son genética y espacialmente homogéneas, puesto que en ellas se distinguen distintos tipos de estructuras que son el objeto de estudio de la biología de poblaciones. En primer lugar, la estructura genética, describe el patrón de distribución de los genotipos; en segundo lugar, la estructura espacial trata de la distribución de individuos dentro de la población; en tercer lugar, la estructura de edades, se centra en la proporción de juveniles y adultos, y por último, la estructura de tamaños, se refiere a la proporción de individuos grandes o menudos. La biología de poblaciones trata pues de explicar el origen de las distintas estructuras para entender cómo interrelacionan entre sí, y evolucionan con el tiempo (Silvertown & Charlesworth 2001). Las disciplinas de estudio que componen a su vez la biología de poblaciones permiten evaluar la estabilidad de las poblaciones para determinar la necesidad de tomar medidas destinadas a su conservación (Figura 1).

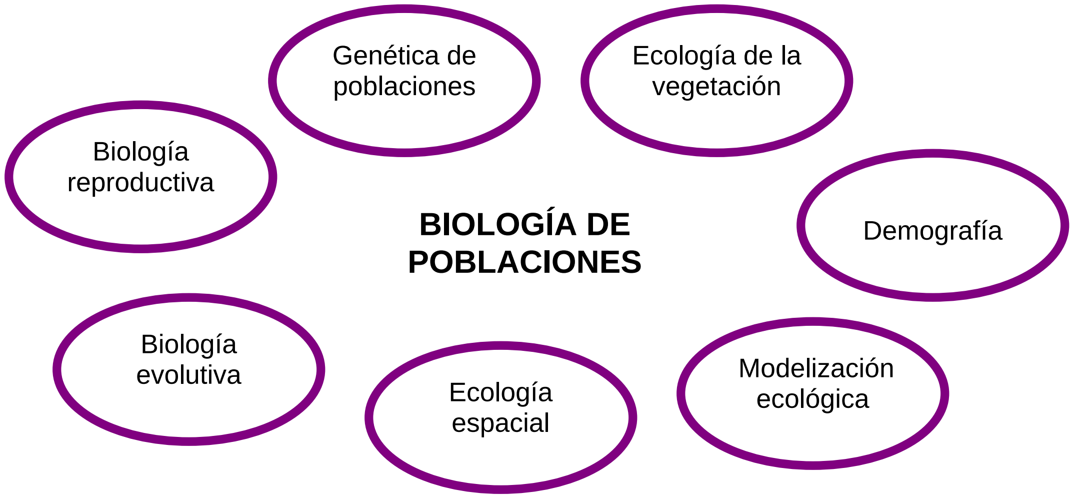


Figura. 1. Disciplinas que constituyen el objeto de análisis para el estudio de la biología de poblaciones de especies amenazadas.

Trabajos previos sobre el estado de conservación de las dos especies de estudio (Martínez *et al.* 2004) ponen de manifiesto la necesidad de estudios más profundos, sobre estos dos endemismos amenazados de las montañas mediterráneas y cuyo reconocimiento taxonómico ha presentado ciertas dificultades en el pasado.

En primer lugar, Martínez *et al.* (2004) señalan que *Pseudomisopates rivas-martinezii* presenta un número de clones aparentemente muy bajo, a falta de comprobación empírica, motivo por el cual la especie podría presentar graves problemas de viabilidad. La baja producción de frutos, escasa capacidad germinativa, presumible baja variabilidad genética, la ocupación de un área relativamente reducida, junto con la fuerte presión existente sobre su hábitat (presión ganadera, fuego, etc.), indica que se enfrenta a graves problemas de supervivencia.

En segundo lugar, Martínez *et al.* (2004) hacen notar que el área de distribución de *Erysimum penyalarense* se ha visto reducida por la desaparición de una de sus dos poblaciones; si bien, el número de individuos de la población restante es elevado y parece tener una buena reproducción. Estos autores sugieren que su crecimiento se ve afectado en las zonas muy pastoreadas, además de por el pisoteo y nitrificación asociados, apareciendo en dichas zonas sólo bajo la protección de otras plantas (como piornos o enebros). Además, hipotetizan que en la reducción histórica del área de ocupación parecen haber influido tanto el aumento del pisoteo, pastoreo y movimiento del terreno, como la creación de infraestructuras de recreo (pistas de esquí y remontes), así como una nitrificación excesiva por excrementos de ganado, que podría además ser la causante de la ausencia del taxón en áreas en principio óptimas para el mismo.

Teniendo en cuenta la información de partida, la hipótesis general de la que parte la presente memoria doctoral plantea que el grado de amenaza actual que presentan las dos especies de estudio podría estar causado por la estocasticidad ambiental, la perturbación de interacciones bióticas o por desequilibrios genéticos (Tabla 1). Para testar dicha hipótesis el principal objetivo de la presente memoria doctoral ha sido el de estudiar las causas concretas de amenaza de *Pseudomisopates rivas-martinezii* y *Erysimum penyalarense*, así como la problemática de su conservación, a través de los rasgos históricos de sus ciclos vitales y la interacción con su entorno.

2. El sistema de estudio

Sobre las especies de estudio: *Pseudomisopates rivas-martinezii*

Caracteres morfológicos. Planta perenne, de largos estolones subterráneos ramificados, que forma rodales compuestos por unos pocos individuos en su mayoría clónicos y de difícil diferenciación. Tallos aéreos erectos de 15-40 cm, leñosos en la base, herbáceos o ligeramente engrosados, simples o escasamente ramificados y con indumento formado por pequeños pelos patentes. Hojas linear lanceoladas de 10-35 × 0,7-1,1 mm, las inferiores subopuestas, el resto alternas, mucronadas, con el nervio central y los bordes engrosados, laxamente hispídulas, no glandulosas, pelos tectores como los de los tallos pero algo más cortos. Inflorescencia en racimo terminal laxo, con indumento semejante al del tallo. Flores zigomorfas con pedicelo de 6-8 mm, mayor que el cáliz, generalmente más corto que la bráctea, erecto en la antesis y fructificación, hispídulo. Cáliz profundamente hendido, con 5 sépalos subiguales, más cortos que el tubo de la corola, unidos en la base, agudos, con pelos tectores como los del tallo. Corola bilabiada, personada y ocluida, de 12-15 mm; de color rosado o blanquecino, con venas purpúreas; exteriormente glandular-pubescente, papilosa en el interior; tubo cilíndrico, prolongado en una giba basal. Androceo didínamo, con 4 estambres. Ovario bilocular glanduloso, estilo recto, persistente, estigma no capitado, dispuesto entre las anteras. Cápsula bilocular, ovoide, glandulosa, con dehiscencia foraminal, con un poro único por lóculo. Semillas de color pardo obscuro, elípticas y con asimetría dorsoventral.

Problemática taxonómica. Este taxon descubierto por Sánchez-Mata en 1988 fue inicialmente descrito como *Misopates rivas-martinezii* Sánchez-Mata. Su inclusión en dicho género se basó en la simetría y ornamentación de las semillas, así como en su número cromosomático n = 7, considerado erróneamente en el protólogo del género *Misopates*. Posteriormente fue combinado en el género *Acanthorrhinum rivas-martinezii* (Sánchez-Mata) Fernández Casas y Sánchez-Mata, por tratarse de una planta perenne; por la cortedad relativa de sus sépalos (largos, estrechos y desiguales en *Misopates*); los dos lóculos, los dos poros y la asimetría de sus cápsulas, y por su número cromosomático de base diferente n = 7 (frente a n = 8 de *Misopates*). Ante la dificultad de mantener la especie en ninguno de estos dos géneros, Güemes propone en 1997 un nuevo género ibérico. Esta nueva consideración se basa en la incapacidad de encontrar reunidos en ninguno de los dos géneros los siguientes caracteres: la cápsula con dos lóculos subiguales; semillas anchamente elípticas, de simetría bilateral y asimetría dorsiventral, sin alas ni crestas longitudinales; sépalos subiguales, estrechos alesnados, más cortos que el tubo de la corola; el indumento de la planta, eglandular y sus tallos rizomatosos. Los estudios filogenéticos realizados por Vargas *et al.* 2004 con marcadores ITS sobre la tribu Antirrhineae, apoyan este tratamiento de género monotípico debido a su historia evolutiva con respecto a los géneros a los que fue previamente asignado.

Hábitat y distribución. En la descripción inicial, basada en los primeros rodales pertenecientes a la población de La Serrota, Sánchez-Mata (1988) apunta que el hábitat general de la especie son los pastizales psicroxerófilos silicícolas oromediterráneos bejarano-gredenses (*Arenario querioidis-Festucetum summilusitanae* Rivas-Martínez, Sánchez-Mata & Fuente; *Hieracio castellani-Plantaginion radicatae* Rivas-Martínez & Cantó; *Sedo-Scleranthetea* Br.-Bl.). El mismo autor resalta también que su origen pudo deberse a un proceso de especiación motivado por las peculiaridades litológicas de La Serrota, donde se encuentran numerosos afloramientos de rocas básicas correspondientes a un gran dique diabásico, ya que en las proximidades de todos los rodales detectados en La Serrota se localizaron vetas importantes de este material. El análisis geoquímico realizado mostró un alto contenido en metales pesados (Cr, Ni, Co) y otros elementos como Zn y Li, siendo los compuestos predominantes SiO_2, Al_2O_3 y Fe_2O_3. No obstante, esta hipótesis queda en entredicho debido al posterior hallazgo de otras poblaciones en localidades cuya composición geológica carece de estas particularidades (Luceño *et al.* 2000; Vargas & García 2008).

Figura 2. Imagen tomada en la población de La Serrota.

En general podemos afirmar que *Pseudomisopates rivas-martinezii* aparece principalmente en las zonas más o menos aclaradas de los piornales de *Cytisus oromediterraneus* (Figura 2) y en pastos supra- y oromediterráneos, sobre suelos ácidos y pobres de tipo ranker. Todas las poblaciones conocidas se hallan en laderas preferiblemente soleadas, con altitudes que van de los 1.300 de la Garganta Lóbrega (Candeleda) hasta los 2.200 m.s.n.m. de La Honda (Villatoro).

Al inicio del presente proyecto de tesis doctoral se conocían 8 poblaciones de este endemismo gredense, repartidas en dos núcleos poblacionales separados entre sí por unos 20 Km de distancia (Luceño *et al.* 2000, Vargas & García 2008) (Figura 3). El de mayor superficie se halla en el macizo central de la Sierra de Gredos, incluido dentro del Parque Regional de la Sierra de Gredos, y está compuesto por 5 poblaciones. El menor se encuentra en la Sierra de La Serrota y está compuesto a su vez por 3 poblaciones. Ambos núcleos se encuentran contemplados en los LIC "Sierra de Gredos" y "Sierra de Paramera y Serrota" respectivamente.

El área de ocupación efectiva de la especie, teniendo en cuenta todas las poblaciones conocidas hasta 2008, es de 617.015 m^2. La población más abundante se encuentra en la Garganta Lóbrega (Candeleda, Ávila) que abarca el 92,61 % de la superficie de la especie. Esta población sufrió un incendio en el año 2005, que arrasó buena parte de la ladera este de la garganta. Se trata de la población que presenta las plantas más vigorosas y abundantes. A continuación le sigue con el 2,29% la población de las Chorreras (Navalperal de Tormes, Ávila), y el 2,16% de La Serrota (Cepeda de la Mora, Ávila). Otras poblaciones menores comprenden, el 1,50% en Los Conventos (Hoyos del Espino, Ávila), el 1,29% de La Honda (Villatoro, Ávila), el 0,07% en La Covacha (Hoyos del Espino, Ávila), el 0,05% de Cerro Pelado (Garganta del Villar, Ávila) y el 0,02% en la Garganta de Chilla (Candeleda, Ávila).

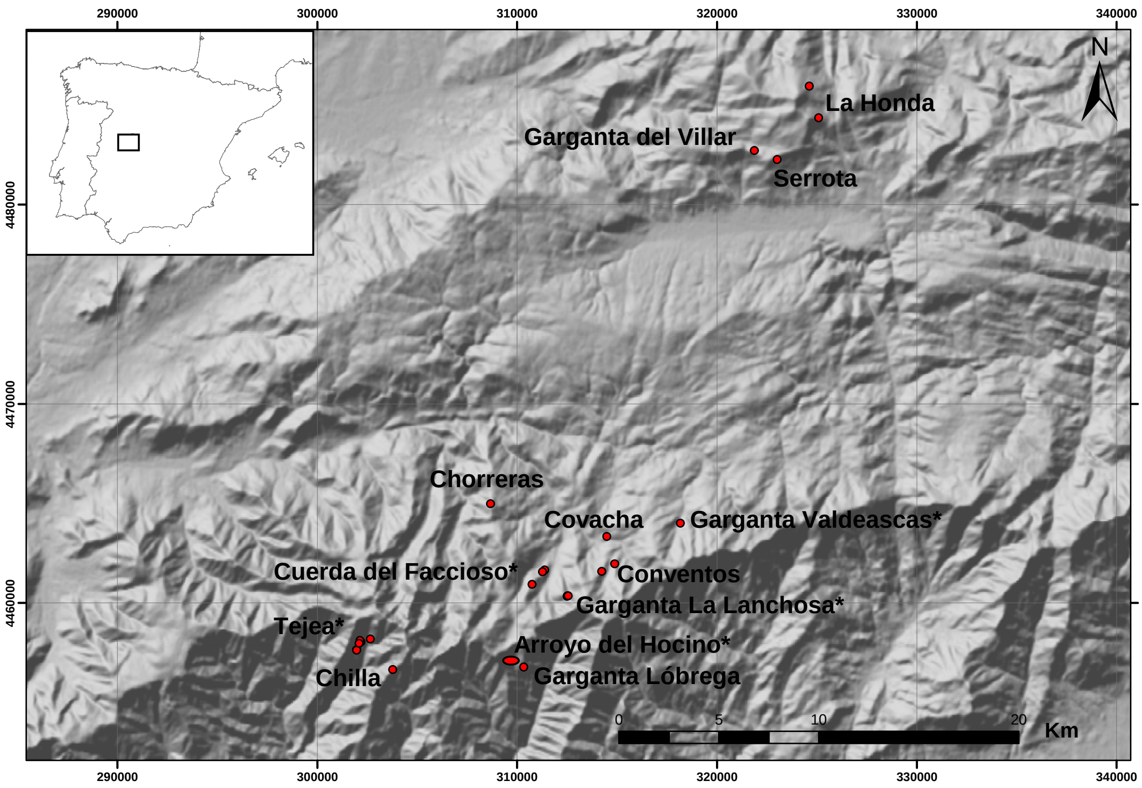


Figura 3. Mapa de localidades de *Pseudomisopates rivas-martinezii*. Figuran señaladas con un asterisco aquellas localidades que no eran conocidas al inicio del presente proyecto de Tesis Doctoral.

La primera población fue hallada por Daniel Sánchez-Mata en 1982, y no fue hasta 1998 cuando los naturalistas Bernardo García Muñoz e Isidro Sánchez Tejedor hallan una nueva población en la Garganta de La Covacha, que empiezan a descubrirse poblaciones fuera de la Sierra de La Serrota. A partir de entonces, B. García Muñoz comienza una búsqueda de nuevas localidades en ocasiones acompañado de su amigo I. Sánchez Tejedor, que da como resultado el hallazgo de las siguientes poblaciones: Los Conventos (1998), La Honda y la Garganta Lóbrega (1999), la Cuerda de las Chorreras (2001), Garganta de Chilla (2005), Cuerda del Faccioso, Cuerda de la Garganta La Lanchosa y Garganta Tejea (2009), Garganta Valdeascas (2010), y finalmente Arroyo del Hocino (2012). Por otro lado, Alfaro Saiz *et al.* (2002) aportan citas en zonas del municipio de Candeleda. No obstante, estas no han podido ser confirmadas con exactitud, si bien tres nuevos rodales fueron hallados a unos 500 m de estas citas.

La notable dificultad de la localización de las poblaciones halladas, debido a su presencia en lugares poco transitables (piornales, pedreras y morrenas) y orientaciones variables (aunque siempre soleadas), hace muy posible su presencia en otras zonas de Gredos. No obstante, Vargas & García (2008) dejan constancia de la prospección de zonas de piornal en busca de la especie, en las que esta no fue hallada. Estas zonas son el macizo principal el tramo entre Los Serranos y el Alto de Las Becedillas; el tramo entre el Puerto del Peón y el Puerto del Pico, y el tramo entre el Puerto del Peón y Los Serranos. En el macizo occidental el tramo entre el Alto de las Becedillas y el Puerto de Tornavacas y la Sierra de Béjar en su práctica totalidad. En el macizo oriental desde el Puerto del Pico al Cerro de Guisando. En la Sierra de La Paramera, la cuerda principal de la Sierra de Villafranca; La Serrota, y la Sierra del Zapatero desde el Puerto de Menga al de La Paramera.

Estado de conservación. *Pseudomisopates rivas-martinezii* es una especie amenazada de extinción, clasificada en la categoría CR - En Peligro Crítico - mediante los criterios de la Unión Internacional para la Conservación de la Naturaleza (UICN) e incluida en la Lista Roja de la Flora Vascular Española (Martínez *et al.* 2004). Se considera a una especie En Peligro Crítico cuando está enfrentando un riesgo extremadamente alto de extinción en estado silvestre en el futuro inmediato. Atendiendo a los conocimientos sobre la especie, dicha clasificación fue fundada en los criterios B y C, siendo estos dos de los cinco criterios posibles según la UICN. De forma que, la clasificación propuesta para esta especie es CR B1 ab(iii)+2ab(iii); C2 a(i), que pasamos a detallar a continuación.

Criterio B1 ab(iii) + 2 ab(iii)

El área de ocupación total de la especie medida en las 8 poblaciones conocidas es de 0,617 Km^2, y la inclusión de poblaciones halladas con posterioridad no superaría los 0,7 Km^2. Por lo tanto, dicho área es muy inferior a los 10 Km^2 que el criterio B2 propone para taxones en peligro crítico. De la misma manera, la extensión de presencia es menor a los 100 Km^2 que propone el criterio B1. En cuanto a los subcriterios considerados, podemos decir que sigue existiendo fragmentación severa en algunas poblaciones en que los rodales se encuentran lo suficientemente distantes entre sí como para que no exista flujo génico entre ellos.

Criterio C2 a(ii)

Aunque no se puede tener certeza del número de individuos, debido a la extensión potencial de los clones, se presume una declinación continua en el número de individuos maduros, y no se estima que estos superen los 250 en total, a pesar de las nuevas poblaciones encontradas. De la misma manera que tampoco se estima que ninguna población contenga más de 50 individuos.

De nuevo, la correcta aplicación de los criterios UICN requiere el seguimiento de variables poblacionales a lo largo de un período razonable de tiempo, tarea que se dificulta debido a la imposibilidad de distinguir individuos en el campo.

Importancia para la conservación. No todas las especies amenazadas tienen el mismo valor para la conservación. Aparte de las especies con interés social o económico, las especies amenazadas poseen diversas características que las otorgan diferentes grados de amenaza. La consideración de *Pseudomisopates* como género monotípico confiere a la especie de estudio un valor especial en cuanto a su conservación, cobrando interés el conocimiento del tiempo y la distancia genética que la separa de sus especies más próximas desde el punto de vista filogenético.

Cabe destacar que, lamentablemente, entre las especies con mayor riesgo de amenaza que encontramos en nuestro país existe una notoria falta de atención hacia los géneros monotípicos con la máxima categoría de amenaza (Bañares *et al.* 2004). En efecto, sorprende que, con todo el dinero invertido en la conservación de subespecies, variedades y poblaciones marginales de especies con una distribución más amplia, los géneros monotípicos amenazados y endémicos de nuestra geografía hayan sido tan ignorados.

A pesar de las nuevas poblaciones encontradas en los últimos años, el nivel de riesgo de este taxon sigue considerándose elevado, dado el desconocimiento de la relación existente entre el número de *genets* y *ramets*.

Metodología

En este apartado se describen el área de estudio y el esquema general de los muestreos realizados. No obstante, la metodología específica utilizada para la consecución de cada objetivo es proporcionada con mayor detalle en el apartado de Material y Métodos de los capítulos correspondientes.

El área de estudio. Las áreas seleccionadas para cubrir los distintos objetivos de estudio para esta especie son las que se detallan a continuación.

La Serrota, enmarcada en los cuadrantes UTM (proyección Datum European 1950, Hemisferio Norte, Huso 30) 30TUK2282 y 30TUK2382, entre los 1850-1960 m.s.n.m. El área forma parte del *Espacio Natural de las Sierras de la Paramera y Serrota.*

La Garganta Lóbrega que se encuentra en los cuadrantes 30TUK0956, 30TUK1055, 30TUK1056 y 30TUK1057, entre los 1300-1950 m.s.n.m. y *Los Conventos* presente el cuadrante 30TUK1461, entre los 1740-1800 m.s.n.m. Ambas poblaciones forman parte del *Parque Regional de la Sierra de Gredos.*

Diseño experimental. A continuación se detalla el diseño experimental utilizado para los distintos objetivos abordados.

Tratamientos de polinización

Se llevaron a cabo tratamientos de polinización en la población de La Serrota (Sierra de la Serrota, Cepeda de la Mora, Ávila) durante los veranos de 2007 y 2008 con el fin de testar: (1) agamospermia, (2) autogamia espontánea, (3) autogamia obligada, (4) polinización cruzada, (5) limitación de polen, y (6) controles de flores sin tratar. Los componentes de éxito reproductivo utilizados para determinar el sistema reproductivo y la limitación de polen fueron: (1) fruit set, (2) seed set, y (3) seed set de semillas viables. Inicialmente se seleccionaron 20 plantas para cada tratamiento en el verano de 2007. Posteriormente, en 2008 se amplió el estudio seleccionando 30 plantas a las que se aplicaron sólo los tratamientos que habían generado resultados el año anterior (tratamientos del 3 al 6). Los resultados de estos tratamientos están recogidos en el capítulo 2.

Muestreos de visitantes florales

Los muestreos se llevaron a cabo en la población de Los Conventos (Sierra de Gredos, Hoyos del Espino, Ávila) durante el verano de 2009. Se seleccionaron 5 rodales, dentro de los cuales 10 plantas fueron muestreadas y se registraron los visitantes florales en períodos de 10 minutos. Se registraron todos los insectos que entraban en contacto con las anteras o estigma de las flores. La diversidad y composición de la comunidad de visitantes florales fue analizada con el fin de determinar la existencia de limitación de polinizadores. Los resultados de estos muestreos están recogidos en el capítulo 2.

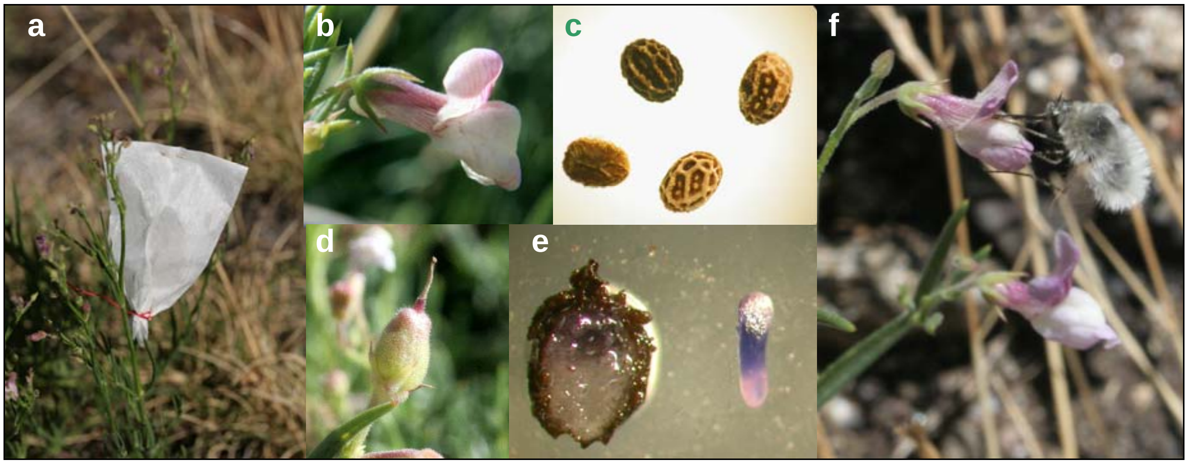


Figura 4. Detalles reproductivos de *Pseudomisopates rivas-martinezii*: (a) ensayos de polinización; (b) flor; (c) semillas; (d) fruto; (e) test de viabilidad; (f) bombílido visitando una flor.

Consecuencias de la clonalidad

La población de La Serrota (Cepeda de la Mora, Ávila) fue seleccionada para llevar a cabo un estudio detallado de los clones durante el verano de 2007. Se llevó a cabo un muestreo anidado, según el cual 10 rodales fueron seleccionados al azar, y dentro de los cuales se seleccionaron aleatoriamente 10 puntos de muestreo. En cada punto de muestreo se tomó la planta más cercana, de la cual se tomó material de hojas, se le efectuaron una serie de medidas (diámetro, número de ramas, número de flores y frutos) y se recolectaron 7 frutos de manera sistemática. Los frutos fueron llevados al laboratorio para recontar sus semillas y medir su viabilidad. A continuación, se midió la cobertura de arbusto en $1m^2$ alrededor de la planta, y se recontó el número plántulas en esta área. Finalmente, la distancia de cada ramet a otro ramet con un genotipo diferente fue establecida una vez obtenidos los datos de marcadores de microsatélites.

Los datos recopilados permitieron analizar: (1) la diversidad de clones y genética, (2) la estructura genética espacial de los clones, y (3) los factores genéticos y ambientales relacionados con la reproducción de la especie. Los resultados de este trabajo están recogidos en el capítulo 3.

Germinación de semillas

La viabilidad y germinabilidad de las semillas de *Pseudomisopates rivas-martinezii* fue estudiada tomando semillas de tres poblaciones de la especie: La Serrota, Los Conventos y La Garganta Lóbrega. Cada tratamiento contuvo 5 réplicas con 25 semillas. Para desentrañar los factores que determinan las germinación de las semillas: (1) se midió la viabilidad de las semillas de las tres poblaciones, (2) se exploró la dormición de semillas mediante tratamientos de estratificación en frío y adición de giberelinas, y (3) los efectos directos e indirectos del fuego fueron investigados mediante la aplicación de tratamientos de shock térmico, adición de cenizas y germinación en oscuridad. Los resultados de este trabajo están recogidos en el capítulo 4.

Sobre las especies de estudio: *Erysimum penyalarense*

E. Amat

Caracteres morfológicos. Planta perenne y policárpica, que consta de una roseta basal simple o ramificada. Indumento de pelos naviculares. Tallos floríferos 5-40 (50) cm –excuído el racimo-, más o menos ascendentes, de ordinario sin ramas lamas laterales. Hojas en rosetas bien definidas en los individuos de cepa congesta y más laxamente dispuestas en los de cepa difusa; hojas de 20-50 (60) x 2-10 (11) mm linear-lanceoladas a subespatuladas, con algún par de dientes en el margen o con éste entero; hojas inferiores más largamente pecioladas que las superiores, que son pecioladas o sésiles. Flores tetrámeras en racimos terminales ebracteados y corimbiformes en la antesis. Pedicelos erecto-patentes de 3,5-8 (11) mm. Sépalos erectos, los laterales casi siempre gibosos en la base, los medianos cumulados en el ápice. Pétalos 16-24 (26) x 4-8 mm, amarillos. Androceo tetradínamo. Nectarios laterales (2), que rodean la base de los estambres correspondientes. Frutos en silicua de 30-80 x (1,3) 1,5-2 mm, lineares, erecto patentes, pubescentes; estilo 3-5 (6) mm;

estigma subcapitado-bilobulado. Semillas de color amarillo-ocráceo uniseriadas, oblongas, aplanadas, a veces con una corta ala apical (Nieto 1996); 2n=48 (Polatschek 1979).

Problemática taxonómica. En 1880 este *Erysimum* del Sistema Central es identificado como *Erysimum ochroleucum* DC. por Willkomm. En 1923 Pau herborizó entre el Paular y la laguna de Peñalara un individuo más robusto que los de Peñalara, humildes y con margen poco dentado, para el que se propuso otra variedad, *E. ochroleucum* DC var. p*enyalarense* Pau. En 1963 Rivas Martínez propone su tratamiento como subespecie, pasando esta a ser *E. ochroleucum* DC subsp. p*enyalarensis* (Pau) Rivas-Martínez y en 1967 lo adscribe a *E. decumbens* (Schleich. Ex Willd.) Dennst. subsp. penyalarensis (Pau) Rivas-Mart. En 1979 Polatschek le otorga valor de especie, pasando a denominarla *E. penyalarensis* (Pau) Polatschek. En 1991, a partir de los trabajos realizados para *Flora Iberica*, Nieto Feliner adopta una clasificación atendiendo a las formas vitales de los *Erysimum*; incluyendo a *E. penyalarense* en el grupo de *E. duriaei*, inclinándose por una coespecificidad de las poblaciones de *E. gorbeanum* del Sistema Ibérico y vascas, poniendo de manifiesto la necesidad de estudios que puedan apoyar su reconocimiento taxonómico. En 1998 López González vuelve a la clasificación tradicional recuperando como nombre válido *Erysimum humile* subsp. *penyalarense* (Pau) Rivas Mart. ex G. López. Dicho autor se basa en el estudio de *Cheiranthus ochroleucus* Haller f. ex. Schleich., basiónimo de *E. ochroleucum* DC. al que estaban supeditadas las plantas de Peñalara. Éste binomen data de 1806, por lo que es prioritario a *Cheirantus decumbens* Schleich. Ex Willd. de 1809. Finalmente, en 2004 López González y Garmendia rectifican sobre el nombre válido para la especie; defienden que *Cheiranthus ochroleucus* Haller fil. ex Schleich., basiónimo de la combinación *E. ochroleucum* (Haller fil. ex Schleich.) DC., es un nombre válidamente publicado, prioritario sobre *Erysimum humile* Pers. Ante la imposibilidad de diferenciar morfológicamente *E. ochroleucum* del grupo de táxones ibéricos relacionados con *E. duriaei* Boiss., proponen que se retome como válida la combinación *Erysimum ochroleucum* subsp. *penyalarense* (Pau) Rivas Mart. Toda esta controversia pone de manifiesto la dificultad de identificación de la especie frente a otras próximas, y la necesidad de discernir su validez taxonómica. Trabajos filogenéticos en curso (Universidad de Granada) ponen de manifiesto la gran diversificación del género *Erysimum*, como muestra de su reciente diferenciación y proceso de especiación. Los datos arrojados por los marcadores moleculares trnF-L (región tabA-D), ndhF, ITS1 e ITS2, descartan la monofilia del grupo *duriaei* en el que tradicionalmente había sido incluida. Por este motivo se puede ya afirmar que *E. penyalarense* no estaría directamente emparentado con *E. ochroleucum*, por lo que debe considerarse como especie con entidad propia y no como subespecie de este otro taxon.

Hábitat y distribución. Su hábitat son los pastizales psicroxerófilos de alta montaña (*Minuartio-Festucion curvifoliae* Rivas-Martínez 1964 corr. Rivas-Martínez, Fernández-González & Loidi 1999). Su distribución conocida se limita a dos poblaciones de la Sierra de Guadarrama (Figura 5). En una revisión reciente, la localidad clásica, situada en el macizo de Peñalara, parece haber desaparecido o bien estaría compuesta de escasos ejemplares que no se han vuelto a encontrar; mientras que, la población de la Cuerda Larga (Figura 6) se considera en aparente buen estado (Martínez *et al*. 2004).

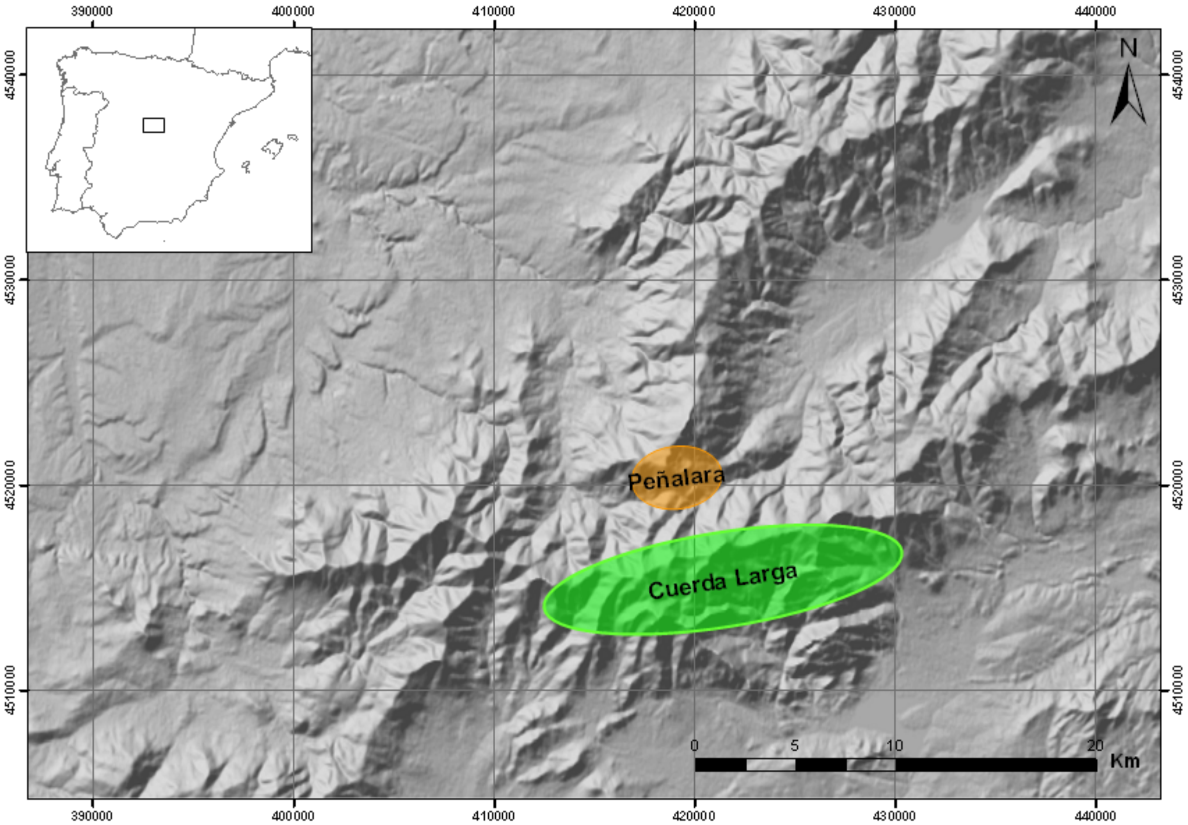


Figura 5. Mapa de localidades conocidas de *Erysimum penyalarense* en la Sierra de Guadarrama al comienzo del presente proyecto. En verde las poblaciones actuales y en naranjas las poblaciones extintas.

Figura 6. Imagen de la población de *Erysimum penyalarense* en la Bola del Mundo.

Estado de conservación. *Erysimum penyalarense* es una especie amenazada de extinción, clasificada en la categoría EN - En Peligro de Extinción - mediante los criterios de la Unión Internacional para la Conservación de la Naturaleza (UICN) e incluida en la Lista Roja de la Flora Vascular Española (Martínez *et al.* 2004). Se considera a una especie En Peligro de Extinción cuando no está en Peligro Crítico pero está enfrentando un riesgo muy alto de extinción en estado silvestre a medio plazo. Atendiendo a los conocimientos sobre la especie, dicha clasificación fue fundada en el criterio B, siendo estos dos de los cinco criterios posibles según la UICN. De forma que, la clasificación propuesta para este especie es EN B2 ab(iii,iv) c(i), que pasamos a detallar a continuación.

Criterio B2 ab(iii,iv) c(i)

El área de ocupación total de la especie medida en la única población conocida de la especie es inferior a los 100 Km^2 que el criterio B2 propone para taxones "En Peligro de Extinción". En cuanto a los subcriterios, se considera que existen no más de 5 poblaciones, en las que se ha observado declinación continua en el área, extensión o calidad del hábitat, y en el número de localidades o subpoblaciones. Esto ha sido debido a la presunta extinción de la localidad clásica, que ha dado lugar a una fluctuación en la extensión de la presencia de la especie en las últimas décadas.

En cualquier caso, de nuevo, la correcta aplicación de los criterios UICN requiere el seguimiento de variables poblacionales (de abundancia y distribución de la especie), a lo largo de un período razonable de tiempo.

Importancia para la conservación. A pesar de que la taxonomía se ha considerado el primer paso a la hora de establecer políticas de conservación eficientes (Leadlay & Jury 2006), ésta constituye un punto de inicio que debe completarse con resultados filogenéticos, genéticos y funcionales (Humphries *et al.* 1995). Si bien la posición taxonómica de *Erysimum penyalarense* ha resultado incierta durante mucho tiempo, su importancia para la conservación reside en el mantenimiento de sus procesos evolutivos y ecológicos. En primer lugar, el aislamiento geográfico de esta especie con respecto a sus parientes más cercanos, su diversidad genética y adaptaciones locales han dado lugar a una especie divergente con valor propio. En segundo lugar, *Erysimum penyalarense* cuenta con un importante papel en la interacción entre plantas y polinizadores debido a la diversidad de la comunidad de polinizadores de esta especie generalista (Amat *et al.*, observación personal). En consecuencia, su conservación cobra especial relevancia debido a sus efectos directos e indirectos sobre la biodiversidad y la conservación de interacciones ecológicas en la Sierra de Guadarrama, uno de los territorios con mayor presión humana de nuestra geografía.

Metodología

En este apartado se describen el área de estudio y el esquema general de los muestreos realizados. No obstante, la metodología específica utilizada para la consecución de cada objetivo es proporcionada con mayor detalle en el apartado de Material y Métodos del capítulo correspondiente.

El área de estudio. El área de estudio se limita a su única población existente, que se extiende, como ya hemos dicho, por las zonas de pastizal psicroxerófilo del macizo de la Cuerda Larga, enmarcadas en los cuadrantes delimitado por las coordenadas UTM (proyección Datum European 1950, Hemisferio Norte, Huso 30) 30T VL1613 (extremo SW), 30T VL1619 (extremo NW), 30T VL2613 (extremo SE) 30T VL2619 (extremo NE).

El área forma parte del actual *Parque Regional de la Cuenca Alta del Manzanares*, que será incluido en el futuro *Parque Nacional de las Cumbres de la Sierra de Guadarrama*, además de formar parte del Lugar de Interés Comunitario *Cuenca del Río Manzanares*, incluido en la Red *Natura*, y haber sido nombrado Reserva de la Biosfera en febrero de 1993.

Diseño experimental. El área de ocupación de la especie fue delimitada utilizando fotografías aéreas a la vez que se recorría la zona a pie para determinar aquellas zonas donde la especie no estaba presente. A continuación se realizó un muestreo aleatorio simple en dicha área, seleccionándose 80 parcelas consistentes en cuadrados de muestreo de 1m^2 que fueron marcados de forma permanente para su monitorización (Figura 7). En dichas parcelas se mapearon los individuos presentes atendiendo a su estadio vital (plántula, adulto no reproductor y adulto reproductor), los cuales fueron seguidos durante las temporadas de 2006 a 2008. A lo largo de estos años, se tomaron también datos de producción de frutos, a partir de los cuales se estimó la producción de semillas.

A continuación se registraron una serie de variables para el estudio del microhábitat: coberturas de herbáceas, leñosas, musgos y líquenes, suelo desnudo y rocas. Así mismo se registró la riqueza y cobertura de las especies acompañantes. Además se tomaron variables de presencia-ausencia de perturbación debida a actividad antrópica y animal, así como una serie de variables ambientales (altitud, pendiente y orientación).

Distribución y abundancia

A partir de dichos datos se estimó la abundancia de *Erysimum penyalarense* utilizando el descriptor estadístico más adecuado, se estudiaron las características de su hábitat y la diversidad de la vegetación acompañante, se estableció una correspondencia entre las especies que con mayor probabilidad aparecen junto a la especie de estudio, se estudió la distribución espacial de la especie, y se exploraron los factores que explican su distribución y abundancia. Los resultados de estos análisis están recogidos en el capítulo 5.


E. Amat

Figura 7. Cuadrado de muestreo en la zona de estudio.

Germinación de semillas

Se llevaron a cabo experimentos de germinación en el campo, utilizando para ello las parcelas con presencia de plantas. Con el fin de explorar los factores que intervienen en la germinación y establecimiento temprano de las plántulas, los resultados de estos experimentos fueron correlacionados con las variables ambientales medidas en cada parcela. Los resultados de estos experimentos están recogidos en el capítulo 6.

3. Justifiación y enfoque de la Memoria Doctoral

3. Justificación y enfoque de la Memoria Doctoral

Las especies elegidas para el presente estudio fueron *Pseudomisopates rivas-martinezii* y *Erysimum penyalarense.* El motivo de dicha elección es que, a nuestro juicio, ambas especies constituyen buenos representantes de los endemismos amenazados de la montaña mediterránea, englobados en el marco biogeográfico común de la Cordillera Carpetana. Además el bajo número de endemismos (c. 19) presentes en un sistema aislado como es el

Sistema Central, en relación a otras montañas ibéricas (Vargas & García 2008), hace necesario el estudio de estos dos táxones por sí mismos. El hecho de que pertenezcan a dos niveles taxonómicos diferentes confiere mayor interés al estudio, ya que esto podría estar reflejando un problemática histórica diferente, una más reciente y otra más antigua. En concreto, estos dos niveles de diferenciación sugieren un origen diferente en el tiempo, pero con un escenario geográfico similar. Adicionalmente, resulta interesante abordar el estudio de una especie presuntamente especialista y con una reproducción mixta (vegetativa y sexual) como es el caso de *P. rivas-martinezii*, frente al de una especie presuntamente generalista como *E. penyalarense*. La diferente biología de ambas especies planteaba el reto de abordar el estudio de cada una de ellas de una manera muy diferente, lo cual contribuye a enriquecer la presente memoria doctoral a través de las distintas estrategias y metodologías empleadas para adaptarse a estas peculiaridades.

Para dotar de una mayor coherencia a la presente memoria doctoral, esta ha sido dividida en dos secciones atendiendo a las dos especies de estudio. Dichas secciones abordan el diagnóstico de sus actuales causas de amenaza, versando sobre aquellos aspectos de la biología de poblaciones de las especies que permiten ilustrar la situación actual de amenaza de las especies estudiadas y aportar así directrices sobre las vías de actuación para su conservación. Ante la imposibilidad de seguir un procedimiento de estudio paralelo para las dos especies, dado lo dispar de su naturaleza, se centró el estudio en aquellos aspectos y factores que parecían críticos para el conocimiento y conservación de cada especie. Con este fin, se adoptaron las herramientas de análisis más apropiadas en cada caso: biología reproductiva, dinámica poblacional, modelización de factores ecológicos explicativos, y genética de poblaciones.

4. Objetivos concretos

Los objetivos concretos, planteados para dar respuesta a la hipótesis general, se establecieron atendiendo a las características de cada sistema de estudio y su problemática de conservación.

Pseudomisopates rivas-martinezii

La problemática de esta especie giraba entorno a la baja viabilidad de sus semillas y los objetivos de investigación se centraron en desentrañar dicha cuestión, como se detalla:

1. Estudiar el sistema reproductivo de la especie con el fin de determinar el grado de independencia en su reproducción sexual.
2. Estudiar la comunidad de visitantes florales para establecer el grado de especialización reproductiva de la especie.
3. Determinar la posible implicación de la limitación de polen, tanto cualitativa como cuantitativa, en la limitada producción de semillas viables.
4. Establecer la estructura genética espacial de los clones para determinar su distribución en el espacio y el alcance de la reproducción vegetativa en la especie.
5. Explorar los factores que promueven la reproducción vegetativa y los efectos de esta en la diversidad genética y la eficacia biológica.
6. Estudiar las condiciones que favorecen la germinación de semillas y en especial su posible adaptación al fuego.

Erysimum penyalarense

La problemática de esta especie se centraba en los factores que habrían llevado a la reducción de su distribución y los objetivos de la investigación se fueron perfilando como sigue:

7. Describir el patrón de distribución espacial de la especie y estimar su abundancia, con el fin de proporcionar una medida más precisa de su nivel de presencia en la Sierra de Guadarrama.
8. Explorar los factores ecológicos que determinan la distribución y abundancia de la especie dentro de su área de ocupación, para contribuir al conocimiento de los requerimientos ecológicos de la especie y facilitar así la adopción de medidas para su conservación.
9. Estudiar los factores que condicionan etapas vitales en la supervivencia de una especie amenazada, como son la germinación de semillas y el establecimiento temprano de plántulas.

5. Contenido de la Memoria Doctoral

5. Contenido de la Memoria Doctoral

La primera sección abarca los capítulos 1 al 3 y está dedicada al estudio de *Pseudomisopates rivas-martinezii*; por su parte, el estudio de *Erysimum penyalarense* se recoge en los capítulos 4 y 5.

El capítulo 1 versa sobre la determinación del sistema reproductivo de *P. rivas-martinezii* (objetivo 1) y las causas de la limitada producción de semillas viables. Para ello se identificó, en primer lugar, el conjunto de visitantes florales (objetivo 2), determinando la abundancia, frecuencia y comportamiento de sus visitas. En segundo lugar, se investigaron los efectos de la limitación de polen (objetivo 3), tanto cuantitativa (limitación de polinizadores y disponibilidad de individuos compatibles), como cualitativa (auto-compatibilidad y depresión por endogamia) en la producción de frutos, semillas y semillas viables.

El capítulo 2 examina las consecuencias - ventajas y desventajas - del extenso crecimiento clónico sobre la eficiencia biológica de *P. rivas-martinezii*, testando la hipótesis de que los bajos niveles de reproducción sexual observados en esta especie autoincompatible pueden deberse al intenso crecimiento vegetativo y otros factores ambientales. Para ello se determinan el nivel de reproducción asexual *vs.* el sexual, se cuantifican los efectos de la clonalidad en la diversidad genética, en la estructura genética espacial y la capacidad de reproducción sexual, a la vez que se determinan los factores ambientales promotores de la clonalidad (objetivos 4 y 5).

El capítulo 3 se centra en el conocimiento de los factores que favorecen la germinación de semillas de *P. rivas-martinezii* (objetivo 6). El estudio del nivel de dormición, a través de la viabilidad de semillas y bajo distintos tratamientos de germinación de semillas, que estimaron los efectos directos e indirectos del fuego, permite conocer las mejores condiciones para la regeneración de la especie en su hábitat natural.

El capítulo 4 describe el patrón de distribución espacial de la especie y la abundancia de *E. penyalarense* dentro de su área de ocupación efectiva (objetivo 7), y lo explica a través de los factores ecológicos que lo determinan (objetivo 8). El estudio observacional aquí presentado tiene por objeto modelizar los factores que explican la abundancia de individuos, centrándose en explorar el impacto que la perturbación humana local produce en la única población existente de la especie.

El capítulo 5 investiga experimentalmente los factores que el establecimiento temprano de plántulas de *E. penyalarense* (objetivo 9), partiendo de la hipótesis de que este puede depender de interacciones intraespecíficas (con semillas y adultos), interespecíficas (con herbáceas, leñosas y animales), e interacciones con factores abióticos de su microhábitat.

A continuación se proporciona una discusión conjunta de los resultados obtenidos en todos los capítulos anteriores, ya que la presentación por artículos científicos puede dificultar el nexo de unión entre los distintos fragmentos del proyecto de tesis doctoral. Se analiza también el grado de cumplimiento de los objetivos concretos previamente establecidos, se proponen posibles líneas de trabajo futuras y se realizan recomendaciones de gestión a la luz de los resultados obtenidos.

Finalmente, se recogen las principales conclusiones que se extraen del trabajo realizado en cuanto a las causas de amenaza de las especies de estudio y las posibles medidas de gestión que podrían adoptarse teniendo en cuenta su biología.

6. Conclusiones

Sobre *Pseudomisopates rivas-martinezii:*

1) Es una especie predominantemente autoincompatible, que sufre de limitación de polen cuantitativa, debido a la baja frecuencia de visitas de polinizadores, y cualitativa, debido a la reducida disponibilidad de individuos compatibles para la reproducción.

2) La limitación de polen en su hábitat natural está motivada por la fuerte estructura genética espacial de sus clones, que dificulta el entrecruzamiento entre individuos.

3) La fuerte clonalidad de la especie se pone de manifiesto a través de una baja riqueza genotípica (PD = 0,23), si bien su alta diversidad genética (He = 0,42) apunta a una alta persistencia de *genets*, que estarían manteniendo la población a pesar del escaso reclutamiento de nuevos individuos.

4) Los factores ambientales que favorecen la reproducción vegetativa frente a la reproducción sexual, son el cierre de la vegetación arbustiva y la herbivoría. Ambos factores reducirían significativamente el nivel de floración, a la vez que promoverían el crecimiento de los clones, dificultando cada vez más el acceso a individuos para la reproducción.

5) Los requisitos para la germinación son en primer lugar un período de frío, seguido de los efectos directos e indirectos del fuego, en segundo lugar. Entre los factores directos del fuego destaca el estímulo químico que ejerce la presencia de ceniza en el medio; mientras que, entre los efectos indirectos se encuentran el aclarado de la vegetación arbustiva que favorece la germinación, y provoca a su vez el intenso rebrote de la especie, promoviendo así una vigorosa fructificación.

6) Su sistema reproductivo mixto le permite adaptarse a la estocasticidad ambiental, a la espera de una perturbación provocada preferiblemente por el fuego, en un entorno de matorrales pirófilos y que tradicionalmente han sido gestionados mediante quemas.

7) Las medidas de conservación para *Pseudomisopates rivas-martinezii* deben ir encaminadas a respetar las perturbaciones naturales de su ecosistema y fomentar el aclarado de la vegetación, así como a controlar la presión de herbívoros, con el fin de permitir la regeneración de la especie. La aplicación de quemas controladas sólo debe llevarse a cabo después de un estricto análisis de la frecuencia e intensidad requeridas.

Sobre *Erysimum penyalarense:*

8) Se distribuye por los pastizales alpinos de *Festuca curvifolia* de la Cuerda Larga (Sierra de Guadarrama). Desciende por las zonas más aclaradas de la cara norte, evitando zonas con densas formaciones de piornal, cervunales, canchales y gleras, así como los rincones más húmedos donde abundan especies como *Festuca iberica*, *Campanula herminii* o *Thymus praecox.*

9) El estudio de la distribución y abundancia de la población de la Cuerda Larga en 2006 indicó que esta se presenta un área de ocupación efectiva de 7,09 Km^2 y que estaba compuesta por al menos 11 millones de individuos, en contraposición a la estimación de trabajos previos que apuntaban a un área superior, pero con una abundancia muy inferior.

10) *Erysimum penyalarense* no es una buena competidora, por lo que se beneficia de la perturbación y es facilitada por especies arbustivas como *Cytisus oromediterraneus* en sus primeras fases de establecimiento. Si bien la actividad humana puede favorecer su expansión a través de la creación de huecos en el piornal, los daños indirectos provocados por ungulados y visitantes resultan negativos para su supervivencia. Por lo tanto, el efecto neto de la actividad humana depende del equilibrio de las distintas actividades desarrolladas en su área de distribución.

11) Las medidas de conservación en *Erysimum penyalarense* deben ir encaminadas a garantizar un nivel de perturbación intermedia, que permita la presencia de zonas en los primeros estadios de sucesión tanto en la Cuerda Larga como en la localidad clásica del macizo de Peñalara. La presión de herbívoros y visitantes en la zona deben ser controlados, y sus efectos deberían ser objeto de un estudio más detallado.

12) Se recomienda la reintroducción de la especie en el macizo de Peñalara con material representativo de la población de Cuerda Larga, siguiendo un muestreo como el realizado en el presente trabajo.

Conclusión general*:*

La perturbación es un proceso natural que forma parte de los ecosistemas mediterráneos de montaña, por lo que muchas especies están adaptadas a ella, e incluso dependen de ella para su regeneración. Predecimos que éste podría ser el caso de muchas otras especies aún no estudiadas y dependientes de un cierto grado de perturbación para su supervivencia.

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Capítulo 1

Pollen quality limitation in the Iberian critically-endangered genus *Pseudomisopates* (Antirrhinaceae)

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Pollen quality limitation in the Iberian critically-endangered genus *Pseudomisopates* (Antirrhinaceae)

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Key words: conservation, mating system, pollen limitation, pollen quality, pollen quantity, *Pseudomisopates rivas-martinezii*.

Abstract

Pollen limitation occurs when sexual reproduction is decreased due to inadequate pollen receipt. Limitation is usually associated with the quality and the quantity of pollen; still, most studies do not discriminate between the two. We used hand-pollination experiments and observations of floral visitors to determine the mating system and limitations to seed production of the Spanish endangered species *Pseudomisopates rivas-martinezii*. We tested for agamospermy, autogamy, obligated autogamy, cross-pollination and supplementation. The response variables considered were fruit set, seed set and viable seed set. Previous studies indicated that: (i) the species exhibits extensive clonal growth, (ii) plants flower profusely in summer, (iii) seeds showed to be highly unviable, and (iv) no seedlings were observed in the field. We found that *P. rivas-martinezii* is predominantly self-incompatible, has an unexpected generalized pollination system considering its occluded corolla, and is limited by qualitative rather than quantitative pollen limitation. These results indicate that, in addition to other presumed environmental factors, the low seed viability of this self-incompatible species is due to pollen quality limitation as a result of reduced mate availability.

Introduction

Pollen limitation, a decrease in potential plant reproduction due to inadequate pollen transfer (Larson & Barrett 2000; Ashman *et al.* 2004; García-Camacho & Totland 2009), may enhance plant population decline (Ashman *et al.* 2004; Ward & Jonson 2005). Recent reviews have shown that pollen limitation is a common phenomenon in plants (Knight *et al.* 2005). Different analysis conducted on seed production studies reported that about 63% of the species investigated exhibited pollen limitation depending on sites or years (Burd 1994; Knight *et al.* 2005).

Pollen limitation in insect-pollinated species comprises two components: low pollen quantity (derived from visitation rate and efficiency) and low pollen quality (affected by self-fertilization, mating between genetically incompatible plants and the degree of relatedness between parents through inbreeding depression). Qualitative factors probably limit seed production as strongly as quantitative factors, since pollen vectors typically disperse pollen locally (Harder & Aizen 2010). However, many pollen supplementation experiments have been unable to distinguish between these two components (Aizen & Harder 2007).

Main factors provoking pollen limitation are: (1) low pollinator visiting frequency (Totland & Sottocornola 2001; Hegland & Totland 2008); (2) low pollinator abundance; (3) the dominance of inefficient pollinators (Elle & Carney 2003); (4) an impoverishment or shift of the pollinator assemblage when different pollinators have complementary effects on plant fitness (Klein *et al.* 2003; Gómez *et al.* 2007; González-Varo *et al.* 2009); (5) pollinators discrimination against particular plant species or flower traits (Fenster *et al.* 2004), such as floral design and display (Thompson 2001); (6) the size and density of the population which affects pollinator attraction and the genetic structure of the population which influences compatible mate availability (Hamrick *et al.* 1991), and (7) limited pollen availability, low pollen-tube survival or zygote death (Busch & Schoen 2008).

The degree of reliance upon pollinators for reproduction may be vital for plant reproductive success. Under pollinator scarcity or low mate availability, self pollen is unable to produce seeds in obligate outcrossers; therefore plants may not yield enough seeds to maintain a viable population. Small populations of many self-incompatible species are threatened for this reason (DeMauro 1993; Byers 1995). Furthermore, species that reproduce clonally as well as sexually may show further reduced sexual reproduction, due to the extension of clones. Under the same circumstances of mate availability, selfers may reach higher seed production (Totland & Schulte-Herbrüggen 2003) and suffer lower inbreeding than outcrossers (Husband & Schemske 1996; Charlesworth & Charlesworth 1997). However, the reduction in offspring number and quality due to lowered mate availability or mating among close relatives can be compensated by higher quality offspring promoted by outcrossing (Vallejo-Marín & Uyenoyama 2004) and the reproductive compensation over multiple breeding seasons in perennial outcrossers (Goodwillie 1999; Larson & Barrett 2000).

In this study we test the occurrence of pollen limitation in *Pseudomisopates rivas-martinezii* (Antirrhinaceae). This species is catalogued as Critically Endangered following the IUCN criteria in the Red List of Spanish Vascular Flora (Martínez Rodríguez *et al.* 2004) and is one of the 19 monotypic genera endemic to Spain (Nieto Feliner 1999) of which 7 are endangered. It shows extensive clonal growth and although plants flower profusely, seeds at many sites seemed to be unviable, as shown by preliminary germinability tests, and no seedlings have been observed in the field (Martínez Rodríguez *et al*. 2004). The specific objectives of this study were to determine the mating system and evaluate the limitations to seed production in *P. rivas-martinezii*. These were assessed by (1) characterizing floral visitors by means of surveys to determine their abundance, frequency of visits and behaviour; (2) investigating the effects of pollen limitation, in both quantity (pollinator limitation and mate availability) and quality (self-compatibility and inbreeding depression) on fruit set, seed set and viable seed set by performing a series of pollination treatments.

Materials and Methods

Study species

Pseudomisopates rivas-martinezii (Antirrhinaceae) is an endangered species and genus endemic to the *Gredos* region, in the Spanish Central Mountain System. Within this region, it is distributed in two main areas separated by 20 Km, one in *Gredos* Mountain Range and the other in *La Serrota* Mountain Range, comprising seven and three known populations respectively (Vargas & García 2008). The species occurs from 1,400 to 1,990 m.a.s.l., inhabiting clearings within *Cytisus oromediterraneus* shrublands. Plants reproduce vegetatively by an underground stolon network, with connections between ramets that can span over one metre. Flowers are apparently occluded (personate) and bloom during July and August. As considerable strength is required for pollinators to enter occluded flowers of the other closely related Antirrhinaceae species, it has been historically hypothesized that bees are the only pollinators (Müller 1929; Sutton 1988), as they exhibit the most suitable body morphology and strength (Gottlieb *et al.* 2005; Vargas *et al.* 2010). Fruiting occurs during August and September. Fruits can contain between 1 and 24 seeds, which are dispersed by barochory.

Mating system

Pollination treatments were performed in *La Serrota* population (one of the three populations located in *La Serrota* Mountain Range, Cepeda de la Mora, Ávila), during August 2007 and 2008. The occupancy area of this population is approximately 14,400 m^2, with a ramet density of 27 ± 4.4 ramets per square metre. The closest populations are located 1 and 2.5 kilometres apart, and no isolated individuals have been found in between. A former study was conducted in 2007 in which six pollination treatments were used to determine the mating system of *P. rivas-martinezii* and the possible reproductive limitations due to pollen quality and quantity. These treatments tested for: (1) *agamospermy*, by emasculating and bagging flowers; (2) *spontaneous autogamy*, by bagging flowers; (3) *obligated autogamy*, by bagging flowers and hand-pollinating them with pollen from the same flower; (4) *cross-pollination*, by bagging flowers and hand-pollinating them with pollen from *c.* 100 plants from *Lóbrega* population (*Gredos* Mountain Range, Candeleda, Ávila), located 20 Km apart; (5) *pollen supplementation*, by supplementing unbagged flowers with pollen from *c.* 100 plants located 5-10 m away, and (6) *control,* flowers left open for natural pollination. Twenty flowers from ten plants (two per plant) were selected for each treatment. In the summer 2008 attention was focused only on those treatments that had a clear effect the previous year, namely: *obligated autogamy*, *cross-pollination*, *pollen supplementation* and *control.* Sample size was increased to thirty flowers from thirty plants per treatment (one per plant) to obtain a more robust estimation. The components of reproductive success used to determine the mating system and test pollen limitation were: (1) fruit set, which was the ratio of fruits to treated flowers; (2) seed set, which was the ratio of seeds to the average number of ovules, and (3) viable seed set, which was the ratio of viable seeds to the average number of ovules. The number of ovules (n=22±3) was obtained separately from a sample of 20 flowers from *La Serrota*. Seed viability and hence an estimate of seed germination, was assessed in 2008 by means of a Tetrazolium test optimized for the species, following the Tetrazolium Testing Handbook (Peters 2000). Seeds were imbibed in water overnight at 21°C, then slightly cut and incubated in 1%TZ at 27 °C for 72 hours. Seeds were finally bisected and viability was accounted for by observing the red colouring of living tissues.

Floral visitor assemblage

Surveys were carried out in five patches in *Los Conventos* population (*Gredos* Mountain Range, Hoyos del Espino, Ávila) during the flowering peak in August 2009. The occupancy area of this population is approximately 12,100 m^2. The goal was to describe the type of insects visiting *P. rivas-martinezii* flowers, rather than to infer any relationship between floral visitor diversity and abundance and pollen limitation intensity. All surveys were performed under similar favourable conditions (sun and no wind). Within each patch 10 plants were chosen for each survey, in which the total number of open flowers and the number of flowers visited during 10-min periods were counted. Surveys were done between 10h40 and 18h35, totalling 2,560 minutes. Any insect observed making contact with anthers or stigma was recorded.

Data analysis

Differences between treatments were analysed using Generalized Linear Models (GLM), including treatment and year as factors. First, the effect on fruit set, seed set and viable seed set was assessed by fitting these variables to a binomial distribution, using *logit* as the link function. The interaction *treatment*year* was not significant for any of these models, therefore data of both years were analysed together. Second, pairwise differences were assessed using *post hoc* likelihood ratio test based on Chi-square distributions. All analyses were performed using JMP version 7.

Two aspects of the floral visitor assemblage visiting *P. rivas-martinezii* flowers are considered in this study: abundance and diversity (Magurran 2004). Abundance of floral visitors was estimated by standardizing the number of visits per open flower and time unit (flowers visited per hour). Floral visitor diversity was assessed by calculating richness (S_{obs}), diversity, evenness, and dominance. S_{obs} was the number of species found visiting flowers in each patch; two asymptotic richness estimates, the incidence coverage estimator (S_{ICE}) and the Michaelis–Menten index (S_{MM}) were calculated using EstimateS software (Colwell 2005). Dominance was calculated as the relative abundance of the most abundant visiting species. Diversity was calculated by the Shannon–Wiener index and Hulbert's PIE (Colwell 2005) by a randomization process using EcoSim (Gotelli & Entsminger 2004). Among-patch differences in floral visitor abundance and composition was tested using a permutational multivariate analysis of variance, PERMANOVA version 1.6 (Anderson 2001), on the basis of Bray-Curtis dissimilarities.

Results

Mating system

The experiments affected all fecundity estimates consistently. There were between-treatment differences in fruit set ($\chi^2 = 39.975$, 5 d.f., $p < 0.001$), seed set ($\chi^2 = 490.799$, 5 d.f., $p < 0.001$), and viable seed set ($\chi^2 = 81.328$, 3 d.f., $p < 0.001$) (Fig. 1, Table 1).

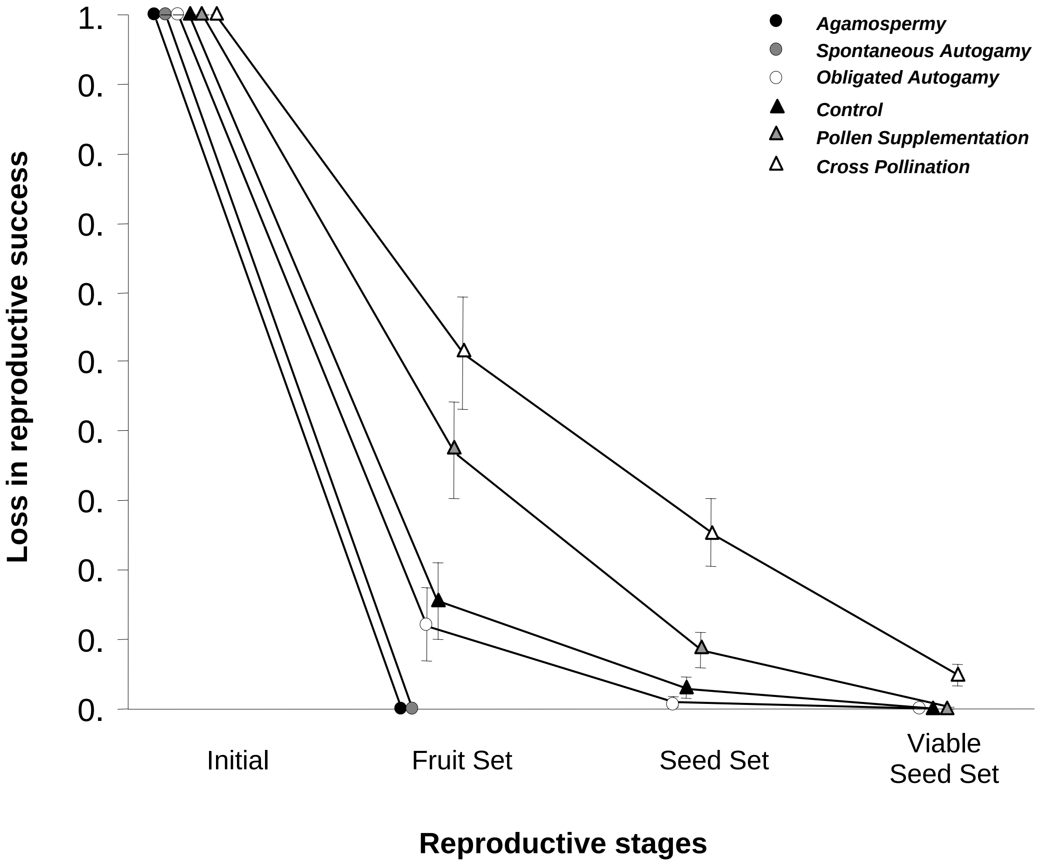

Fig.1. Reproductive success at the different reproductive stages in *Pseudomisopates* as a result of pollination treatments in 2007 and 2008. *Autogamy* and *spontaneous autogamy* are only given for 2007. *Obligated autogamy*, *cross-polination*, *pollen supplementation* and *control* are averages across the two years.

Flowers treated for *agamospermy* and *spontaneous autogamy* in 2007 produced no fruits (Fig. 1, Table 1), being significantly different to all other treatments (Table 1). Flowers from *obligated autogamy* showed very low fecundity, producing 22% less fruits and 44% less seeds than controls, as well as no viable seeds (Fig. 1, Table S1). These differences were only significant in seed set (Table 1).

Cross-pollinated flowers showed the highest fecundity, producing 53% more fruits and 46% more seeds than control flowers (Fig. 1, Table S1). Although no differences were observed with *pollen supplementation* in fruit set, they differed in further seed stages (Table 1). Significant differences between *cross-pollination* and all other treatments were found at all stages (Table 1).

	Agamospermy	**Spontaneous autogamy**	**Obligated autogamy**	**Cross pollination**	**Pollen supplementation**	**Control**
Fruit set						
Agamospermy						
Spontaneous autogamy	0					
Obligated autogamy	4.188*	4.188*				
Cross pollination	21.522***	21.522***	14.929***			
Pollen supplementation	14.464***	14.464***	7.144**	2.093		
Control	6.245**	6.245**	0.465	11.192***	4.295*	
Seed set						
Agamospermy						
Spontaneous autogamy	0					
Obligated autogamy	7.983**	5.535*				
Cross pollination	202.425***	141.750***	274.526***			
Pollen supplementation	66.396***	45.697***	66.638***	101.342***		
Control	21.935***	15.133***	8.464**	220.810***	32.115***	
Viable seed set						
Obligated autogamy						
Cross pollination			44.469***			
Pollen supplementation			1.387	36.905***		
Control			$1.926\cdot10^{-8}$	44.469***	1.387	

Significance levels indicated as *P < 0.05, **P < 0.01, ***P < 0.001

Table 1. Results of the *post hoc* Chi squared test comparing the effects of the pollination treatments on fruit set, seed set and viable seed set in *Pseudomisopates*.

Pollen supplemented flowers had higher fecundity than *control* flowers, producing 30% more fruits and 39% more seeds (Fig. 1, Table S1). However, in 2007 pollen-supplemented flowers produced a similar fruit set to the *control* treatment; whereas, in 2008 it showed a four-fold result compared to the *control* (Table S1). This difference was mainly due to the between-year variation in fruit set for the *control* treatment, which yielded a 68% lower fruit set the second year.

Floral visitor assemblage

Insects from 6 orders, 16 families and 26 species were recorded (Table 2). Most species belonged to the order Hymenoptera (12 species), although the most abundant species were Coleoptera. Excluding the three most dominant species (*Mylabris* sp., *Megachile* sp.1 and *Melighetes* sp.1), which comprised 63.7% of all visits, most species made less than 5% of visits. *Mylabris* sp. constituted 34.41% of visits, with an average visitation rate of $7.01 \cdot 10^{-4}$ visits per flower per hour and an average of 2.1 flowers visited per plant. The solitary bee *Megachile* sp.1 carried out 18.43% of visits, with an average visitation rate of $4.72 \cdot 10^{-5}$ visits per flower per hour and an average of 8 flowers visited per plant. *Melighetes* sp.1 carried out 10.91% of visits, with an average visitation rate of $2.39 \cdot 10^{-4}$ visits per flower per hour and an average of 1.5 flowers visited per plant.

On the day surveyed, the average number of flowers visited per plant was 2.54 ± 4.46, whereas the proportion of flowers visited per plant was 0.12 ± 0.19. The average number of flights per insect within a plant was 1.12 ± 2.10. Abundance, as shown by the visitation rate was 0.002 ± 1.16 visits per flower per hour. The observed species richness (S_{obs}) in the population was 26 ($CI_{95\%}$ = 20.8-31.19). The expected floral visitor richness according to asymptotic estimates was S_{ICE} = 41.54 and S_{MM}=40.6; these values suggest that floral visitor assemblages were richer than inferred from our sampling. Hulbert's PIE index was 0.83 for the population (83% probability of two randomly selected flower visitors belonging to different species) and Shannon-Wiener H was 2.28. Dominance was 0.70, with few species scoring a relative abundance higher than 5%.

Patches did not vary significantly in floral visitor abundance (Kruskal-Wallis, $\chi^2 = 3.5378$; d.f. = 4; p = 0.4721). Pollination assemblage composition varied significantly among patches (PERMANOVA, F = 2.2078, p = 0.01). Although differences were mainly due to slight variations in the abundance of *Mylabris* sp. and *Megachile* sp.1.

	Order	Family	Species	Total	% Visit
1	Coleoptera	Meloidae	*Mylabris* sp.	224	34.41
2		Mordellidae	Mordellidae	2	0.31
3		Nitidulidae	*Melighetes* sp. 1	71	10.91
4			*Melighetes* sp. 2	1	0.15
5	Diptera	Bombyliidae	*Hemipenthes* sp.	1	0.15
6		Scathophagidae	Scathophagidae	17	2.61
7		Syrphidae	*Eupeodes corollae*	3	0.46
8	Heteroptera	Miridae	*Hadrodemus* sp.	2	0.31
9	Hymenoptera	Apidae	*Anthidium* sp.	18	2.76
10			*Amegilla* sp.	13	2
11			*Anthophora* sp.	10	1.54
12			*Bombus pascuorum*	2	0.31
13		Chalcididae	Chalcididae	4	0.61
14		Formicidae	*Formica* sp.	2	0.31
15		Halictidae	*Halictus* sp.	28	4.3
16			*Lasioglossum* sp. 1	18	2.76
17			*Lasioglossum* sp. 2	14	2.15
18		Megachilidae	*Megachile* sp. 1	120	18.43
19			*Megachile* sp. 2	28	4.3
20			*Hoplitis* sp.	15	2.3
21	Lepidoptera	Hesperiidae	*Thymelicus* sp.	1	0.15
22		Lycaenidae	*Lampides boeticus*	4	0.61
23		Nymphalidae	*Melanargia lachesis*	25	3.84
24		Nymphalidae	*Erebia* sp.	2	0.31
25		Sphingidae	*Macroglossum stellatarum*	21	3.23
26	Thysanoptera	-	*Gen* sp.	5	0.77

Table.2. Floral visitors of Pseudomisopates at *Los Conventos* population.

Discussion

Self-incompatibility

Pollination experiments showed that *P. rivas-martinezii* is predominantly self-incompatible and depends on pollen vectors for sexual reproduction. Indeed, fecundity after self-pollination and agamospermy was much lower than after cross-pollination or open pollination, and yielded no viable seeds (Fig. 1, Table 1). This result has been previously reported on the closely related *Antirrhinum* (Antirrhinaceae), in which many species are considered to be self-incompatible (Tseng 1938; Sutton 1988) but show different levels of self compatibility (East 1940; Mateu-Andrés & Segarra-Moragues 2004; Carrió *et al.* 2009). The total absence of fruits produced by agamospermy and spontaneous autogamy indicates that, as in other Antirrhineae species (Sutton 1988), this species is not apomictic, nor does it have a mechanism for spontaneous self-pollination.

Pollen quantity and quality limitation

Seed production in *P. rivas-martinezii* appears to be limited by the quantity of pollen grains reaching stigmas. This self-incompatible species depends on pollinator visits and is therefore subject to pollen quantity limitation, which is herein supported by the fact that flowers supplemented with pollen from the same population set significantly more fruits and seeds than controls (Fig. 1, Table 1). Furthermore, our control results showed that fruit set varied between the two years (Table S1), indicating that pollen limitation fluctuates in time. The fourfold lower fruit set yielded by controls relative to supplementation in 2008 may suggest a decrease in number of pollinator visits, a lower load of compatible pollen than the previous year or less favourable environmental conditions. Moreover, the lower fruit set for most pollination treatments in 2008, indicates that environmental changes may be a major factor determining this fluctuation between the two years.

In addition, the low fecundity shown by self-pollinated flowers gives evidence for limited pollen quality. Flowers that were cross-pollinated with plants from a different population yielded the highest results (Fig. 1, Table 1). Differences between supplemented and out-crossed flowers were significant at the seed set and viable seed set stages, but not at the fruit set stage. These differences did not seem to come from bagging, since fruit set was equivalent and no apparent additional damage to flowers was observed on unbagged flowers. Also supplemented flowers yielded higher results than controls which were not bagged either. These findings suggest that the main factor explaining reduced sexual reproduction could be due to limited mate availability promoted by clonal growth in the study area, which leads to low quality pollen reaching stigmas in this predominantly self-incompatible species. Other self-incompatible species such as *Centaurea corymbosa* (Hardy *et al.* 2004) or *Maianthemum bifolium* (Honnay *et al.* 2006) have shown that viable seed set is limited by mate availability. Furthermore, it is very unlikely that *La Serrota* population could naturally receive pollen grains from surrounding populations. Therefore, this study provides experimental evidence of a small population of a clonally-reproducing, self-incompatible species, that suffers from inefficient pollination and is threatened with extinction, as are many others under these circumstances (Les *et al.* 1991; Silvertown 2008).

The low viable seed set (10%) yielded by potentially high quality pollen used in cross-pollination, indicates that there must be other factors limiting seed viability. These could include limited resources, resource allocation between vegetative and reproductive growth and accumulation of deleterious mutations. Comparisons with other species may help understand the influence of these factors, even if results for *P. rivas-martinezii* could be very different in the absence of genetic problems. In addition, most studies do not account for seed viability, but only fruit set or seed set. In this sense, seed set can reach up to 49% with cross-pollination in *P. rivas-martinezii*, which is equivalent to that shown by other outcrossing perennials (Wiens 1984). Although many outcrossing perennials have shown even lower seed sets: *Linaria vulgaris* (1.6%) (Saner *et al.* 1994); *Decendra candensis* (2-7%), *Erythronium albidum* (6-11%) (Schemske 1978) or *Genista versicolor* (25%) (Gómez & García 1997). In addition, seed viability is very variable across species, with *Linaria vulgaris* reaching up to 50% (Nadeau & King 1991) or *Veronica* spp. reaching up to 100% (Juan *et al.* 1995). Moreover, seed viability can also vary between years (Chambers 1989), and studies in progress have shown that this is the case in *P. rivas-martinezii* (Chapter 3). Therefore, although a 10% is a very low value of viable seed set, it might be enough for a species that produces thousands of flowers and can compensate seed production across years.

Pollinator limitation

Pollinator limitation may arise via low pollinator visitation rates and low pollinator efficiency (Gómez *et al.* 2007). Although an exhaustive survey was not performed for *La Serrota* population, a similar pollinator assemblage was observed, as expected from the equivalent ramet density and disposition of patches in both populations (Table S2). The visitation rate in *Los Conventos* population (0.002) was low compared to other specialized Iberian species, such as some *Antirrhinum* species which showed 0.15 to 0.31 visits per hour (Vargas *et al.* 2010). In contrast, the generalist *Erysimum mediohispanicum* showed values between 33.3 and 95.5 visits per hour depending on the population (Gómez *et al.* 2007).

About 41% of visits were carried out by bees, which are very effective pollinators (Dafni *et al.* 2005), but their visitation rate was extremely low (below 0.0002 visits per flower per hour). Among low efficiency pollinators, beetles carried out about 46% of all visits. This low efficiency was dramatically exemplified by *Mylabris* sp. (which damaged 3.22% of flowers) and a number of different unidentified and unrecorded insect larvae. Low efficiency pollinators were as abundant as efficient pollinators. Therefore, efficient pollinator abundance may swamp out negative fitness effects of inefficient pollinators (Lau & Galloway 2004). Nevertheless, floral herbivory on plant reproduction should be further investigated, for it could be a relevant factor reducing seed production and pollinator visitation rates (Sánchez-Lafuente 2007).

The diversity in morphology, size and behaviour of floral visitors suggested a generalized pollination system. This finding contradicts the previous idea that the occluded flower of *P. rivas-martinezii* would require specialized visitors as in other Antirrhineae species. For instance, in *Antirrhinum* species bees accounted for over 90% of visits (Vargas *et al.* 2010); pollinator assemblage of *Linaria aerugineae* was composed mainly of bees (Herrera *et al.* 2009), and these were the only pollinators recorded in *L. vulgaris* (Stout *et al.* 2000) and *L. lilacina* (Sánchez-Lafuente 2007). The diverse floral visitor assemblage of *P. rivas-martinezii* indicates that the corolla is not fully occluded and that a type of semi-generalist personate flower can be assumed. Although opening the corolla to a more generalized pollinator assemblage may increase visitor diversity, extreme generalization may decrease overall efficiency (Gómez *et al.* 2007; Perfectti *et al.* 2009). The question remains as to whether additional factors affect visitation rates given that *P. rivas-martinezii* received a lower pollinator rate than *Antirrhinum* despite its semi-occluded flower.

Implications for conservation

Self-incompatible species may reduce seed production due to low pollinator efficiency (Schemske & Horvitz 1984; Vaughton & Ramsey 2010), mate availability (Byers 1995; Campbell & Husband 2007; Busch & Schoen 2008) and stigma clogging with self pollen (Proctor *et al.* 1996). In this sense, *P. rivas-martinezii* yields a very low quantity of seeds by hand self-pollination compared to pollen supplementation and hand cross-pollination and should be unable to produce enough self-seeds to maintain a viable population. Furthermore, mate availability may be extremely lowered by clones colonizing large surfaces and forming populations with a single or very few genotypes in clonally-reproducing species (Charpentier *et al.* 2000). Studies in progress indicate that the spatial genetic structure created by clones in *P. rivas-martinezii* limits successful pollination (Chapter 2). Therefore, the balance and compensation across years between sexual and clonal reproduction will determine population viability, since it could be ultimately compromised by clonal reproduction alone.

It is important to conserve populations with high genetic diversity to allow successful cross pollination, increase the number of S alleles and allow adaptation to environmental stochasticity. Severe reductions in population size should be tackled by controlling excess grazing by large herbivores and maintaining habitat heterogeneity, with clearings that will allow the recovery and gene flow within populations. For this reason, reinforcements are not recommended before genetic data and knowledge on population dynamics are available for this regionally rare, but locally abundant species. Conservation measures should focus on understanding the factors that enhance one kind of reproduction or the other and evaluating whether present conditions of the plant are able to assure its sustainability in the long run.

Concluding remarks

In conclusion, the low sexual reproductive success in *P. rivas-martinezii* is mainly a consequence of low mate availability (pollen quality) and secondarily of insufficient pollination (pollen quantity). Environmental stochasticity and resource limitation are other undergoing factors, as shown by the low seed viability and the variation in treatments across years. Although *a priori* floral morphology suggested a more specialized pollination system, a more generalized system can be inferred from the ample floral visitor assemblage observed on actual surveys, rather than on pollination syndromes (Aigner 2004). A low visitation rate of relevant pollinators, due to the long distance flights required to reach compatible mates, may be behind the causes of the observed pollen limitation. Further spatial genetic analysis would reveal the underlying mating opportunities among genotypes.

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Appendix

Table. S1. Fruit set, seed set and viable seed set resulting from pollination treatments.

	Agamospermy	**Spontaneous autogamy**	**Obligated autogamy**			**Cross pollination**			**Pollen supplementation**			**Control**		
	2007	2007	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean	2007	2008	Mean
N	20	13	11	30		11	28		20	30		16	30	
Fruit set	0	0	0.182	0.100	0.141	0.545	0.500	0.523	0.300	0.400	0.350	0.313	0.100	0.207
Seed set	0	0	0.136	0.061	0.099	0.477	0.503	0.490	0.258	0.231	0.245	0.155	0.197	0.176
Viable seed set	-	-	-	0	0.000	-	0.097	0.097	-	0.004	0.004	-	0.000	0.000
No. of flowers[a]	20	20	11	30	41	11	28	39	20	30	50	16	30	46
No. of fruits	0	0	2	3	5	6	14	20	6	12	18	5	3	8
No. of seeds	0	0	6	4	10	63	155	218	34	61	95	17	13	30
No. viable seeds	-	-	-	0	0	-	30	30	-	1	1	-	0	0

[a] Initial number of flowers treated were 20 in 2007 and 30 in 2008. Flowers lost during the experiment are not included.

Table S2. List of floral visitors of *Pseudomisopates* for a *La Serrota* population. This pilot study includes 510 minutes of observation, from 50 plants located in 5 patches.

	Order	**Family**	**Species**	**Total**	**% Visit**
1	Coleoptera	Meloidae	*Mylabris* sp.	24	32.00
2		Nitidulidae	*Melighetes* sp.1	28	37.33
3			*Melighetes* sp.2	1	1.33
4	Diptera	Scathophagidae	*Scatophagidae*	3	4.00
5	Heteroptera	Miridae	*Hadrodemus*	2	2.67
6	Hymenoptera	Chalcididae	Chalcididae	7	9.33
7		Formicidae	*Formica* sp.	2	2.67
8		Halictidae	*Halictus* sp.	5	6.67
9			*Lassioglosum* sp.	1	1.33
10	Thysanoptera	-	*Gen* sp.	2	2.67

Capítulo 2

Clonality as a survival strategy in the critically-endangered plant genus *Pseudomisopates*

Una versión de este capítulo se encuentra en proceso de revisión

para su publicación en Journal of Heredity

Clonality as a survival strategy in the critically-endangered plant genus *Pseudomisopates*

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Key words: canopy closure, clonal growth, fitness, herbivory, Mediterranean, *Pseudomisopates rivas-martinezii,* reproductive success, SSR.

Abstract

Clonal growth can be a double-edged sword for endangered species, since the short-term insurance against extinction may incur a longer-term hazard of creating small inbred populations with low fecundity. In the present study, we quantify the advantages and disadvantages of clonal growth regarding the fitness of the central Iberian, monotypic, endangered genus *Pseudomisopates*. Preliminary studies showed that the species is self-incompatible and exhibits extensive clonal growth with plants flowering profusely. However, seeds at many sites seemed to be unviable, and no seedlings have been observed in the field. A fully replicated nested sampling design was conducted to explore genetic (using SSRs) and environmental factors potentially affecting seed viability, such as: (1) clonal and genetic diversity; (2) spatial genetic structure, and (3) environmental factors (shrub cover and grazing). Generalized Linear Mixed Models were fitted relating genetic and environmental variables to fitness variables (seed viability and flower display). Results indicate that the relatively low genotypic diversity of the population (0.23), as quantified by SSRs, and the strong spatial genetic structure observed are the result of intense clonal growth. This clonal

growth is enhanced by unfavourable environmental conditions, such as canopy closure and grazing. Under these circumstances, both flower display and mate availability decrease, thus hindering sexual reproduction. Indeed, a mixed reproductive system (clonal and sexual) to escape environmental stochasticity is crucial for the survival of a species inhabiting a disturbance-prone ecosystem.

Introduction

Clonal growth is very widespread in plants, with about 66.5% of central European flora exhibiting some kind of clonal growth (Klimes *et al.* 1997). Clonal growth allows plants to grow and spread horizontally, and is more frequent in aquatic plants, plants prone to disturbance, rare and endangered species, alien plants, and at the edge of species geographical ranges (Silvertown 2008). The rate of clonal versus sexual reproduction in natural populations has crucial effects on demography and genetics (Eriksson 1989; Arnaud-Haond *et al.* 2007), since a certain level of genetic recombination enhances genotypic diversity and the ability to adapt (Balloux *et al.* 2003). Nevertheless, the advantages of clonality are many and reduce the costs of sexual reproduction, allowing translocation of resources between ramets in heterogeneous environments (Jónsdóttir & Watson 1997; Price & Marshall 1999), demographic benefits such as increased probability of survival and fecundity (Harper & White 1974), or rapid increase in size and mobility.

But clonal growth could be a double-edged sword for an endangered species because the short-term insurance against extinction might incur a potentially longer-term hazard of creating small inbred populations with low fecundity. In fact, small populations of many clonal species may give the appearance of abundance, though being genetically depauperate (Sydes & Peakall 1998; Eckert & Barret 1993). In many cases, sexual reproduction can be almost permanently suppressed by environmental conditions such as canopy closure (Kudoh *et al.* 2001), vegetation succession (Piquot *et al.* 1996), mowing (Schaal & Leverich 1996) or temperature (Woodward 1990). This situation may lead to lowered genetic diversity or inbreeding, especially in small populations of self-incompatible species, in which pronounced clonal growth may result in reduced mate availability and plants may not receive enough compatible pollen, thus leading to reduced sexual reproduction or even extinction (Honnay &

Bossuyt 2005; Charpentier *et al.* 2000). However, evidence for this lowered fitness in clonal populations is still scarce (Honnay *et al.* 2006, Wolf *et al.* 2000).

Nonetheless, some authors have suggested that the advantages of clonal growth may be larger than the disadvantages imposed by increased geitonogamy and inbreeding depression (Eckert 2000). Indeed for many species living in heterogeneous habitats or depending on disturbance asexual reproduction might be the best strategy to ensure survival. The clonal nature of many species represents an advantage for colonizing and competing successfully in a range of habitats. Thus, they have become important colonizers of disturbed or man-made habitats, including spoil heaps, and are also dominant in early stages of succession of recently disturbed habitats or early stages of secondary succession (Prach & Pyšek 1994). The evolutionary maintenance of clonality in self-incompatible species will depend on the relative benefit of producing higher quality offspring promoted by outbreeding versus the reduction in offspring number due to lowered mate availability (Vallejo-Marín & Uyenoyama 2004) and the reproductive compensation over multiple breeding seasons (Goodwillie 1999; Larson & Barrett 2000).

The present study focuses on the advantages and disadvantages of clonal growth in the Iberian endangered species *Pseudomisopates rivas-martinezii* (Plantaginaceae). Although plants flower profusely in this species, most seeds seem to be unviable, as shown by preliminary germination tests, and the apparent absence of seedlings at sites monitored over a period of five years (Martínez *et al.* 2004; Amat *et al.* 2011). The hypothesis that the low levels of sexual reproduction observed on this self-incompatible species may be a consequence of the extensive clonal growth and certain environmental factors was tested. Our aims were to: (1) determine the level of sexual vs. asexual reproduction; (2) evaluate the effects of clonality on the genetic structure and the sexual reproduction ability, and (3) determine the environmental factors that enhance clonality. These were assessed by estimating the genotypic and genetic diversity, the genetic structure, as well as the spatial genetic structure using SSRs. Furthermore, the relation of genetic and environmental factors (canopy closure and grazing), with reproductive and fitness variables (flower display and seed viability) were explored.

Materials and methods

Study species

Pseudomisopates rivas-martinezii (Plantaginaceae, tribe Antirrhineae) is an endangered species endemic to the Gredos region, in central Spain. It has been classified as Critically Endangered following the IUCN criteria in the Red List of Spanish Vascular Flora (Martínez *et al.* 2004). It is one of the 19 monotypic genera endemic to Spain (Nieto Feliner 1999), of which 5 are critically endangered. The species occurs in the *Cytisus oromediterraneus* shrublands from 1,400 to 1,990 metres above sea level. Populations preferably occupy clearings within this habitat, where plants show higher vigour than in the dense shrubland. Two main population areas have been found separated by 20 Km, one in *Gredos* Mountain Range and the other in *La Serrota* Mountain Range, comprising seven and three populations respectively (Vargas & García 2008). Plants are long-lived and ramets typically resprout each year. Excavation of plants revealed that plants multiplied vegetatively by an underground stolon network, with connections between ramets that can span over one metre. The species is predominantly self-incompatible and insect pollinated (Amat *et al.* 2011). Flowers bloom from July to August and fruiting occurs between August and September. Fruits can contain from 1 to 24 seeds, which are dispersed by barochory.

Study area and sampling design

One population from *La Serrota* Mountain Range was selected for this study. *La Serrota* population (*Cepeda de la Mora, Ávila*), in particular, was sampled during the growing season of 2007. The habitat consists of *Cytisus oromediterraneus* shrubland interspersed with granite rocks in a smooth steep hill. Plants grow from 1850 to 1960 m. At higher altitudes plants occur in the shrubland, on the loose soil and between the granite rocks. At lower altitudes the shrubland has been cleared for pasture and plants grow in pasture grassland. A total number of 21 plant patches, occupying 13,336 m^2, were found and delimited using a GPS receiver (Garmin Etrex Vista). A Nested Sampling Design was conducted, 10 patches were randomly selected, and 10 sample points were randomly allocated per patch. The nearest plant to these sample points was sampled for leaf material. As a result, 100 ramets (10 from each patch) were analyzed. This sample size was adjusted considering the area of the population, the previous estimate of just 164 genets in this population and the extensive clonal growth of individuals which expand over metres via underground stolons (Martínez *et al.* 2004). About 60 mg of leaf from each ramet were collected and preserved dried in silica-gel. Finally, a data set of potential drivers on the species fitness was compiled. Thus, the diameter, the number of branches, flowers and fruits were measured from each sampled plant. Each of these plants were taken as the centre of a 1m^2 quadrat in which shrub cover was estimated to the nearest 1%, and ramet density and number of seedlings were counted.

Seed viability test

A total number of seven fruits were systematically sampled (when present) from each ramet, and all seeds where counted and tested for viability. As a result 554 seeds from 51 individuals were analyzed. Seed viability, and hence an estimate of seed germination, was assessed by means of a Tetrazolium test optimized for the species, following the Tetrazolium Testing Handbook (Peters 2000). Seeds were imbibed in water overnight at 21 °C, then slightly cut and incubated in 1%TZ at 27 °C for 72 hours. Seeds were finally bisected and viability was estimated by observing red colouring of living tissues.

DNA extraction and microsatellite protocol

DNA was extracted from leaf material, using the QUIAGEN DNeasy Plant Mini Kit. DNA samples were analysed with 7 polymorphic nuclear microsatellite markers (SSRs) (Table 1) originally genotyped from *Antirrhinum* ESTs (B. Davies, University of Leeds, UK, unpublished data) using SPUTNIK (http://cbib.u-bordeaux2.fr/pise/sputnik.html), and with primers designed using PRIMER3 (http://primer3.sourceforge.net/). A total of 77 markers were tested on *P. rivas-martinezii*, and those amplifying for the species were screened on samples from all known populations, in order to choose the most polymorphic loci. Forward primers were labelled with IRD-800 fluorescent marker. PCR was carried out under the following conditions depending on the primer pair: 5-7 ng of template DNA, 0.2-0.4 µl of each 10 mM primer, 2-2.5 µl of 25 mM MgCl2, 0.1 µl of 2mM dNTPs, 0.5 U *Taq polymerase*. The PCR was run for four minutes for an initial denaturation at 94°C, followed by 30 cycles of 30 sec at 94°C, 40 sec at 55-60°C (depending on the primer combination), and 1.20 min at 72°C. A final extension at 72°C for 10 min to ensure quantitative terminal transferase activity of the *Taq polymerase*. PCR products were separated on a 6.6% denaturing polyacrylamide gel (40% acrylamide) on a *Li-Cor 4200* DNA sequencer. PCR products were sized with a ladder run next to the amplified microsatellites. Fragments amplified by microsatellite primers were scored as present or absent using GeneImagIR version 4.0.

Locus	Primer sequences (5’-3’)	Repeat structure	Product length (bp)	Number of alleles	TA (°C)
MSAT 35	F- CCTTGGCCTTTCTCTCTCCT R- CCAAGCATCCTTTCGGAATA	CTT(9)	236	2	60
MSAT 53	F- TCGACGATGGTGAAGATGAC R- CCCTGAAACGAGAGCGTAAG	TAA(10)	268	2	58
MSAT 61	F- CTCGCCCTCTTATCCTCAAA R- TTCGTTGCTGTTGACATGGT	TCT(9)	150	5	60
MSAT 63A	F- CAAGGATTTGTTGGGAAGGA R- ACTAACCCCGGCTTATACGG	AAC(8)	243	4	60
MSAT 63B	F- ACCTCAATTTGGGCACTGAT R- GGTGGAGTTGCTCTTCTTGC	GAA(10)	379	5	60
MSAT 69	F- CACATGTAACCCACCGAAAAG R- GGGACCTTCACCAGTACCAA	GT(12)	404	3	58
MSAT 77	F- ACCTCGACGTCAACTTCCAC R- GAGGTTGGGCTTGGGAATAC	GCA(11)	250	2	55

Table 1. Data for the 7 microsatellites loci: primer sequences, repeat-structure, PCR product length and annealing temperature (T_A) and number of alleles amplified per locus for *Pseudomisopates rivas-martinezii*.

Analysis of clonal diversity

Clonal diversity was evaluated by calculating the genotypic richness ratio $PD = G\text{-}1/N\text{-}1$, i.e. the proportion of distinguishable genotypes, where *G* represents the number of multilocus genotypes (MLGs) and *N* represents the number of sampled ramets (Dorken & Eckert 2001). This estimator ranges from 0 for monoclonal populations to 1 when all samples belong to distinct genotypes. All calculations were performed using GENCLONE version 2.0 (Arnaud-Haond & Belkhir 2007).

The discriminative power of the polymorphic markers used to differentiate the genotypes (MLGs) present in the sample was explored by: (1) plotting the number of loci versus the number of MLGs detected (Vandepitte *et al.* 2010) (Figure S1), and (2) constructing a histogram of genetic distances among MLGs (Figure S2).

Analysis of genetic diversity and structure

Genetic diversity was measured as mean number of observed alleles per locus (n_a), mean allelic richness per locus (R_S), mean private allelic richness per locus (R_P), mean gene diversity or expected heterozygosity (H_S), observed herozygosity (H_O) and inbreeding coefficientents (F_{IS}). Calculations were performed using FSTAT v.2.9.3. (Goudet 1995). Outcrossing frequencies (O_R) were estimated as $(1\text{-}F_{IS})/(1\text{+}F_{IS})$ according to Allard *et al.* (1968). Differences in heterozygosity between shrubland and grassland were explored with a Kruskal-Wallis test using STATISTICA 6.0.

Compatible first-degree parentage relationships between accessions were assessed with GIMLET (Valière 2002), although no clear relations were identified. In order to visualize the genetic distances between genotypes, a factorial correspondence analysis (FCA) was carried out using GENETIX 4.05.2 (Belkhir *et al.* 1996-2004).

Hierarchical structure of genetic variation was examined by an analysis of molecular variance (AMOVA) using ARLEQUIN version 3.1 (Excoffier *et al.* 2005) to describe the genetic structure and variability among patches in the population. The total genetic variation was partitioned: (1) between patches, and (2) between patches within habitats (pastureland vs shrubland). The AMOVA was performed at the ramet-level, *ie*. including all samples. The analysis at the genet-level considering only one individual per genotype could not be performed due to the high level of clonality, which lead to an insufficient number of genotypes per patch (one to six, see below), making it unfeasible to partition the variance between patches. Although *F*-statistics and related techniques were developed assuming sexual reproduction in randomly mating populations (McLellan *et al.* 1997), and even if ramet-level analysis would lead to pseudoreplication, this analysis still provides insights into population structure and is relevant considering the spatial scale of the sampling scheme.

Analysis of spatial structure

First, the spatial distribution of equal genotyped ramets was tested using the aggregation index (*Ac*) and the extension of clones in the population was measured using the clonal sub-range, which is the longest geographic distance between ramets sharing the same MLG (Arnaud-Haond & Belkhir 2007). Second, the spatial genetic structure between patches was examined using the Mantel test implemented in IBDWS version 3.16 (Jensen *et al.* 2005). The correlation between the triangular matrix with pairwise geographical distances between patches and the triangular matrix with pairwise F_{ST} values between patches was calculated. Finally, spatial autocorrelation analyses were used to confirm the scale-dependence of clonal diversity. The average genetic distance or kinship coefficient *Fij* (Loiselle 1995) between pairs of individuals within specific ranges of geographic distance was plotted against 10 spatial distance classes (21, 63, 107, 178, 219, 299, 358, 455, 545, 660 metres) among which the number of individual pairs compared was evenly distributed. The statistic was calculated at both the ramet and genet level. This ratio is dependent upon the rate of decrease of pairwise kinship coefficients between individuals with the logarithm of the distance in two dimensions, and can be used to compare the extent of spatial genetic structure (SGS) among populations or species (Vekemans & Hardy 2004). All calculations were performed at the ramet and genet level using SPAGEDI version 1.3.a (Hardy & Vekemans 2002).

Analysis of the influence of genetic and environmental factors on fitness

Generalized Linear Mixed Models were fitted relating environmental variables to reproductive and fitness variables (flower display and seed viability). These followed the hypothesis that flowering would be affected by environmental factors, whereas seed viability would be affected by genetic factors. Fixed effects quantify the overall effects of canopy closure, plant size and grazing in the first model, and minimum distance to a different genotype and flower display in the second model; random effects quantify the variation across patches of the fixed-effect parameters. Because the response variables (number of flowers and mean viable seeds) were count data following a negative binomial distribution, analyses were performed using the package ‘glmm.admb’ (Otter Research Ltd, Sidney, BC, Canada) written for R 2.1.1 (R Development Core Team, 2008).

Results

Clonal diversity

Redundant multilocus genotypes (MLGs) were always located within the same patch (Fig. 1) and considered as belonging to the same clone. Therefore, the total number of distinct genets considered was 23, and ranged from one in some of the smallest patches to six in the largest

(Table 2), with 8 being sampled only once (35%) and 15 more than once (65%). The genotypic richness (*PD*) was 0.23, indicating that only 23% of the 100 ramets sampled belonged to distinct genotypes.

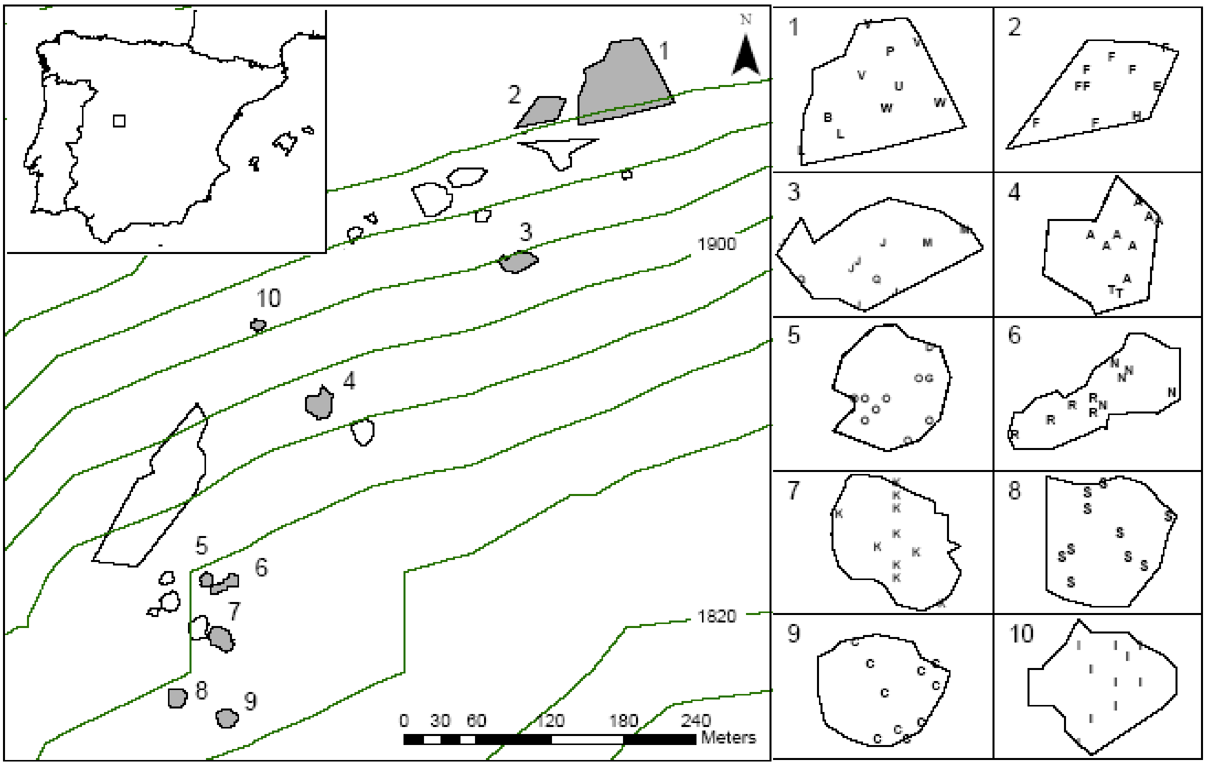

Figure 1. Patches of *P. rivas-martinezii* at La Serrota population. Disposition of genotypes within sampled patches (in grey) are represented on the right. Patches 5 to 9 are located in pastureland, whereas the rest are located in the shrubland.

Genetic diversity and structure

A total of 23 alleles were amplified. The most frequent allele appeared at a frequency of 0.96, where as 48% appeared at a frequency of between 0.3-0.62, and another 48% with a frequency below 0.2 (Table S1). The number of alleles per locus (n_a) was low, ranging from an average number of 1.43 to 2.29. The number of alleles and the mean allelic richness per locus (R_S) were highest on patch 5 and lowest on patches 7, 8 and 9. Private allelic richness (R_P) was almost insignificant for most patches. However, the number of polymorphic loci and the average heterozygosities were quite high, except on patches 7, 8 and 9, indicating a high distribution of genetic variation among patches (Table 2). The expected heterozygosity (*He*) ranged from 0.226 (patches 7, 8, 9) to 0.43 (patch 3). The observed heterozygosity (*Ho*), however, was significantly higher from *He* in almost all patches. Average expected heterozygosity for the population was 0.42 and average observed heterozygosity was 0.51. All patches showed surplus of heterozygosity, except for patch 6, and the absence of most homozygote combinations for four of the seven loci.

Patch	N	N_G	PL	n_a	R_S	R_P	Ho	He	Fis	O_R
1	10	6	71.4	2.000	2.000	0.000	0.444	0.360	-0.251	1.672
				(0.816)	(0.816)	(0.000)	(0.346)	(0.244)		
2	10	3	71.4	1.714	1.700	0.000	0.571	0.314	-0.905	20.000
				(0.488)	(0.480)	(0.000)	(0.468)	(0.245)		
3	10	3	85.7	2.000	1.998	0.000	0.571	0.427	-0.364	2.143
				(0.816)	(0.814)	(0.000)	(0.406)	(0.211)		
4	10	2	85.7	2.000	1.998	0.143	0.571	0.349	-0.698	5.625
				(0.000)	(0.003)	(0.378)	(0.392)	(0.202)		
5	10	3	85.7	2.286	2.227	0.000	0.414	0.311	-0.356	2.105
				(0.756)	(0.718)	(0.000)	(0.426)	(0.230)		
6	10	2	71.4	2.143	2.143	0.000	0.357	0.395	0.100	0.818
				(0.900)	(0.900)	(0.000)	(0.226)	(0.272)		
7	10	1	42.9	1.429	1.429	0.000	0.429	0.226	-1.000	n/a
				(0.535)	(0.535)	(0.000)	(0.495)	(0.260)		
8	10	1	42.9	1.429	1.429	0.286	0.429	0.226	-1.000	n/a
				(0.535)	(0.535)	(0.488)	(0.495)	(0.260)		
9	10	1	42.9	1.429	1.429	0.143	0.429	0.226	-1.000	n/a
				(0.535)	(0.535)	(0.378)	(0.495)	(0.260)		
10	10	1	71.4	1.714	1.714	0.143	0.714	0.376	-1.000	n/a
				(0.488)	(0.375)	(0.378)	(0.452)	(0.238)		
Mean		**2.3**	**67.1**	**1.814**	**1.807**	**0.072**	**0.493**	**0.321**	**-0.647**	**5.394**
S.D.		**1.6**	**17.9**	**0.316**	**0.307**	**0.101**	**0.109**	**0.074**	**0.401**	**7.343**

Table 2. Sample size (N), number of genets (N_G), percentage of polymorphic loci (PL), alleles per locus (n_a), mean allelic richness per locus (R_S), mean private allelic richness per locus (R_P), average expected (He) and observed (Ho) heterozygosities, inbreeding coefficients (F_{IS}), outcrossing frequencies(O_R). Standard deviations in parenthesis. Diversity estimates are calculated at the ramet-level. Data not available (n/a).

In the factorial correspondence analysis (FCA, Figure 2), the first factor (axis 1) essentially separates shrubland from pastureland (from left to right), with the exception of Patch 2 which is closely related to pastureland patches, despite their geographic isolation (Fig. 1). Consequently, when the upper level in the AMOVA was shrubland versus pastureland, *P.*

rivas-martinezii samples were significantly differentiated within groups ($F = 0.171$, p-value = 0.0068, Table 3) suggesting that habitat differences is a source of genetic differentiation.

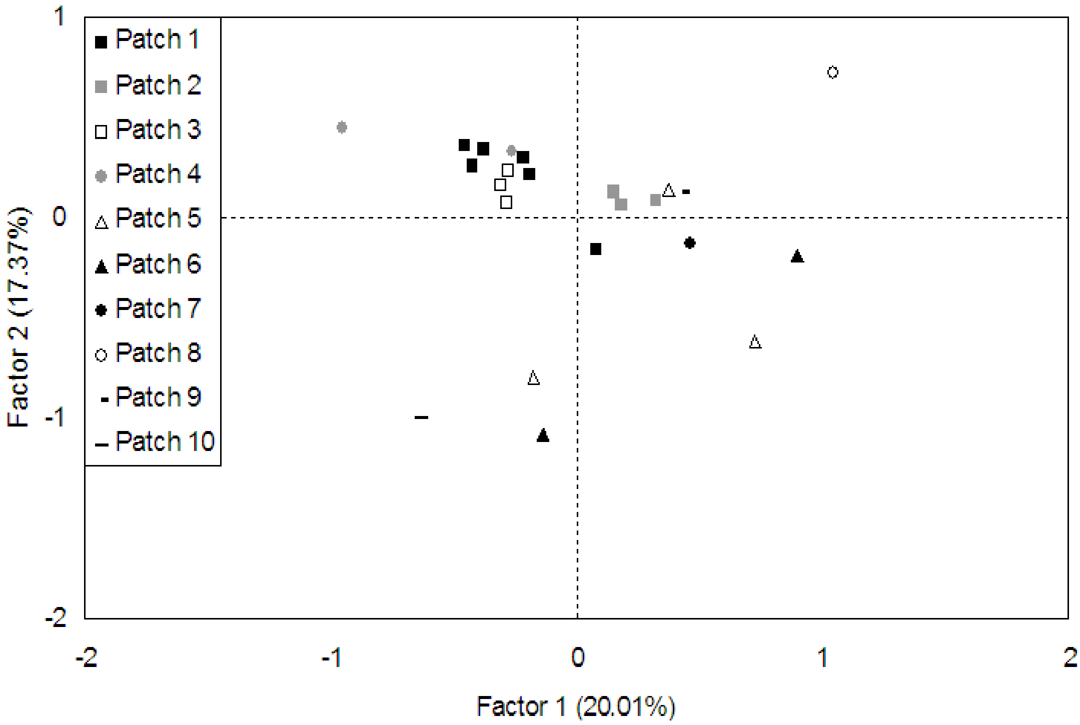

Figure 2. Factorial correspondence analysis representing genetic relationships between *P. rivas-martinezii* genets. The two first factors are plotted, explaining 20.01% (horizontal axis) and 17.37% (vertical axis) of the total variation, respectively. Patches 5 to 9 are located in pastureland, whereas the rest are located in the shrubland.

Indeed, shubland patches showed significantly higher values for observed heterozygosities (K-W, H = 7.16, p = 0.007). The rest of the variation was mainly found within patches (F = 0.446, p-value < 0.0001, Table 3). Pairwise F_{ST} between patches showed significant differentiation ($p < 0.05$), with values ranging from 0.061 (between patches 5 and 7) and 0.617 (between patches 4 and 8) (Table S2).

Source of variation	d.f.	SS	Variance	% variation	Fixation indices	p-value
Among patches	9	143.83	0.75	40.09	0.401	<0.0001
Within patches	188	210.89	1.12	59.91		
Among habitats	1	46.45	0.35	17.09	0.171	0.0068
Among patches within habitats	8	97.37	0.56	27.55	0.332	<0.0001
Within patches	188	210.89	1.12	55.36	0.44637	<0.0001

Table 3. Summary of ramet-level AMOVA for *P. rivas-martinezii*.

Spatial structure

The aggregation index indicated a clumped distribution for the population, with 73% of ramets sharing the same MLG with their closest neighbour (*Ac* = 0.7305, p = 0.0000, 1000 permutations) and a clonal sub-range of 35.85m. On the whole, the spatial distribution of genets was mainly patchy, although some patches showed some level of intermingling. Thus, this perennial species combines both guerrilla and phalanx growth forms, with guerrilla predominating in the shrubland (*Ac* = 0.5897, p = 0.0000, 1000 permutations) and phalanx in the pastureland (*Ac* = 0.8354, p = 0.0000, 1000 permutations) (Fig.1). Furthermore, patches showed dominance of one large clone, since the proportional area that genotypes would be occupying, according to the clonal repetitions observed, showed a right-skewed distribution (Fig. S3a), and the largest clone area was strongly correlated ($R^2 = 0.95$) to the total patch area within each patch (Fig. S3b).

No relationship was observed between genetic distance (measured by F_{ST}) and geographical distance as indicated by the Mantel test (Z = -22.71, r = 0.211, p = 0.104). The finer spatial autocorrelation analysis of microsatellite genotypes showed the clonal structure of the population. At the ramet level, a significant positive autocorrelation was found among ramets located up to 115m. Beyond this distance coancestry values were not significantly different to zero up to 140m, after which a negative autocorrelation between ramets was observed (Fig. 2a). At the genet level, results were very different, and no significant correlation was observed along the fluctuating pattern observed (Fig. 2b). Accordingly, the *Sp* value reflected the

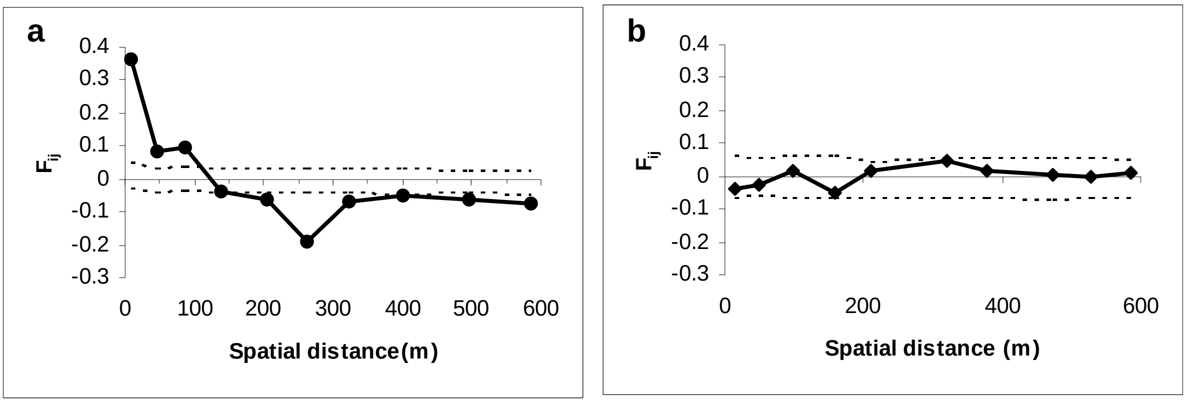

Figure 3. (a) Ramet level correlogram showing kinship coefficients of *P. rivas-martinezii* at different spatial distance classes. (b) Genet level correlogram produced with central coordinates for redundant genets. Dotted lines delimit 95% confidence intervals around the null hypothesis of randomly spatially distributed genets.

observed spatial autocorrelation at the ramet level (*Sp* = 0.1643), whereas it was not even calculated at the genet level, given the random distribution of genets in the sample.

Influence of genetic and environmental factors on fitness

Flower display ranged from 0 to 334 per ramet, with average flower production being 32.52 ± 57.40. The estimated fruit set was 13.91% ± 16.64%. Out of the 554 seeds tested only 11.19% were viable seeds. Considering the number of ovules per flower the seed set was 0.07 ± 0.13, and the viable seed set was 0.0013 ± 0.0046. The proportion of ramets grazed by cattle was 0.21 ± 0.41 and shrub cover around ramets was on average 16.35% ± 25.15.

The fitness components (number of flowers and viable seeds) were correlated with genetic and environmental variables (Table S3). First, once the difference in diameter (β_{diameter} = 0.1152) was taken into account, an increase in shrub cover (β_{shrub} = -0.0137) and the presence of grazing (β_{grazing} = -0.7707) both decreased flower display. Second, the mean number of viable seeds produced was slightly increased by the number of flowers (β_{flowers} = 0.0034), but the main predictor was the increasing distance to a different genotype ($\beta_{\text{distgenot}}$ = -0.0336), which lowered seed viability.

Discussion

High genetic diversity despite clonality

The existence of very few and unique genotypes within patches is a common result for clonal plant species (Loveless & Hamrick 1984; Ellstrand & Roose 1987). The level of genotypic richness found in *P. rivas-martinezii* (*PD* = 0.23) was low compared to that shown by other clonal species. Indeed, reviews of SSRs on clonal species by Honnay & Jacquemyn (2008) yielded a mean value of *PD* = 0.44 ± 0.08 for all species included. Nevertheless, the number of unique genets is likely double in this population, considering that only half of the population area was included in the sample.

Clonal species have been demonstrated to be as genetically diverse as non-clonal species (Ellstrand & Roose 1987; Hamrick & Godt 1990; Widén *et al.* 1994; Richards *et al.* 2004). Indeed, *P. rivas-martinezii* is more genetically diverse than expected based on the mean values reported by Hamrick and Godt (1990) for dicots, long-lived herbaceous perennials, species with regional ranges and temperate species. These species have fewer than 50% polymorphic loci at the species level, whereas *P. rivas-martinezii* has 67.1% polymorphic loci at the population level. In addition, this species fulfils the assumption of monophyly and therefore loci polymorphism due to hybridization is ruled out (see Carrio *et al.* 2010). Closely related self-incompatible species of *Antirrhinum* in the Iberian Peninsula are less genetically diverse, with about 50% of polymorphic allozyme loci and a expected heterozygosity of 0.029 to 0.470 (Jimenez *et al.* 2002; Carrió *et al.* 2010). Again, the heterozygote maintainance and the absence of most homozygote combinations is a common characteristic of clonal and self-incompatible species (Evans *et al.* 2003; Halkett *et al.* 2005; Stoeckel *et al.* 2006). Certainly, diverse colonizing cohorts and high genet persistence (Eriksson 1993; Watkinson & Powell 1993) must be maintaining the population even if seed recruitment is low (Watkinson & Powell 1993).

The uniqueness of genotypes among patches is reflected on the strong genetic differentiation, as indicated by the fixation index (Table 3) and the pairwise F_{ST} (Table S2), although differentiation would be more due to differences in allele frequencies rather than unique alleles (Table 2). Differentiation among patches could be due to past fragmentation of the population and colonization from seed; whereas the stronger differentiation within patches points towards a limited dispersal of both pollen and seeds. In addition, differentiation between pastureland and shrubland could be the result of habitat differences, intense grazing and growth form. Pastureland patches have phalanx ramets that are more affected by grazing. Whereas, the spreading guerrilla ramets in the shrubland enhance successful pollination (Handel 1985), as shown by the significantly higher heterozygosity in shrubland patches.

Strong genetic spatial structure as a result of clonality

Clonal reproduction resulted in a clumped distribution of ramets overall. Hence, the population showed dominance of a few large clones, and a majority of either small or intermediate clones (Fig. S3). The lack of isolation by distance suggests limited gene flow between patches. The finer scale spatial structure analysis, however, revealed strong non-random mating among ramets up to a distance of 115m (Fig. 3a), but complete random mating among genets (Fig. 3b). The strong spatial genetic structure observed is the result of intense clonal growth and an indication of a long time since the last disturbance took place. The random genetic structure among genets throughout the area is not too common amongst clonal plants which tend to show some level of spatial autocorrelation (Vekemans & Hardy 2004), although it has been previously reported in some species, such as oaks (Alfonso-Corrado *et al.* 2004), the seagrass *Zostera marina* (Reusch *et al.* 1998) and the saltmarsh *Spartina alternifolia* (Travis *et al.* 2004). This structure is rather related to the self-incompatible mating system of the species, by which flowers need necessarily be fecundated by pollen from a different genet. Thus, seed viability is likely to be affected by this spatial genetic structure of clones, which lowers compatible mate availability by proximity, as suggested by the pollen quality limitation reported by previous studies (Amat *et al.* 2011).

Advantages and disadvantages of clonality

Our results support the hypothesis that reproductive success and fitness could be eventually reduced by environmental conditions. Indeed, shrub canopy closure and herbivory could enhance clonality, which in turn reduce mate availability due to the spatial genetic structure created by clones. Under these circumstances, plants reduce their investment into sexual reproduction and enhance clonal spread. Translocation of resources and reproductive investment is commonplace between ramets in many clonal species (D'Hertefeldt & Jónsdóttir, 1994). In some cases, flowering individuals may only be observed at initial stages or originate from plants that have persisted in shaded places until gap formation (Kanno & Seiwa 2004). Many clonal herbaceous forest plants show increased sexual vigour in gaps. For instance, management practices which affect the degree of canopy closure, affected plant density and sexual reproduction in *Maianthemum dilatatum* (Lezberg *et al.* 2001); differences in fruit size and seed number in *Trillium erectum* were associated with a lower carbon allocation in populations with more intense canopy closure (Routhier & Lapointe 2002), and suppressed flowering and monoclonal populations were reported in closed canopy habitats in *Uvularia perfoliata* (Kudoh *et al.* 1999). In addition, in many clonal species, grazing or mowing prevents sexual reproduction and resources are allocated towards clonal spread (Kerley *et al.* 1993; Schaal & Leverich 1996). The higher ramet density on intensely grazed patches of *P. rivas-martinezii* could be a response to grazing. Moreover, herbivores, through selectively feeding on herbaceous species, are considered to increase the resources available to woody species, increasing shrub cover under grazing (Walker *et al.* 1981; Skarpe 1990). Finally, we can ascertain that increased shrub cover and grazing reduce the investment of plants into sexual reproduction. Under these circumstances clones may gain a relative benefit in terms of clonal spread, since they are more likely to persist by clonal reproduction under less favourable environmental conditions.

At the same time, the increased survival opportunity created by clonality will diminish mate availability due to the spatial genetic structure created by clones. Evidence shows that this must be a common situation in this species, since although fruit-set was higher than in other clonal species (Eriksson & Bremer 1993; Honnay *et al.* 2006), the percentage of viable seeds was very low. Moreover, results indicate that the mean number of viable seeds decreases by larger distances to ramets with a different genotype, indicating that the spatial genetic structure created by clonal growth imposes a physical mating barrier as observed in other clonal species, e.g. *Rubus saxatilis* (Eriksson & Bremer 1993), *Calystegia collina* (Wolf *et al.* 2000), *Maianthemum bifolium* (Honnay *et al.* 2006) and *Convallaria keiskei* (Araki *et al.* 2007). Thus, these results support previous hand pollination studies and floral visitor observations which showed that the low sexual reproductive success in *P. rivas-martinezii* was mainly a result of pollen quality limitation probably due to low mate availability, and secondarily of pollen quantity limitation due to inefficient pollination (Amat *et al.* 2011).

Habitat disturbance and conservation

The ultimate goal of conservation is to ensure the survival of populations, whilst maintaining their evolutionary potential (Frankham *et al.* 2004). For rare and endangered species this has relied on conserving their ecosystems (Franklin 1993; Lindenmayer *et al.* 2007). However, in disturbance-prone species, conservation strategies might require soil disturbance and opening of the canopy, as assessed by traditional management practices, for successful seedling recruitment.

Genetic diversity allows populations to adapt to a changing environment and is vital for assessing suitable conservation strategies (Hamrick 1983; Falk & Holsinger 1991; Frankham *et al.* 2002). Increasing the number of individuals per population would be effective in preventing genetic loss and the chance of local extinction by stochasticity. However, in the case of clonal species that germinate in response to disturbance and are later overgrown by shrubs, resprouting and seeding play a crucial role (Keely & Fotheringham 2000; Ojeda *et al.* 2005). Besides, high heterozygosity observed in this population suggests that it is unlikely to be suffering from inbreeding depression and that it might be valuable for conservation, although erosion of allelic richness at self-incompatibility loci may be limiting the reproductive capacity of populations (Young *et al.* 1999).

Clonality is limited by disturbance (Silvertown 2008), so that many plants in disturbance prone ecosystems show a mixed reproductive system that allows them to adapt to environmental stochasticity. *Pseudomisopates rivas-martinezii* inhabits the clearly anthropogenic landscape of Gredos Range, which includes a combination of forest patches, pastures, and widespread shrublands, mostly generated during the transition between the 17th and 18th centuries AD, when forest management activities, including fire and intense grazing, caused the progressive deforestation and consequently the expansion of the current fire-prone shrubland (López-Merino *et al.* 2009). Accordingly, germination studies have shown that the species exhibits specificity to fire. A positive response to the effects of fire, both directly (79% higher germinability after ash treatments) and indirectly (higher resprouting in burned sites and 74% lower germinability in darkness) has been observed (Chapter 3). Moreover, seed viability varies across years, so that certain compensation could be taking place (Chapter 3). Therefore, despite being critically-endangered, clonality represents the best strategy for the survival of this species till the next disturbance takes place.

Fire is a natural process in many Mediterranean ecosystems and has been implemented successfully in managing endangered species (Fensham & Fairfax 1996), as in the iconic *Sequoiadendron giganteum* (Swetnam *et al.* 2009). Nevertheless, incorporating fire as a management tool for increasing the abundance of an endangered or rare species must be taken with caution since fire may negatively affect plant communities as a whole, and the precise effects on the species must be monitored (Pendergrass *et al.* 1999). Consequently, further knowledge on population dynamics and response to the disturbance caused by fire in *P. rivas-martinezii* is essential for conservation.

In contrast, disturbance by grazing affects complete ramets and patches. Recruitment will remain suppressed, and populations threatened by lack of replacement and loss of genetic flexibility. To maintain *P. rivas-martinezii* populations in the long term, lengthy periods of grazing rest and controlled grazing pressure will be necessary to allow both new recruits and mature individuals to reproduce.

Concluding remarks

In conclusion, *Pseudomisopates rivas-martinezii* exhibits a mixed reproduction system, which allows it to benefit from different strategies and to escape from environmental stochasticity. Sexual reproduction is the main factor determining the formation of new genets and is important at determining the population genetic structure. In turn, clonal growth is one of the main factors determining population growth and spatial genetic structure. Although seed viability is affected by the spatial genetic structure of clones, which lowers compatible mate availability, seed production may be enough to compensate across years as shown by the maintenance of genetic diversity. Furthermore, under adverse environmental conditions of canopy closure and intense grazing in which sexual reproduction and genetic diversity may be reduced, clonality allows this species to escape extinction in a successional shrubland environment.

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Appendix

Table S1. Number of alleles, heterozygosity and F_{is} at each microsatellite locus for *P. rivas-martinezii* patches in the study site.

Locus	Number of alleles	H_{obs}	H_{sn}	F_{is}
MSAT 35	2	0.072727	0.079257	0.082386
MSAT 53	2	0.500000	0.509174	0.018018
MSAT 61	5	0.759259	0.699846	-0.08489
MSAT 63A	4	0.690909	0.639257	-0.0808
MSAT 63B	5	0.709091	0.64686	-0.0962
MSAT 69	3	0.333333	0.599881	0.444334
MSAT 77	2	0.333333	0.475738	0.299334

Table S2. Patch pairwise differentiation. All F_{st} values are significant (p-value <0.0001).

PATCHES	1	2	3	4	5	6	7	8	9	10
1	0									
2	0.277	0								
3	0.148	0.255	0							
4	0.204	0.407	0.146	0						
5	0.361	0.271	0.293	0.476	0					
6	0.302	0.378	0.323	0.474	0.297	0				
7	0.448	0.365	0.374	0.563	0.061	0.380	0			
8	0.465	0.460	0.443	0.617	0.384	0.380	0.426	0		
9	0.536	0.307	0.374	0.548	0.277	0.474	0.368	0.549	0	
10	0.312	0.396	0.315	0.374	0.483	0.383	0.532	0.532	0.557	0

Figure S1. Box plot describing the genotypic resolution of microsatellites in *Pseudomisopates rivas-martinezii* containing 100 sampling units genotyped using 7 microsatellites (1000 permutations). Box edges show the minimum and maximum number of genotypes, and the central line shows the average number of genoptypes identified. The graph shows that a set of seven loci allows an accurate determination of the number of genotypes in the sample.

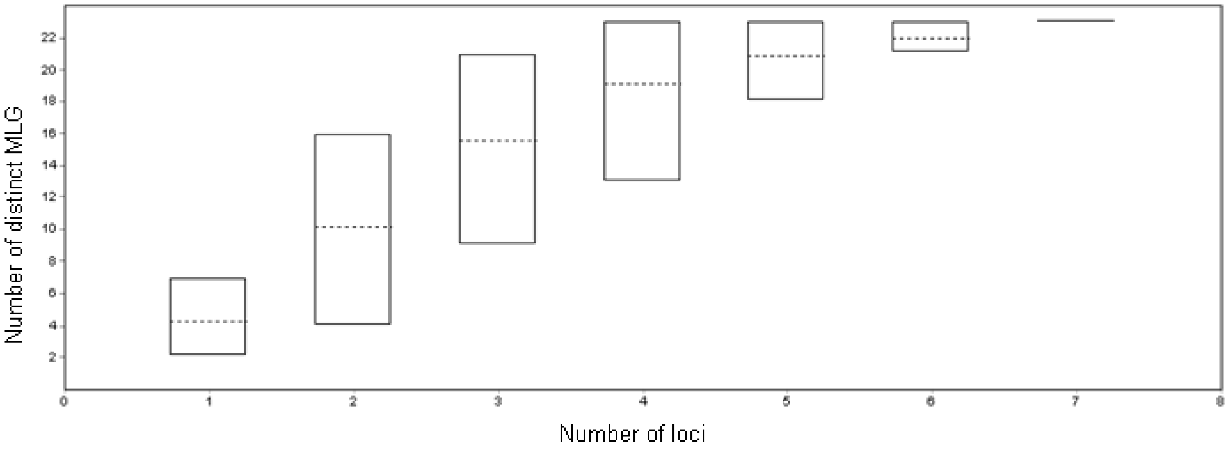

Figure S2. Frequency distribution of the pairwise number of alleles differences between MLGs for *Pseudomisopates rivas-martinezii* (100 permutations). Neither identical MLG nor somatic mutations are expected. The dashed line represents the P_{sex} threshold below which distinct MLGs would have originated from the same zygote.

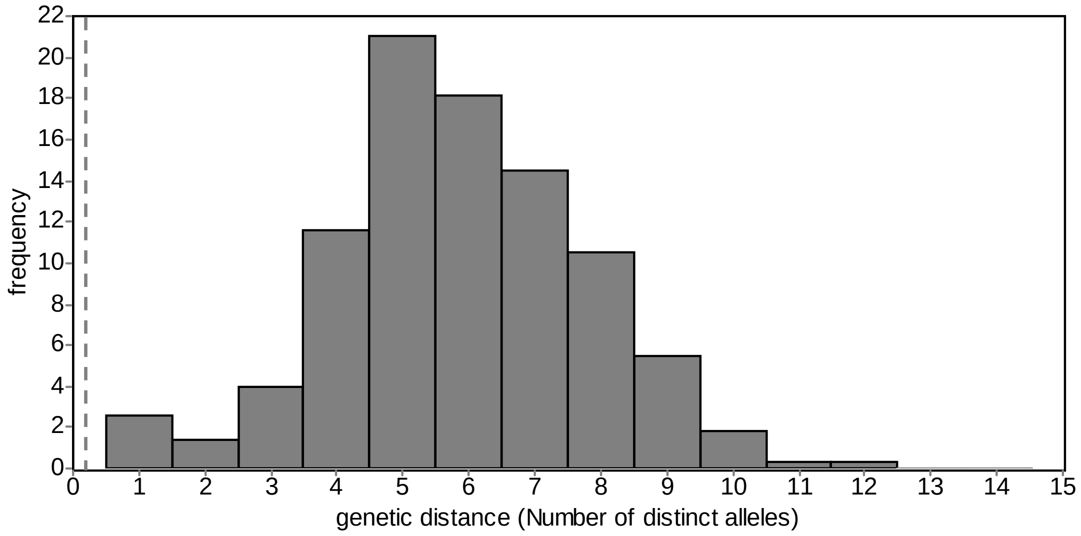

Figure S3. (a) Proportional area potentially occupied by the different genotypes, according to the clonal repetitions observed in the sample. A-W: genotype names. (b) Scatterplot showing the relation between patch area and the area of the largest genet per patch.

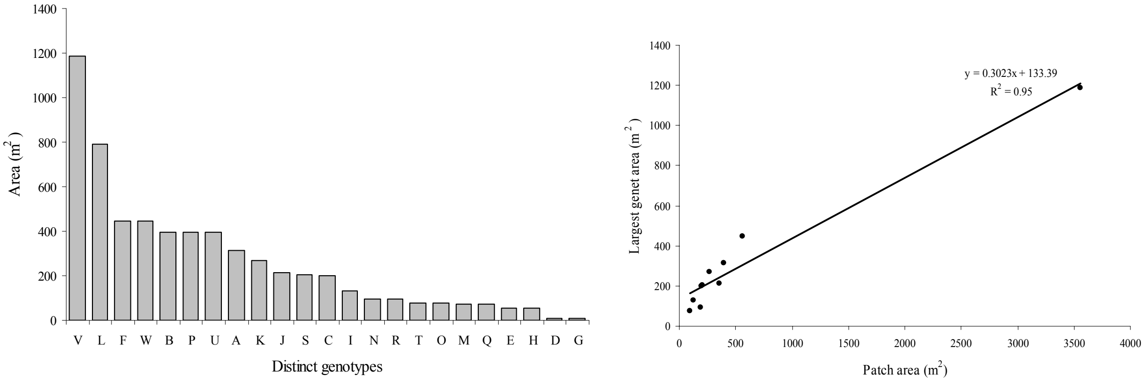

Table S3. Results for the Generalized Linear Mixed Model relating *Pseudomisopates rivas-martinezii* reproductive (flower display) and fitness variables (mean number of viable seeds) to environmental and genetic factors.

Flower display	**Viable seeds**
Model flowers ~ shrub + diameter + grazing Family: nbinom alpha = 0.9019 Zero inflaction: p = 0.0459 Fixed effects: Log-likelihood: -397.983 Formula: flowers ~ shrub + diameter + grazing (Intercept) shrub diameter grazing 1.443600 -0.0137 0.1152 -0.7707 Random effects: Grouping factor: patch Formula: ~1 Structure: Diagonal matrix (Intercept) 0.4563 Number of Observations: 100 Number of Groups: 10	Model meanviable ~ flowers + distgenot Family: nbinom alpha = 0.4199 Zero inflaction: p = 0.0017 Fixed effects: Log-likelihood: -52.4835 Formula: meanviable ~ flowers + distgenot (Intercept) flowers distgenot -1.0401 0.0034 -0.0336 Random effects: Grouping factor: patch Formula: ~1 Structure: Diagonal matrix (Intercept) 0.0052 Number of Observations: 100 Number of Groups: 10

Capítulo 3

Factors controlling seed germination of the Iberian critically-endangered *Pseudomisopates* (Antirrhinaceae)

Manuscrito inédito

Factors controlling seed germination of the Iberian critically-endangered *Pseudomisopates* (Antirrhinaceae)

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Abstract

Many seeds are dormant when shed from the mother plant. This unique characteristic of plants poses challenges in conservation and many different treatments have traditionally been used to break dormancy. When germination only occurs under certain circumstances, recruitment may be insufficient and the viability of plant populations may be threatened. A marked dormancy was previously identified in the Iberian critically-endangered species *Pseudomisopates rivas-martinezii*. The present study aimed to determine the magnitude of dormancy by estimating seed viability under different germination treatments: heat, ash, stratification, gibberelin addition and darkness. Our results indicate that there were significant differences in viability across plant populations and treatments. Maximum seed germination was obtained under ash addition, although cold stratification alone gave a considerable enhancement. However, gibberelic acid did not improve germination and darkness had a diminishing effect. In conclusion, a cold period is required for the species to germinate, coupled with the effects of fire: (1) ash, (2) vegetation clearings, and (3) resprouting, which are major factors triggering seed germination. Although these conditions

are widely found in the field, we hypothesize that a more dramatic situation may have tackled this species before the increase of human-mediated fires in the last millennia.

Introduction

Seed germination is a critical stage in the life cycle of sexually reproducing plants (Caswell 1989). Dormancy can prevent germination when conditions are suitable for germination, but the probability of survival and growth of the seedling is low. There are three main different types of seed dormancy (Baskin & Baskin 1998): (1) morphological dormancy, due to an undeveloped embryo; (2) physical dormancy, caused by seed or fruit coat impermeability to water, and (3) physiological dormancy, where inhibitors limit germination enzymes and hormones, so that a chemical change needs to take place in the seed. Physiological dormancy is the most common and allows a more flexible response to the environment than the other two types of dormancy.

When germination only occurs under certain circumstances, recruitment may be insufficient to compensate mortality or maintain genetic diversity, threatening the viability of plant populations (Keith 1996). Therefore, this unique characteristic of plants poses challenges in conservation and many different treatments have traditionally been used to break dormancy, including temperature, scarification of the seed coat or the application of hormones, mainly gibberellins, or other chemicals that have been proven to be effective (Bewley & Black 1994). Environmental factors such as chilling and light promote a decrease in the concentration of the germination inhibitor, abscisic acid (ABA), which in turn induces the synthesis of gibberellins (GA). These changes in ABA:GA balance, eventually result in wall expansion to permit radicle emergence (Finkelsten *et al.* 2008). Fire is frequent in most Mediterranean ecosystems, and has shaped their composition, ecology and evolution in time. For instance, fire-prone communities in the Californian chaparral and South African fynbos are dominated by species which exhibit remarkable similarities in their germination response to fire elements, such as charred wood and smoke. This germination behaviour has arisen independently in distantly related families and is interpreted as convergent evolution (Keeley & Bond 1997). Moreover, fire has also long been part of man’s activity since it has been widely used as a management tool in natural ecosystems. As such, it can affect soil, water, vegetation, wetland, air and cultural aspects, therefore a correct evaluation and cost-benefit analysis among these components has to be achieved in order to make a proper use of fire in managing ecosystems (DeBano *et al.* 1998). In Mediterranean-type ecosystems, many plant species are adapted to germinating soon after the occurrence of fire (Lloret *et al.* 1999). Fire plays a key role in breaking seed dormancy in some of the most species-rich families of the Mediterranean: *Fabaceae*, *Poaceae*, *Asteraceae* and *Cistaceae* (Clemente *et al.* 1996; Pérez-Fernández & Rodríguez-Echevarría 2003; Quintana *et al.* 2004). These plants can behave as resprouters, seeders or both when exposed to fire (Keely & Fotheringham 2000; Konstantinidis *et al.* 2005; Ojeda *et al.* 2005). Under highly seasonal conditions resprouters seem to be the dominant form, whereas seeders are more dominant in milder Mediterranean climates (Ojeda *et al.* 2005).

Species may benefit from direct or indirect factors of fire. Direct effects include: (1) high temperatures generated by the fire, which stimulate the germination of serotinous (Cowling & Lamont 1987) and hard seeded species (Keeley 1991; González-Rabanal & Casal 1995; Pérez-Fernández & Rodríguez-Echevarría 2003); (2) smoke derived gasses (Baxter *et al.* 1994; Dixon *et al.* 1995; Keeley & Fotheringham 1997); (3) chemicals contained in charred or ashed wood (Brown 1993; Enright *et al.* 1997), or (4) the release of nitrogenous compounds in the soil from plant biomass combustion (Humphreys & Craig 1981; Thanos & Rundel 1995; Pérez-Fernández & Rodríguez-Echevarría 2003). Indirect effects are related to the changes that occur in the environment after fire that enhance site quality for germination and recruitment. These include: (1) formation of vegetation clearings, which benefits some species by decreasing competition and increasing levels of light, thus favouring growth and survival (Valverde & Silvertown 1995; Menges & Hawkes 1998); (2) acceleration of the mineralization of organic matter, making inorganic nutrients more readily available (Wells *et al.* 1979; Certini 2005), or (3) soil sterilization which alters microbial pathogens populations (Wicklow 1988; Ahlgren 1974).

The aim of this study is to determine the factors that influence seed germination in *Pseudomisopates rivas-martinezii* (Antirrhinaceae). This endangered species shows extensive clonal growth and although plants flower profusely, seeds at many sites seemed to be unviable, as shown by preliminary germinability tests, and no seedlings have been observed in the field (Martínez *et al.* 2004). To unravel the factors triggering seed germination: (1) the level of seed viability in three populations of the species was determined, (2) the presence of dormancy was investigated by applying cold stratification and gibberellins, and (3) the direct and indirect effects of fire were studied by applying heat, ash and darkness.

Materials and Methods

Study species

Pseudomisopates rivas-martinezii (Scrophulariaceae) is an endangered species and monotypic genus endemic to the Gredos Mountain Range, in the Spanish Central Mountain System. It has been classified as Critically Endangered following the IUCN criteria in the Red List of Spanish Vascular Flora (Martínez *et al.* 2004). The species occurs from 1,400 to 1,990 m in *Cytisus oromediterraneus* shrublands, one of the most common habitats of Gredos. In this habitat, the species mostly occupies clearings among shrubs, where plants show higher vigour than plants growing in dense shrublands. There are two main distribution areas located 20 Km apart from each other, comprising five and three populations respectively (Vargas & García 2008). The species is self-incompatible (Amat *et al.* 2011) and reproduces vegetatively by an underground stolon network, with connections between ramets that can span over one metre. Flowers bloom during July and August. Fruiting occurs during August and September. Fruits can contain between 1 and 24 seeds, which are dispersed by gravity.

Study areas

In Mediterranean mountains the natural vegetation above tree line is a shrubland dominated by several fabaceous species (*piornal*). The dominant species in our study areas are *Cytisus oromediterraneus, Genista cinerascens or Echinospartum barnadesii.* This area has a Mediterranean Continental climate type with a distinct dry summer period, a mean annual rainfall of about 897 mm and an average annual temperature ranging from -1 to 15 ºC.

Seeds were collected during the dispersal period of *P. rivas-martinezii*, at the end of the summer 2009, and stored in paper bags at room temperature. Seeds from a total of three populations (comprising both centres of distribution) were collected for study. The first one, is located in the *Serrota* Mountain (Cepeda de la Mora, Ávila, Spain) comprised an area of approximately 14,400 m^2. The dominant shrub species was *C. oromediterraneus*. The second population is located in *Conventos* (Hoyos del Espino, Ávila, Spain) comprised an area of approximately 12,100 m^2. Vegetation consisted of *C. oromediterraneus* and *E. barnadesii* shrubland. *P. rivas-martinezii* plants were distributed in patches of different sizes throughout the area of both populations. The third population is located in *Garganta Lóbrega* (Candeleda, Ávila, Spain) comprised an area measuring approximately 100,500 m^2. The dominant shrub species was *C. oromediterraneus*. This last site was burnt in October 2005 and *P. rivas-martinezii* has intensely occupied the recovering shrubland.

Germination experiment

Seed germination in mountain species often requires the application of dormancy-breaking treatments (Körner 2003). Thus, in this study most seeds were pretreated with a cold-wet stratification period of two months at 4 ºC. Next, all seeds were surface sterilised with 2% sodium hipochloride. Germination experiments were conducted in plastic Petri dishes (9 cm diametre) lined with glass beads and covered with filter paper disks, and moistened with either distilled water or an ash solution, according to the test. Germination experiments were carried out in an incubator equipped with cool-white fluorescent tubes under a 16h light/8h dark photoperiod at a constant temperature of 20 ± 1ºC. These experimental conditions were chosen to emulate the environmental thermoperiods and photoperiods that a seed would experience on the soil surface in spring. This season is characterized by melting snow and rainfalls that provide enough soil moisture to activate seed germination. We considered the following treatments:

High Temperature Treatment (HIGHTEMP). Seeds were dry heated in an oven for 5 min at 110ºC prior to stratification. This treatment was selected to simulate the extreme conditions potentially encountered by seeds on the surface or near the surface of the soil during fire and test seed survival (Christensen & Muller 1975; DeBano *et al.* 1979).

Low Temperature Treatment (LOWTEMP). Seeds were dry heated in an oven for 5 min at 70ºC prior to stratification. This treatment simulates the conditions potentially encountered by seeds in the soil during fire (Christensen & Muller 1975; DeBano *et al.* 1979).

Ash Treatment (ASH). The effect of ash on germination was studied by watering seeds with a suspension of ash (Keeley & Fotheringham 2000). This solution was obtained from the combustion of leaves and branches from several representative species from the shrubland. Biomass was burned until there was no plant material left. The proportion of combusted species used was about 60% shrubs and 40% grasses, simulating the proportion found in its natural habitat. Ash was diluted with distilled water to a concentration of 10 g of ash per litre of water.

Gibberelic Acid Treatment (GA). The breakdown of dormancy by gibberellins was studied by imbibing a set of untreated seeds in a solution of Gibberelic Acid (GA_3) at a concentration of 150mM, pH 6.5.

Stratified Gibberelic Acid Treatment (STR_GA). Seeds that had been previously cold-wet stratified were imbibed in a solution of Gibberelic Acid (GA_3) at a concentration of 150mM, pH 6.5.

Darkness Treatment (DARK). Petri dishes were wrapped in foil paper to avoid light from reaching seeds. This treatment was selected to simulate the effect of shrub closure on seeds, since it has been seen to affect flowering display (Chapter 2).

Stratification (STR). Seeds were wet-cold stratified and incubated with distilled water at 4ºC during two months.

Control (CONTROL). Untreated seeds were incubated with distilled water.

Each treatment was represented by 5 replicates containing 25 seeds each and the germination rate was expressed as a percentage. The germination criterion was defined as the emergence of the radicle and was recorded every two days over a period of 60 days. At the end of the period, non-germinated seeds were individually checked for viability (embryo and endosperm intact). When treated seeds were in apparent good condition, viability was accounted for by a Tetrazolium test, so that final results could be adjusted considering non-viable seeds. Eight treatments were conducted to test the different effects of fire in the ecosystem, the presence of dormancy and finally the effect of darkness.

Data analysis

Differences in seed viability among populations were first evaluated by a Generalized Linear Model comparing the categorical variable population and the binary response seed viability. Seed viability for each population and treatment was estimated as the proportion of germinated and non-germinated but viable seeds, as shown by the Tetrazolium test. Differences in seed germination of viable seeds were analysed with a Generalized Linear Mixed Model, fit by the Laplace approximation, studying the relation between the categorical variable treatment and the binary response number of germinated seeds, with population as random factor. Pairwise differences were assessed using the non-parametric Chi-squared test. Analyses were performed using the package 'lme4' written for R (R Development Core Team 2008) and JMP version 7.

Time to germination was strongly right-censored, since 87% of the viable seeds did not germinate irrespective of the treatment applied (i.e. 13% of the observations were censored). Therefore, data were analysed by Survival Analysis. Differences in time to germination were assessed by a regression failure-time analysis studying the relation between treatment nested within population, and day of germination, using the log-normal distribution. The germination index number of days for 50% of the total number of seeds germinated (T_{50}) was also calculated. Analyses were performed with JMP version 7.

Results

Seed viability varied between populations ($\chi^2 = 75.51$; $p < 0.0001$) and was significantly higher in *Lóbrega* (0.45) than in *Serrota* (0.29; $\chi^2 = 64.77$; $p < 0.0001$) and *Conventos* (0.31; $\chi^2 = 43.47$; $p < 0.0001$); where as *Serrota* and *Conventos* were not significantly different from each other ($\chi^2 = 0.14$; $p = 0.14$).

Treatment	Germination level ± SE				Germination timing	
	Serrota	Conventos	Lóbrega	Total	T_{50}	Parameter ± SE
Intercept						2.56±0.08
ASH	0.70±0.07	0.20±0.06	0.19±0.05	0.34±0.04	14	0.19±0.18
STR	0.30±0.05	0.10±0.04	0.15±0.04	0.19±0.02	12	-0.04±0.17
STR_GA	0.23±0.06	0.09±0.06	0.11±0.05	0.16±0.03	6	0.02±0.11
HIGHTEMP	0.24±0.11	0.03±0.03	0.10±0,04	0.10±0.03	18	0.89±0.37
LOWTEMP	0.13±0.06	0.07±0.05	0.08±0.03	0.09±0.02	10	-0.18±0.11
DARKNESS	0.08±0.05	0.02±0.02	0.07±0.04	0.05±0.02	6	0.32±0.27
GA	0.05±0.03	0.05±0.04	0.00±0.00	0.03±0.01	34	-0.53±0.21
CONTROL	0.00±0.00	0.00±0.00	0.03±0.02	0.01±0.01	38	0.59±0.03

Table 1. Among-treatment differences in germination level (proportion of germinated seeds) for *Pseudomisopates rivas-martinezii* populations, and in germination timing as indicated by the T_{50} and the parameters of the failure-time analysis regression. ASH: ash addition; STR: stratification; STR_GA: stratification with GA_3; HIGHTEMP: preheating at 110ºC with stratification; LOWTEMP: preheating at 70ºC with stratification; DARKNESS: no light; GA: GA_3 addition; CONTROL: untreated seeds.

There were significant between-treatment differences in germination rates (Fig. 1, Table 1, Appendix). ASH treatment had the highest germination rate ($\beta_{ASH} = 1.479 \pm 0.381$; $p = 0.0001$), but there were also significant differences between treatments, largely due to low results of GA ($\beta_{GA} = -1.486 \pm 0.615$; $p = 0.0001$) and CONTROL ($\beta_{CONTROL} = -2.207 \pm 0.791$; $p = 0.005$). The population effect had a standard deviation of 0.627, indicating that there is a certain level of variation between populations. This variation is due to the significantly higher germination rates observed in *Serrota* population (GLM, $p < 0.0001$). However, since interaction between treatment and population was not significant (Appendix), and results are equivalent in all populations, data for treatments across populations have been pooled together for further analysis and figures.

Maximum seed germination was obtained with ASH, although STR alone gave a considerable enhancement in germination rate. However, differences were significant between these treatments ($\chi^2 = 11.64$; $p < 0.0006$). STR had significantly a higher germination rate than the CONTROL ($\chi^2 = 29.14$; $p < 0.0001$).

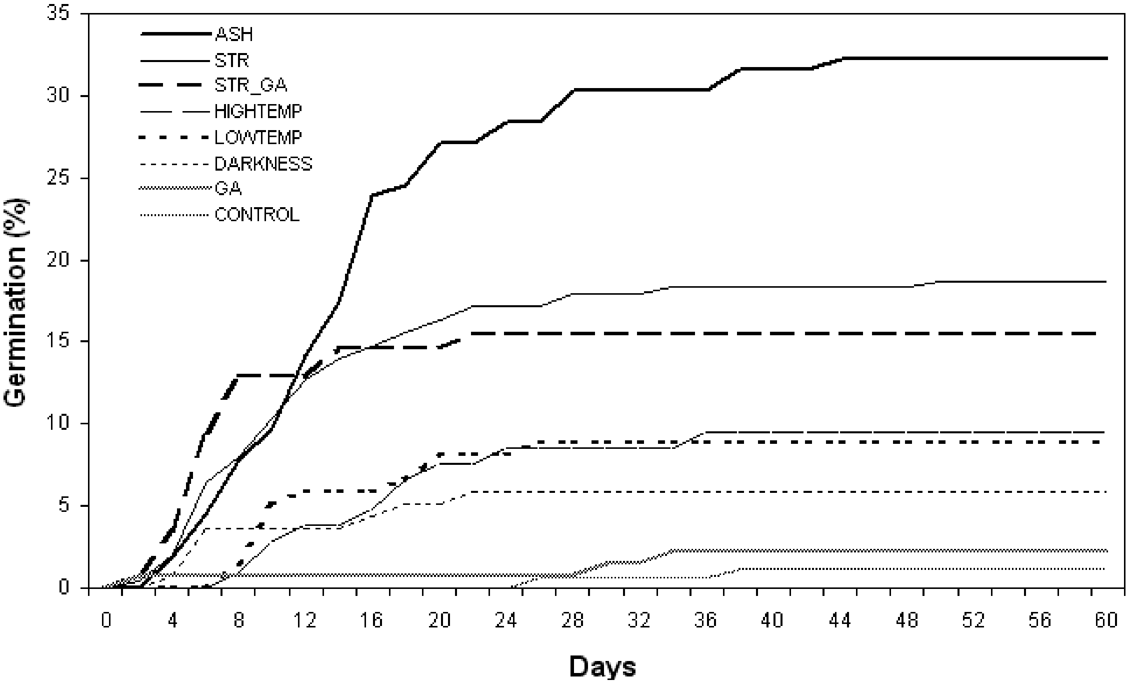

Figure 1. Accumulated germination of *Pseudomisopates rivas-martinezii* in response to germination treatments, expressed as percent of viable seeds germinated. ASH: ash addition; STR: stratification; STR_GA: stratification with GA_3; HIGHTEMP: preheating at 110ºC with stratification; LOWTEMP: preheating at 70ºC with stratification; DARKNESS: no light; GA: GA_3 addition; CONTROL: untreated seeds.

No significant differences were observed between the two heat treatments ($\chi^2 = 0.04$; p = 0.837). LOWTEMP had significantly lower germination than STR ($\chi^2 = 4.774$; p = 0.028), as did HIGHTEMP ($\chi^2 = 3.099$; p = 0.078).

Gibberellic Acid (GA_3) did not produce any stimulatory effect on germination compared to controls. Germination of seeds from the GA treatment was not significantly different to that of CONTROL seeds ($\chi^2 = 1.15$; p = 0.284). Nor was the germination of STR_GA seeds significantly different to STR ($\chi^2 = 0.7$; p = 0.403). DARKNESS also had a diminishing effect in germination compared to STR seeds, even if it had been stratified itself ($\chi^2 = 14.13$; p = 0.0002).

Finally, only 13% of the viable seeds (N = 1047) had germinated at the end of the experiment. Survival analysis revealed significant between-treatment differences in germination timing (Log-Rank, χ^2=29.59; $p < 0.0001$; Wilcoxon, $\chi^2 = 26.41$; $p < 0.0004$), with CONTROL seeds taking the longest time to germinate and STR_GA and DARKNESS seeds taking the shortest (Table 1). Treatment within population significantly affected germination timing ($\chi^2 = 56.844$; $p < 0.001$), whereas population did not ($\chi^2 = 3.031$, $p = 0.2196$).

Discussion

The different levels of viability observed in the three populations may be a consequence of different levels of mate availability among populations. A previous study reported that lowered mate availability decreased levels of seed viability due to high clonality in this self-incompatible species (Chapter 2). The higher abundance, density and vitality of plants in *Lóbrega* population promoted by the fire occurred in 2005, may have increased genetic diversity, mate availability and therefore seed viability. However, this higher viability did not result in higher germination rates.

The present study gives evidence of the high dormancy found in seeds of *P. rivas-martinezii* as previously reported (Martínez *et al.* 2004), with a germination of just 1% on completely untreated seeds (CONTROL, Table 1). A stratification treatment is required for seed germination, as reported for many other mountain species (Körner 2003). In high mountain Mediterranean species cold-wet stratification increased germination, although a relatively high number of these species seemed to be ready to germinate without any treatment (Giménez-Benavides *et al.* 2005).

Surprisingly, the effect obtained by applying gibberellic acid (GA_3) was not significantly different to controls. Gibberelins applied to seeds have been proved very effective in breaking the dormancy of many species that otherwise would require other stimuli that increase gibberelin biosynthesis, such as cold stratification or exposure to light (Derkx & Karssen 1994). In particular, GA_3 has been found to be active in breaking dormancy in other closely related Antirrhinaceae species, such as *Chaenorrhinum minus* (Arnold *et al.* 1996) or *Linaria vulgaris* (Bielefeld 1987). However, since sensitivity to different gibberellins seems to be highly species-specific (Arnold *et al.* 1996), a positive response to a different gibberellin in *P. rivas-martinezii* could be expected.

Darkness lowered germination ability, even if seeds had been wet-cold stratified. This response is congruent with the spatial pattern of the species in its natural habitat, where it preferably occupies clearings and shows diminished flowering by shrub closure (Chapter 2). Although usually light filtrates through canopy (Saatkamp *et al.* 2011), the behaviour observed may increase the chance of seedling establishment in clearings or more open sites. It also indicates that seeds will not probably need to be buried for germination. Reduced or lack of germination in darkness is a common result observed in another Antirrhinaceae species, *Linaria tursica* (Valdés & Díaz 1996), amongst other Iberian endemics (Cabello *et al.* 1998). However, Valdés & Díaz (1996) reported an increase in germination of seeds on soil in contrast to filter paper under darkness conditions.

Interestingly, although preheating did not lower seed viability, seeds showed a significant decrease in germination following heat treatments. This result indicates that although the heat doses applied (70º C and 110º C for 5 min) were not lethal for *P. rivas-martinezii* seeds, its germination was not stimulated either. Hence, seeds that survive fire may subsequently germinate under different stimuli. Species in fire-prone environments can show different germinating behaviours. Some may germinate readily without treatment; some may be stimulated by heat, others by charred wood or by other environmental factors, such as light or sowing medium (Keeley 1987).

Maximum seed germination was obtained by applying ash. Although the chemical compounds of ash were not identified, ash effects on *P. rivas-martinezii* germination are probably chemically-mediated. Species that require the presence of charred wood to break dormancy are also likely to be dependent on the pH of the medium (Sanders *et al.* 1981; Keeley & Fotheringham 1998). However, *P. rivas-martinezii,* which grows in natural acidic soils, exhibited high germination rates on the alkaline medium of ash. Similarly, some *Cistus* species that grow in naturally acidic soils display high germination rates in both the acid medium smoke and the alkaline medium of charred wood. This behaviour suggests that chemicals other than pH seem to have an effect on the seed dormancy of these species (Pérez-Fernández & Rodríguez-Echevarría 2003). Charred wood stimulus is far more specific than heat at timing seedling establishment and some species are almost completely dependent on it; it determines the abundance of these species the first year after burning and in some cases even their disappearance until the next fire (Keeley & Keeley 1987).

Despite aboveground die-back, wildfires may have minimal impact on the survivorship of *P. rivas-martinezii* populations due to deeply buried stolons. Plants have been proven to resprout and flower abundantly after the fire occurred in 2005 in *Lóbrega* population, thus increasing seedling recruitment in postfire years (Bernardo García, personal observation). This behaviour is widely extended in Mediterranean-climate regions, in which fire stimulates flowering of plants with deeply buried bulbs, corms or rhizomes (Horton & Kraebel 1995; Keeley & Bond 1997). Because soil seed banks may not build up between fires, on some sites resprouting is the predominant mode of reestablishment after fire. For instance, postfire recovery studies on Californian chaparral species have shown all species to be present the first growing season as vigorous resprouters, with seedlings being absent. However, the resprouts flowered and fruited abundantly, leaving abundant seedling recruitment in the second growing season (Keeley & Keeley 1984). This ability of some species to use both the sexual and vegetative methods to regenerate has been interpreted as an evolutionary response to fire (Trabaud 1984).

Pseudomisopates rivas-martinezii seeds seem to display physiological dormancy, since seed coat is permeable, seeds required a period of cold stratification, and GA_3 did not increase germination. The response shown by this species suggests that around 20% of the seeds will germinate readily upon wetting by winter rains if seeds remain near the soil surface, but another 15% will remain dormant until exposed to ash. Most seeds will remain dormant, if buried below the level of light penetration or under dense canopy, since germination is inhibited in the dark. Similar results have been reported in other Californian chaparral and coastal sage species (Keeley 1987).

The increased viability and vigorous resprouting observed in *Lóbrega* population, together with the clear trigger effect of ash on germination of *P. rivas-martinezii* indicates that we are dealing with a postfire species. Consequently, fire is not only a chemical stimulus for germination in this species, as results indicate that darkness inhibits germination. Thus, the reduction in competition provoked by fire would be another trigger to germination. These results suggest that fire has both direct and indirect effects on this species, since: (1) it is a chemical stimulus for germination; (2) it creates clearings for seeds, and (3) it enhances resprouting.

In conclusion, *Pseudomisopates rivas-martinezii* seems to shows a specificity to fire. The palynological record of the Gredos Range showed that before the 12th century AD, *Pinus sylvestris* forests were dominant. Whereas, the current landscape of the Gredos Range is clearly anthropogenic, and includes a combination of forest patches, pastures, and dense shrubby formations, mostly generated during the transition between the 17th and 18th centuries AD, when forest management activities, including fire and intense grazing, caused a progressive deforestation and the expansion of the current fire-prone shrubland (López-Merino *et al.* 2009). In this sense, despite being critically-endangered the question remains as to whether this plant is now favoured by human-mediated fire and underwent more critical stages in the past. Consequently, the conservation of this species may rely on the continuity of traditional land management practices.

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Appendix

Results for the Generalized linear mixed models relating treatment to germination, with population as a random factor, for *Pseudomisopates rivas-martinezii* populations. Germination corresponds to percent of viable seeds germinated. ASH: ash addition; STR: stratification; STR_GA: stratification with GA_3; HIGHTEMP: preheating at 110 ºC with stratification; LOWTEMP: preheating at 70 ºC with stratification; DARKNESS: no light; GA: GA_3 addition; CONTROL: untreated seeds.

Generalized linear mixed model fit by the Laplace approximation, without interaction.

Formula: germ ~ treatment + (1 | population)

```
  AIC    BIC   logLik   deviance
  784.8  830.6  -383.4   766.8
```

```
Random effects:
 Groups     Name          Variance   Std.Dev.
 population (Intercept)   0.39367    0.62743
Number of obs: 1200, groups: population, 3
```

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-2.1626	0.4958	-4.361	1.29e-05 ***
LOWTEMP	-0.2175	0.4576	-0.475	0.63457
ASH	1.4787	0.3811	3.88	0.000104 ***
CONTROL	-2.2069	0.7912	-2.789	0.005280 **
STR	0.5816	0.3781	1.538	0.123969
STR_GA	-1.4859	0.6151	-2.416	0.015708 *
DARKNESS	-0.8727	0.5198	-1.679	0.093168 ·
STR_GA	0.2141	0.4315	0.496	0.619842

\-\-\-

Signif. codes: 0 *** 0.001 ** 0.01 * 0.05 ·

Generalized linear mixed model fit by the Laplace approximation, with interaction.

```
Formula: germ ~ treatment + (1 | population) + (1 | treatment:population)

   AIC    BIC   logLik   deviance
  786.6  837.5  -383.3    766.6

Random effects:
 Groups              Name        Variance Std.Dev.
 treatment:population (Intercept) 0.020461 0.14304
 population           (Intercept) 0.380040 0.61647
Number of obs: 1200, groups: treatment:population, 24; population, 3

Fixed effects:
```

	Estimate	Std.Error z	value	Pr(>|z|)
(Intercept)	-2.1747	0.4986	-4.362	1.29e-05 ***
LOWTEMP	-0.2098	0.4737	-0.443	0.657896
ASH	1.4914	0.4001	3.727	0.000193 ***
CONTROL	-2.2039	0.8021	-2.748	0.006001 **
STR	0.5964	0.3966	1.504	0.132595
GA	-1.4706	0.6289	-2.338	0.019365 *
DARKNESS	-0.8588	0.5344	-1.607	0.108069
STR_GA	0.2327	0.4495	0.518	0.6047

```
---
Signif. codes:  0 *** 0.001 ** 0.01 *  0.05 ·
```

Capítulo 4

Effects of human activity on the distribution and abundance of an endangered Mediterranean high-mountain plant (*Erysimum penyalarense*)

Este capítulo se encuentra aceptado para su publicación

en Journal for Nature Conservation

Effects of human activity on the distribution and abundance of an endangered Mediterranean high-mountain plant (*Erysimum penyalarense*)

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Key words: conservation, endemism, *Erysimum penyalarense*, human disturbance, plant distribution.

Abstract

The Iberian mountains are important biodiversity hotspots that face great immediate threats like other species-rich regions on Earth. Preservation of natural communities has historically been based on protecting them from physical disturbance. One of the most important causes of disturbance in natural communities today is human activity. *Erysimum penyalarense* is an endemic and endangered Mediterranean high-mountain herb. In this study, we explore the effect of environmental factors (microhabitat characteristics) and human activity on its abundance and distribution within the only remaining population of the species. Our results indicate that the species is much more abundant than previously thought. The only population known benefited from human disturbance but was negatively affected by animal disturbance and shrub encroachment. Therefore, the net effect of human activity on the species depends on the balance between the different activities carried out within its distribution range, suggesting that intermediate levels of disturbance could help preserve some endangered plants.

Introduction

The Iberian mountains are important biodiversity hotspots (Médail & Quézel 1999). This is the result of climatic, topographic, evolutionary, paleogeographical and historical factors (Quézel 1985; Vargas 2003). Although these factors determine the diversity of plant species, the persistence and establishment of plant populations is critical in the maintenance of this diversity, and has gained importance as a result of global climate change and expected changes in species distribution (Pauli *et al*. 2007). Furthermore, these diverse mountain ecosystems have traditionally been exposed to a significant level of disturbance due to human activity. Assessing appropriate conservation strategies in mountain ecosystems is vital for the preservation of biodiversity in the future. Indeed, for the management of endangered species, an understanding of the factors that determine their distribution and abundance is of paramount importance in order to predict future changes and establish conservation strategies.

Plants are unevenly distributed due to the interaction between evolutionary histories and environmental factors, with climate exerting the dominant control on the distribution of vegetation at large scales (Woodward 1987). At a smaller scale, however, plant distribution may be controlled by many other factors, such as: land use and habitat, soil properties, topography, interspecific competition, herbivory, dispersal ability or inbreeding (Saunier & Wagle 1967; Louda & Rodman 1996; Passos & Oliveira 2002; Gómez 2005, Frei *et al*. 2012; Herrero *et al*. 2012; Pellisier *et al*. 2012). Regardless of these factors, conservation of natural communities has historically been based on protecting them from physical disturbance. Fortunately, the idea that disturbance is an unusual occurrence that disrupts the ordinary processes of a community is changing in both ecologists' and conservationists' minds. This has led to a better understanding of mechanisms of species co-existence and has influenced management practices (Gurevitch *et al*. 2006). Disturbance is, no doubt, an important component of natural ecosystems and plays a crucial role in plant ecology, influencing dramatically the composition, structure, biomass, net primary productivity and turnover rate of plant communities (Picket & White 1985; Nishimura 2006).

One of the most important causes of disturbance in natural communities today is human activity, which includes disturbances caused by land management, domestic animals, spoil heaps and pollution, amongst others. The highly diverse Mediterranean regions (Myers 2000) are no exception to this and face greater immediate threats than any other species-rich regions on Earth due to the stress exerted by their climate (Rundel *et al*. 1998). Indeed, human activities often alter soil characteristics that displace and remove species, and promote the establishment of weedy or ruderal species, due to both increases in nutrients and reduced competition from neighbouring plants (Grime 2002). However, land management may also create landscape heterogeneity that will favour species biodiversity in many different taxa, from birds to mammals, arthropods and plants (e.g. Pulido & Díaz 1992; Benton *et al*. 2003).

The present study analyzes the distribution and abundance of *Erysimum penyalarense* (Pau) Polatschek. This endangered Mediterranean high-mountain species has only one remaining population. The specific aims of this study were to: (1) estimate the abundance of *E. penyalarense*, (2) study characteristics of its habitat and vegetation, (3) illustrate the spatial distribution pattern of the species using interpolation techniques of geographical information systems (GIS) which emphasize the analysis spatially, and (4) explore the environmental factors that determine the abundance of the species using Generalized Linear Models, in order to determine the kind of disturbance to which the species will be most sensitive.

Materials and Methods

Study species and distribution area

Erysimum penyalarense (Pau) Polatscheck (Brassicaceae) is an endangered Mediterranean species endemic to the *Guadarrama* Mountain Range in central Spain. The species inhabits the Mediterranean high mountain grasslands of *Festuca curvifolia* and is catalogued as Endangered (EN) following the IUCN criteria in the Spanish Atlas of Endangered Flora (Martínez *et al*. 2004). It is a 15-25 cm tall, perennial polycarpic herb. Adults have a basal rosette or group of rosettes with lanceolate leaves that produce a few to around a hundred flowers on a variable number of reproductive stalks. Flowers are hermaphroditic, slightly protandrous, with four bright yellow petals and a tetradynamous androecium (four long and two short stamens). The species is a generalist plant that reproduces sexually by cross-pollination, which is carried out by insects. It flowers in early summer (June-July) and bears fruits in siliques in late summer (August-September). Seeds are dispersed by barochory.

The species is known historically from two populations located in the *Guadarrama* Mountain Range (Figure 1). This range is part of the Spanish Central Mountain System and runs southwest-northeast to the north of the Madrid province. The population occurs in a protected area that has recently been established as National Park. Currently, there is just one remaining population in *Cuerda Larga* where plants are locally abundant. The classical population, located in the *Peñalara* Mountain, seems to have become extinct, since it has not been found during the last 25 years (Fernández González 1987). The place has been exposed to intense restoration activities carried out in the *Peñalara* Regional Park during the last decades. The ski resort was closed down in 1999 and vegetation restoration has been carried out in disturbed areas.

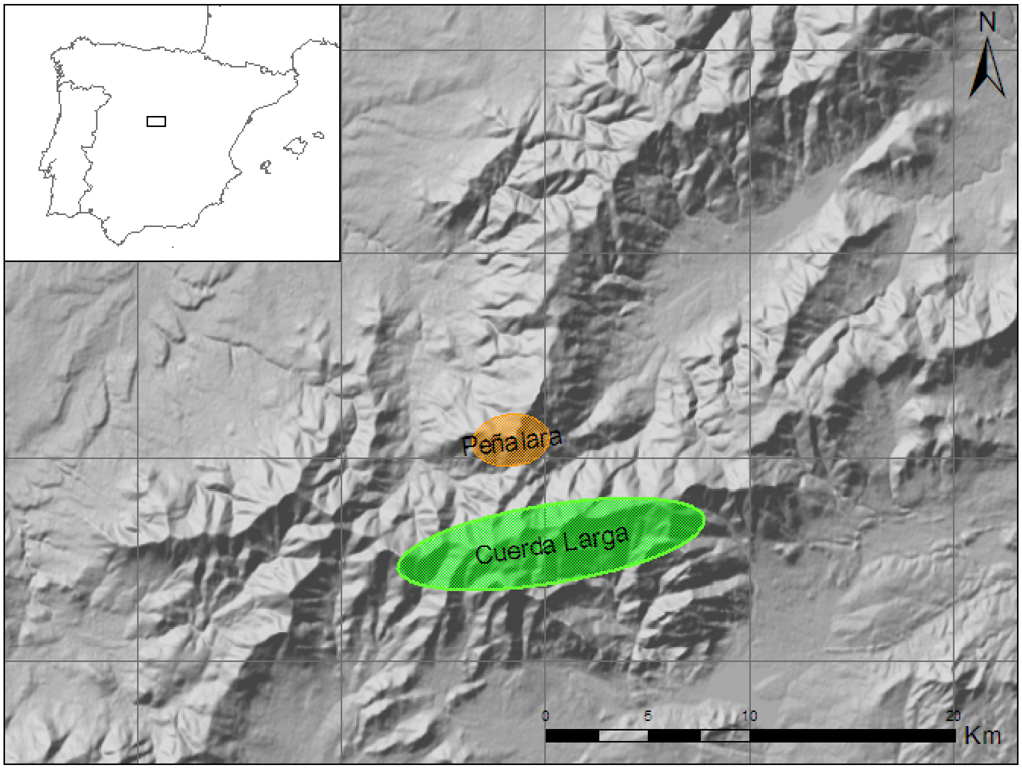

Figure 1. Known geographical distribution of *Erysimum penyalarense* at the beginning of this study, showing actual distribution in green and extinct populations in orange.

Study area

The only existing population of the species in *Cuerda Larga* was studied. This area has traditionally been managed for livestock grazing and leisure activities, with part of the land being occupied by ski resorts. Annual rainfall at this site is about 1,400 mm, with a very pronounced summer drought that comprises less than 10% of the annual precipitation (data from *Navacerrada* weather station 1,890 m). Snow cover remains between 120 and 140 days per year (Palacios *et al*. 2003). Annual average air temperature is 6.4 ºC, whereas the mean monthly temperature ranges from -0.6 ºC in January to 16.3 ºC in August (data from *Navacerrada* weather station, www.aemet.es). These mountains are predominantly composed of Paleozoic granites and sediments (360-290 mya). The dominant vegetation, above the tree line of pine forests (*Pinus sylvestris*), is shrubland (*Cytisus oromediterraneous*), which finally gives way to xeric mountain grassland of *Festuca curvifolia* at higher altitudes.

Estimation of abundance

The effective occupancy area (Pisanu *et al*. 2009) of the species was delimited by using aerial photos, and surveying the *Cuerda Larga* to exclude unsuitable sites or those where the plant was not present. A simple random design was conducted within the delimited area, where eighty 1m^2 permanent quadrats were selected, in which the number of individuals was recorded according to their vital stage. The stage classes recorded were: seedlings, adults and reproductive adults. Juveniles were hard to assign, since the size of rosettes depends very much on microhabitat characteristics and was not considered to be reliable as an indication of age. The estimation of abundance was eventually performed excluding seedlings.

Habitat and vegetation variables

To estimate microhabitat characteristics, the cover of the following set of variables was estimated to the nearest 1% per quadrat, using a 10 x 10 grid, with all variables adding up to 100%: (1) HC: cover of herbaceous plants; (2) SH: shrub cover; (3) ML: moss and lichens; (4) SO: bare soil cover; and (5) RO: rock cover. In addition, (6) RI: richness of the accompanying plant species and (7) CO: cover of each plant species recorded per quadrat was also estimated (see Appendix 1 for details on species). Furthermore, we recorded four more qualitative variables related to human activity, by evaluating the presence-absence of: (8) HD: human disturbance caused by works *i.e.* ski lanes, spoil heaps from infrastructures or road edges; (9) HU: human use *i.e.* walk paths or litter; (10) AD: animal disturbance *i.e.* trampling, grazing or droppings caused by cattle, sheep, horses and to a lesser extend the Spanish ibex (*Capra pyrenaica*), and (11) GR: grazing on *E. penyalarense*. Finally, a set of environmental variables was recorded in each quadrat: (12) ALT: altitude; (13) SL: slope, and (14) ASP: aspect.

Data analysis

Estimation of abundance

The dispersion of *E. penyalarense* abundance data between plots within its occupancy area was determined by the Standardized Morisita Index of Dispersion (I_P) in order to estimate plant abundance. This index is one of the best dispersion measures, since it is independent of population density and sample size (Myers 1978). Plant abundance (plants per square metre) was fitted to the Negative Binomial distribution according to the dispersion of data, and goodness of fit to this distribution was calculated with the U-statistic (Krebs 1998). Analyses were performed using *Ecological Methodology v.6.1.* (Krebs 1998).

Habitat and vegetation

Habitat characteristics were first explored and diversity of accompanying plants in the *E. penyalarense* quadrats was described by means of: (a) species richness, calculated as the number of species found in the sample (S_{obs}), and two asymptotic richness estimates, the incidence coverage estimator (S_{ICE}) and the Michaelis–Menten index (S_{MM}) using EstimateS software (Colwell 2005); (b) evenness was calculated using the Smith & Wilson index using Ecological Methodology v.6.1. (Krebs 1998); (c) the Hulbert’s PIE diversity index (Colwell 2005) calculated by a randomization process using EcoSim (Gotelli & Entsminger 2004), and (d) dominance, calculated as the relative abundance of the most abundant species in the sample.

Among-quadrat differences in plant abundance and composition were tested using a permutational multivariate analysis of variance with distance matrices, using the 'adonis' function (McArdle & Anderson 2001; Anderson 2001) in the library vegan of R 2.1.1 (R Development Core Team 2008). A Correspondence Analysis was used to relate the abundance of community species to *E. penyalarense* presence using STATISTICA. Species with a 5% cover or below were removed from the analysis. In addition, relation of species richness and abundance to environmental variables was explored with General Linear Models using R 2.1.1 (R Development Core Team 2008).

Spatial distribution pattern

The effective occupancy area and the abundance data were integrated into a GIS to produce a relative abundance map using ArcGis 10. The density contour map was created using the IDW interpolation technique, which works on the basis of spatial autocorrelation, assuming that the values of the variables assigned to unsurveyed locations are an average of the values for the contiguous sampled sites, weighted by the distance to these sites. This technique weighs distance to the closest sites and is inversely proportional to the distance between unsampled and sampled points (Isaaks & Srivastava 1989). There are many reasons why plant populations might be expected to exhibit spatial autocorrelation (soil properties, clonal growth or competition), making interpolation a valid procedure (Isaaks & Srivastava 1989). Although plant abundance may vary at a local scale within the occurrence area due to microhabitat characteristics, global statistics may detect the large-scale trend in abundance, making interpolation useful (Fortin & Dale 2005). Finally, spatial autocorrelation in plant abundance across the effective occupancy area was tested by means of Moran's I coefficient with 300 permutations (Rangel *et al.* 2010).

Factors correlating with plant abundance

We evaluated the effects of microhabitat (HC, SH, ML, SO, RO, RI, CO, ALT, SL, ASP) and disturbance (HD, HU, AD, GR) characteristics on plant abundance of *E. penyalarense,* using spatially-explicit generalized linear models. Different spatial structures were assumed (Exponential, Gaussian, Linear, Rational Quadratic and Spherical). Spatial GLM models were then compared with non-spatial GLM models. Analyses were performed using the library nlme of R 2.1.1 (R Development Core Team 2008). However, since no significant differences were found between spatial and non-spatial models (Table S2), data were analyzed using non-spatial models. Thus, a Generalized Linear Model with a Negative Binomial distribution and log link function was fitted using SPSS 18. Due to the high number of independent variables, all variables were explored separately, and the most ecologically informative combinations were explored in models including no more than five variables. The best fitting models were finally selected according to the Akaike's information criterion (AIC) (Table S3).

Results

Estimation of abundance

The population shows a clumped distribution as indicated by the Standardized Morisita Index ($I_P = 0.55$). Plants were present in just 29 plots out of the 80 plots sampled. Consequently, the abundance of the population was described by the Negative Binomial distribution (mean = 4.24; k = 0.1273; N = 80). This was the most suitable descriptor as shown by the U-statistic, since U < 2 S.E (U = -2.83; Standard error of U = 63.13). The effective occupancy area comprised 7.09 Km^2 (9 Km^2, using a 1 x 1 Km grid, according to IUCN criteria) in which the density of individuals per m^2 was 4.24 ± 2.65 with 95% confidence. This outcome suggests an overall population size of at least 11 million individuals. According to the frequency of appearance (36% of plots), we would expect the species to actually occupy 2.57 Km^2 within the estimated area.

Habitat and vegetation

The only existing population of *Erysimum penyalarense* is distributed across the *Cuerda Larga* and the *Maliciosa* mountains. It can be found from the highest elevation at *Cabezas de Hierro* (2,346 m), descending along the cleared areas of its northern face down to *Puerto de Navacerrada* (1,880 m), avoiding the dense *Cytisus* shrubland on the southern face, the *Nardus stricta grasslands* and the screes. The effective occupancy area is composed of 50.59% ± 2.76 plant cover (of which 31.90% ± 2.28 were herbaceous species, 16.84% ± 3.18 were woody species, 2.14% ± 0.45 were mosses and lichens); 41.65% ± 2.80 stones and rocks, and 7.49% ± 1.57 bare soil. Shrub cover was negatively related to animal disturbance (Appendix 4; GLM: β_{AD} = -18.865; t-value = -3.048; p = 0.003). Grazing pressure by domestic and wild ungulates in the area was quite strong, since evidence of animal presence (droppings, walk paths or grazing) was recorded in 60% of the quadrats sampled. However, damage to *E. penyalarense* was only recorded in 3.8% of quadrats. Overall, anthropic evidence within quadrats was 21.25% ± 0.05, with 10% ± 0.03 due to human use and 11.25% ± 0.04 due to human disturbance.

The plant community was composed of 54 species ((S_{obs}) $IC_{95\%}$ = 49.64-58.64) (Appendix 1), belonging to 18 families and 48 genera. The most represented families were Poaceae (12 spp.), Asteraceae (11 spp.), Caryophyllaceae (5 spp.), Crassulaceae, Brassicaceae and Plantaginaceae (4 spp.). However, the expected species richness according to asymptotic estimates was S_{ICE} = 64.05 and S_{MM} = 56.89, suggesting that the community composition was probably richer than that inferred from our sampling. Hulbert's PIE index was 0.80 ($IC_{95\%}$ = 0.69-0.87) for the population and the evenness index was 0.20. Dominance was 0.39, with few species scoring a relative abundance higher than 3%. In particular, the most dominant species, according to their relative cover, were *Juniperus communis* (0.39), *Festuca curvifolia* (0.16), *Cytisus oromediterraneus* (0.10) and *Deschampsia flexuosa* (0.09) (Appendix 1).

Richness of accompanying species was significantly and positively related to slope (GLM: β_{SL} = 0.055; t-value = 2.907; p = 0.005) and negatively related to shrub cover (GLM: β_{SH} = -0.028; t-value = -2.801; p = 0.007); whereas the cover of these species was significantly and negatively related to human disturbance (GLM: β_{HD} = -30.399; t-value = -3.049; p = 0.003) and animal disturbance (GLM: β_{AD} = -13.318; t-value = -2.074; p = 0.041). There were significant among-quadrat differences in plant composition depending on *Erysimum* presence (ADONIS, F = 2.58, p < 0.01). The percentage cover of *Agrostis castellana, Armeria caespitosa, Campanula herminii, Cerastium ramosissimum, Festuca iberica, Jurinea humillis, Leucanthemopsis pallida* and *Thymus praecox* accounted for most of the variation in the CA solution, as they were strongly correlated with dimension 1, which extracted 100% of total inertia (Table 1). Presence of *E. penyalarense* was positively correlated to the presence of *J. humilis (0.11), A. castellana (0.08), A. caespitosa (0.08), C.*

herminii (0.08) and L. pallida (0.05), and the almost absence of *F. iberica (0.21), C. ramosissimum (0.08),* and *T. praecox (0.08).*

Acompanying speces	Coordinates Dim. 1	Inertia Dim.1
Agrostis castellana	-0.04101	**0.08365**
Agrostis truncatula subsp. truncatula	-0.014182	0.010405
Armeria caespitosa	-0.048703	**0.080225**
Biscutella intermedia subsp. pauana	0.015407	0.008028
Campanula herminii	0.062032	**0.084212**
Cerastium ramosissimum	0.075242	**0.084476**
Criptograma crispa	0.010658	0.003051
Cytisus oromediterraneus	-0.010761	0.005644
Deschampsia flexuosa	0.006776	0.004751
Festuca curvifolia	-0.013087	0.026065
Festuca iberica	0.058836	**0.213498**
Jasione crispa	-0.002484	0.000528
Juniperus communis subsp. alpina	-0.023412	0.059433
Jurinea humilis	-0.070073	**0.117228**
Leucanthemopsis pallida	-0.044124	0.054228
Luzula hispanica	0.033724	0.031677
Paronychia polygonifolia	-0.014182	0.005202
Pilosella vahlii	0.020749	0.020557
Rumex acetosella	0.013305	0.010741
Sedum brevifolium	0.009974	0.011676
Senecio pyrenaicus subsp.carpetanus	-0.024662	0.00968
Silene ciliata	-0.000401	0.000012
Thymus praecox	0.040493	**0.07503**

Table 1. Correspondence analysis for accompanying plant species of *Erysimum penyalarense.* Variables with a contribution to the inertia greater than 0.07 in bold.

Spatial distribution and abundance pattern of E. penyalarense

Erysimum penyalarense was most abundant on the furthest edges of its occupancy area, especially on the western end (Figure 2). Significant autocorrelation measured by Moran's I was not biologically significant, since it was only found at the second distance class (from 320 to 860 m) for two of the variables tested, *E. penyalarense* abundance (I = 0.163; p = 0.007) and human disturbance (I = 0.274; p = 0.003). But also at the fourth distance class (from 1400 to 2000) for human disturbance (I = 0.105; p = 0.023).

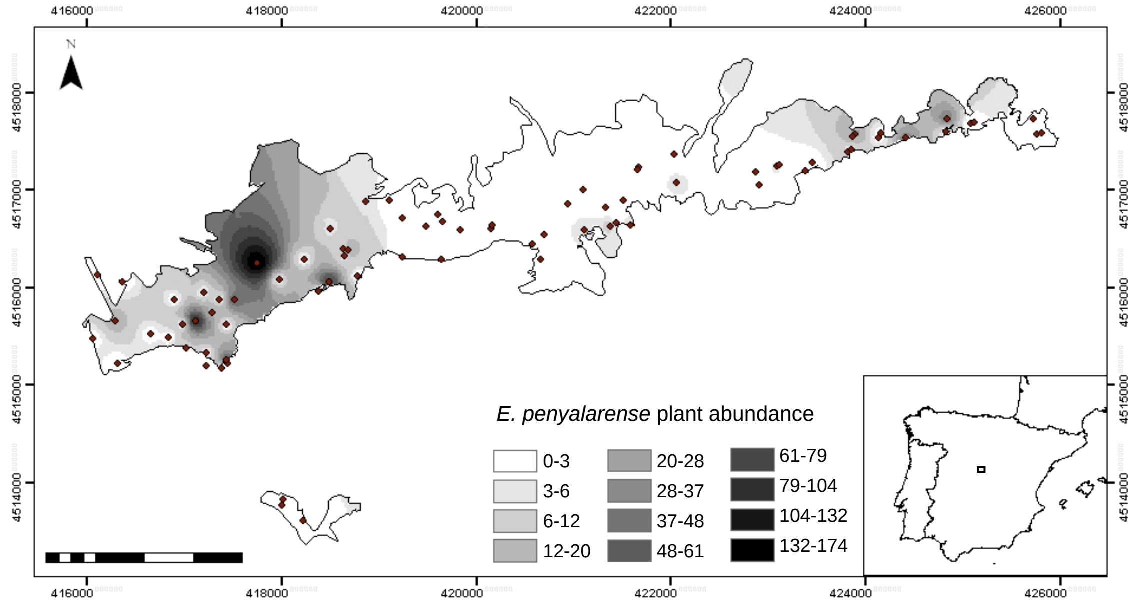

Figure 2. General distribution pattern of relative abundance of *Erysimum penyalarense* in Spain obtained using the inverse distance weighted (IDW) interpolation method.

Human activity was the main factor explaining the difference in abundance among quadrats (Table 2). Thus, we found that in all selected models, human disturbance was positively related to plant abundance, whereas human use and animal disturbance were negatively related to plant abundance (Table 2). Finally, shrub cover was also negatively related, but its effect was marginally significant. Additionally, the best models also included slope, which was negatively related to abundance, and aspect, with north-oriented quadrats showing higher abundance, although these two variables were not significant (Table 2).

Plant abundance variables	Coeff ± Std Error	95% Lower	95% Upper	Wald Chi^2	p-value
MODEL 1					
Intercept	1.242 ± 0.4560	0.348	2.136	7.416	0.006
Human disturbance	1.578 ± 0.1537	-1.801	-1.199	95.32	<0.000
Human use	-2.101 ± 0.4314	1.255	2.946	23.716	<0.000
Shrub cover	-0.007 ± 0.0025	0.328	0.806	21.579	<0.000
Animal disturbance	-0.567 ± 0.1220	-0.012	-0.002	8.02	0.005
Slope	-0.009 ± 0.0049	-0.018	0.001	3.144	0.076

Plant abundance variables	Coeff ± Std Error	95% Lower	95% Upper	Wald Chi^2	p-value
MODEL 2					
Intercept	1.177 ± 0.4544	0.286	2.068	6.709	0.01
Human disturbance	1.578 ± 0.1477	-1.867	-1.288	114.105	<0.000
Human use	-2.063 ± 0.4304	1.220	2.907	22.981	<0.000
Shrub cover	-0.532 ± 0.1202	-0.011	-0.011	6.488	<0.011
Animal disturbance	-0.008 ± 0.0020	0.297	0.768	19.614	<0.000
MODEL 3					
Intercept	1.369 ± 0.4922	0.404	2.068	7.738	0.005
Human disturbance	1.637 ± 0.1548	-1.941	-1.288	111.939	<0.000
Human use	-1.871 ± 0.4436	1.001	2.907	17.785	<0.000
Animal disturbance	-0.443 ± 0.1283	0.192	0.695	11.941	0.001
Shrub cover	-0.006 ± 0.0024	-0.01	-0.001	5.343	0.021
Aspect					
[aspect=N]	0.199 ±0.1699	-0.134	0.532	1.372	0.241
[aspect=E]	-0.145 ±0.2433	-0.622	0.332	0.355	0.551
[aspect=S]	-0.118 ±0.2138	-0.537	0.301	0.303	0.582
[aspect=W]	-	-	-	-	-

Table 2. Results for the best generalized linear models selected by the AIC procedure. Redundant parameters marked with dash.

Discussion

Population size and microhabitat requirements

The number of individuals of *Erysimum penyalarense* is much greater than that of prior estimates (43 times higher), although it occupies a smaller area (69% lower) (Martínez *et al.* 2004). The study site contained a considerable sample of the grassland species of the *Guadarrama* mountain range (Fernández González 1987; Luceño & Vargas 1991) and showed dominance of some few species. Richness of accompanying species was increased by slope as a result of microhabitat heterogeneity, and reduced by shrub cover. This shows that the control exerted by ungulates over shrubs may promote high plant diversity. Surprisingly the proportion of woody species (49.27%) was almost as high as that of herbaceous species (51.73%), the dominant species being *Juniperus communis*. This result corroborates the observations of Sanz-Elorza *et al*. (2003) which indicate a clear regression of the high mountain grassland in the *Peñalara* mountain (*Guadarrama* mountain range), where it is being overgrown by patches of *Juniperus* and *Cytisus* as shown by aerial photos of 1957 and 1991. Therefore, *E. penyalarense,* as well as the rest of the Mediterranean high mountain species, could eventually be overgrown by the advancing shrubland of *Cytisus* and *Juniperus* due to climate change and abandonment of traditional land managing practices. Indeed a certain amount of pressure from herbivores could be contributing to the maintenance of this high mountain grassland, since shrub cover was negatively related to animal disturbance. The true effects of herbivores on the community would require further investigation.

The species exhibits a clumped distribution, probably due to the short distance dispersal of its seeds, as in other *Erysimum* species (Gómez 2007), as well as to microhabitat requirements for recruitment. The low colonization ability shown is a reported characteristic for narrow endemic species (e.g. Fiedler 1987; Byers & Meagher 1997). Narrow endemic species often differ significantly from their widespread congeners for a number of attributes, for instance, they occur at higher altitudes, on steeper slopes, surrounded by higher rock cover and in more open vegetation. The lower aboveground competition met under these circumstances may have played an important role in the differentiation of narrow endemic Mediterranean species (Lavergne *et al*. 2004). Accordingly, the study species is not as abundant in the cooler or fresher places where *Festuca iberica, Campanula herminii* or *Thymus praecox* occur. Instead, it preferably occupies drier environments of the high mountain grassland. Indeed, the species may well be more adapted to stressful conditions and be unable to compete for resources in more productive sites.

Effects of disturbance on population performance

Mountain ecosystems are harsh environments in which species richness is generally controlled by environmental stress intensity, with disturbance and competition playing a secondary role by fine-tuning plant communities' diversity (Kammer & Möhl 2002). Soil disturbances which occur naturally, can also be artificially favoured by human activity, and be a way to introduce alien species and destroy habitats in Mediterranean ecosystems (Gaertner *et al.* 2009). For instance, human disturbance in the study are has introduced species from other habitats, such as *Puccinellia festuciformis* (González Bernáldez 1997), which must have been carried along with the sand used to build the infrastructures around the ski resort, despite being a protected area. Other Mediterranean ecosystems, where human agriculture and other activities have long created soil disturbance, contain a large and successful group of disturbance-prone weedy species (Naveh 1967). Nevertheless, species respond differently to disturbance effects. In this study, *E. penyalarense* was more abundant in the more human disturbed and frequented sites within its effective occupancy area. These sites are found on the furthest edges of its distribution, and show disturbance events which include turn over of the soil at road edges, ski lanes and infrastructures. The common occurrence of the species in such disturbed habitats indicates that we are dealing with a disturbance-prone plant species that declines under strong competition. Many *Erysimum* species (Blanca *et al.* 2009; Nieto 1996), as well as other endangered species (e.g. *Centaurea horrida* in Farris *et al.* 2009), are benefited by disturbance.

Animal disturbance in the area was another source for population decrease. The reduction in our study species due to animal evidence, however, was not a result of grazing, since only 3.8% of plots showed some level of grazing on just a few flowering stalks. Therefore, we hypothesize that the negative effect of humans and animals must be caused by trampling, which could cause indirect plant damage. In fact, trampling has been proven to affect, not only individual performance, but also population dynamics, spatial structure, habitat distribution and niche width of *Erysimum mediohispanicum*, *E. baeticum bastetanum* and *E. popovii*, with plants growing under shrubs suffering less damage than those on open sites and over 50% of seedlings dying due to ungulate trampling (Gómez 2005; Fernández & Gómez 2012). Similarly, *E. penyalarense* can often be seen growing under shrubs. Although animal urine and droppings can be an additional source of nitrogen in the soil, the poor competition ability of *E. penyalarense* must impede its proliferation under these circumstances, whereas it is able to grow abundantly on presumably nitrogen rich severely disturbed areas in the absence of competitors.

In conclusion, human use had a significantly higher negative effect than the positive effect observed for human disturbance on the abundance of *E. penyalarense*. Animal disturbance also showed a negative effect, but this was not as distressing as that caused by humans. Overall, the negative effect of human use and animal disturbance together cancels out the positive effect of human disturbance, so that the net effect results in a decrease of the population ($\beta_{\text{NET EFFECT}} = -1.09$). Things get even worse when considering the total impact of such disturbance events on the site. Considering the total area of 7.1 Km^2, results have shown that 60% of the area is affected by animal disturbance and 10% by human use. Overall, 64% of the area is affected by these two types of disturbances, whereas only 11% is affected by human disturbance. As a result, a population composed by 11 million individuals would decrease by 2.4 million individuals (22% of the population) due to the negative effects of disturbance. Eventually, moderate frequencies or intensities of disturbance should promote maximum species richness as expected by the intermediate disturbance hypothesis (Roxburg *et al.* 2004; Connell 1978; Fox 1981).

Implications for management

Temporary changes of resources due to disturbance events, which are accompanied by reduced competition from neighbouring plants, are directly responsible for enhanced establishment of *E. penyalarense*. However, although a certain pressure of herbivores could be contributing to the maintenance of mountain grassland, an excess of animal trampling could have an overall negative effect. Such a negative effect adds to that caused by human trampling, although this effect is often confined to the proximity of paths. Nevertheless, managers should be cautious in order not to exceed the carrying capacity of this fragile ecosystem.

The observed performance leads us to consider its potential presence in surrounding mountains or disturbed areas along its mountain range. However, its absence from other localities, in principle suitable for occurrence, needs further investigation (species distribution modelling, field exploration and genetic diversity). Consequently, we hypothesize that the extinction of the species in its classical locality could be the result of the intense restoration activities carried out in the *Peñalara* Regional Park during the past decades. The closing down of the ski resort and the works for vegetation restoration carried out in disturbed areas may have been the cause for displacing this species from its classical location.

The results of this study have important implications for the design of conservation strategies for *E. penyalarense*. Thus, considering the requirements of this species, we believe that: (1) reintroduction in its classical location is advised; (2) domestic livestock must be strictly controlled; (3) human use must be controlled and confined to marked paths, and (3) intermediate levels of disturbance must be allowed in its natural ecosystem.

Concluding remarks

Erysimum penyalarense shows a rather pioneer behaviour, in that it is not a good competitor and in that it benefits from disturbance. Although human opening of clearings favours its expansion and the species shows some protection from grazing, both animal disturbance and human use appear to strongly affect the species through plant damage. Therefore, the net effect of human activity on the species depends on the balance between the different activities carried out within its distribution range. Both domestic livestock and human use must be strictly controlled in protected areas. Finally, we suggest that an intermediate level of disturbance could help preserve some endangered plants.

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APPENDIX

Table S1. Species sampled in quadrats in the study area of *Erysimum penyalarense*.

SPECIES	FAMILY	ENDEMICITY	DISTRIBUTION	LIFE FORM	SPECIALIZATION	RELATIVE ABUNDANCE
Agrostis castellana Boiss. & Reuter	Poaceae	Non endemic	Mediterranean	*H*	Mountain	0.0051
Agrostis truncatula Parl. *subsp.* ***truncatula***	Poaceae	Iberian Peninsula	Iberian Peninsula	*H*	Mountain	0.0109
Arabis hirsuta (L.) Scop.	Brassicaceae	Non endemic	Eurosiberian	*T*		0.0011
Armeria caespitosa (Gómez Ortega) Boiss.	Plumbaginaceae	Guadarrama range	Iberian Peninsula	*C*	Mountain	0.0044
Biscutella intermedia Gouan subsp. ***pauana*** A. González & G. López	Brassicaceae	Central System	Iberian Peninsula	*C*	Mountain	0.0047
Bromus hordeaceus L.	Poaceae	Non endemic	Eurosiberian	*T*		0.0007
Campanula herminii Hoffmanns & Link	Campanulaceae	Iberian Peninsula	Iberian Peninsula	*C*	Mountain	0.0084
Cerastium ramosissimum Boiss.	Cariofilaceae	Non endemic	Mediterranean	*T*	Mountain	0.0022
Chaetopogon fasciculatus (Link) Hayek	Poaceae	Non endemic	Mediterranean	*T*		0.0022
Criptograma crispa (L.) R. Br. ex Hooker	Adiantaceae	Non endemic	Eurosiberian	*G*	Mountain	0.0065
Cytisus oromediterraneus Rivas Martínez & al.	Leguminosae	Iberian Peninsula	Iberian Peninsula	*F*	Mountain	0.0985
Deschampsia flexuosa (L.) Trim.	Poaceae	Non endemic	Subcosmopolitan	*H*		0.0908
Digitalis purpurea L.	Plantaginaceae	Non endemic	Eurosiberian	*H*	Mountain	0.0004
Erophila verna (L.) Chevall.	Brassicaceae	Non endemic	Subcosmopolitan	*T*		0.0004
Erysimum penyalarense (Pau) Polatschek	Brassicaceae	Guadarrama range	Iberian Peninsula	*C*	Mountain	0.0120
Festuca indigesta Boiss. subsp. ***curvifolia*** Rivas Martínez & al.	Poaceae	Iberian Peninsula	Iberian Peninsula	*H*	Mountain	0.1639
Festuca iberica (Haeckel) Kerguélen	Poaceae	Iberian Peninsula	Iberian Peninsula	*H*	Mountain	0.0338
Gagea soleirolii F.W. Schultz	Liliaceae	Iberian Peninsula	Iberian Peninsula	*G*	Mountain	0.0011
Gentiana lutea L.	Gentianaceae	Non endemic	Eurosiberian	*H*	Mountain	0.0015
Hieracium carpetanum Willk.	Asteraceae	Iberian Peninsula	Iberian Peninsula	*H*	Mountain	0.0004
Jasione crispa (Pourret) Samp. subsp. ***centralis*** (Rivas Martínez) Tutin	Campanulaceae	Iberian Peninsula	Iberian Peninsula	*C*	Mountain	0.0196
Jasione laevis Lam.	Campanulaceae	Non endemic	Eurosiberian	*C*	Mountain	0.0007

SPECIES	FAMILY	ENDEMICITY	DISTRIBUTION	LIFE FORM	SPECIALIZATION	RELATIVE ABUNDANCE
Juniperus communis L. *subsp.* ***alpina*** (Suter) Čelak	Cupressaceae	Non endemic	Eurosiberian	*C*	Mountain	0.3943
Jurinea humilis (Desf.)	Asteraceae	Non endemic	Mediterranean	*H*	Mountain	0.0055
Koeleria caudata (Link) Steudel subsp. ***crassipes*** (Lange) Rivas Martínez	Poaceae	Iberian Peninsula	Iberian Peninsula	*H*	Mountain	0.0015
Leontodon carpetanus Lange	Asteraceae	Iberian Peninsula	Eurosiberian	*H*		0.0018
Leucanthemopsis pallida (Miller) Heywood	Asteraceae	Iberian Peninsula	Iberian Peninsula	*H*	Mountain	0.0051
Linaria saxatilis (L.) Chaz.	Plantaginaceae	Iberian Peninsula	Iberian Peninsula	*H*		0.0007
Luzula hispanica Chrtek & Krísa	Juncaceae	Iberian Peninsula	Iberian Peninsula	*H*	Mountain	0.0058
Micropyrum tenellum (L) Link	Poaceae	Non endemic	Multi-regional	*T*		0.0011
Minuartia recurva (All.) Schinz & Thell.	Cariofilaceae	Non endemic	Multi-regional	*C*	Mountain	0.0044
Mucizonia lagascae (Pau) M. Laínz subsp. ***sedoides*** (DC.) D.A. Webb.	Crassulaceae	Iberian Peninsula	Iberian Peninsula	*C*	Mountain	0.0015
Narcisus bulbocodium L.	Amaryllidaceae	Iberian Peninsula	Iberian Peninsula	*G*		0.0007
Nardus stricta L.	Poaceae	Non endemic	Eurosiberian	*H*	Mountain	0.0018
Paronychia polygonifolia (Vill.) D.C.	Cariofilaceae	Non endemic	Eurosiberian	*C*	Mountain	0.0033
Phyteuma hemisphaericum L.	Asteraceae	Non endemic	Eurosiberian	*H*	Mountain	0.0004
Pilosella vahlii (Froel.) F.W. Sch. & Sch. Bip	Asteraceae	Iberian Peninsula	Iberian Peninsula	*H*	Mountain	0.0091
Plantago alpina L.	Plantaginaceae	Non endemic	Eurosiberian	*H*	Mountain	0.0011
Poa bulbosa L. var. ***vivipara*** Borckh.	Poaceae	Non endemic	Multi-regional	*H*		0.0004
Puccinellia festuciformis (Host) Parl.	Poaceae	Non endemic	Mediterranean	*H*		0.0004
Ranunculus bulbosus L. subsp. ***aleae*** (Willk.) Rouy & Fouc.	Ranunculaceae	Non endemic	Eurosiberian	*H*		0.0007
Rumex acetosella L.	Polygonaceae	Non endemic	Subcosmopolitan	*H*		0.0091
Sedum amplexicaule D.C.	Crassulaceae	Non endemic	Mediterranean	*C*		0.0004
Sedum album L.	Crassulaceae	Non endemic	Mediterranean	*C*		0.0004
Sedum brevifolium D.C.	Crassulaceae	Iberian Peninsula	Iberian Peninsula	*C*	Mountain	0.0007
Senecio boissieri D.C.	Asteraceae	Non endemic	Boreo-alpine	*H*	Mountain	0.0171

SPECIES	FAMILY	ENDEMICITY	DISTRIBUTION	LIFE FORM	SPECIALIZATION	RELATIVE ABUNDANCE
Senecio jacobea L.	Asteraceae	Non endemic	Eurosiberian	*H*		0.0007
Senecio pyrenaicus L. subsp. ***carpetanus*** (Willk.) Rivas Martínez	Asteraceae	Iberian Peninsula	Eurosiberian	*H*	Mountain	0.0058
Silene ciliata Pourret	Cariofilaceae	Iberian Peninsula	Iberian Peninsula	*H*	Mountain	0.0233
Solidago virga-aurea L.	Asteraceae	Non endemic	Eurosiberian	*H*		0.0004
Spergula morisonii Boreau	Cariofilaceae	Non endemic	Multi-regional	*T*		0.0004
Taraxacum Weber gp. ***officinale***	Asteraceae	Non endemic	Subcosmopolitan	*H*		0.0007
Thymus praecox Opiz	Labiatae	Non endemic	Eurosiberian	*C*	Mountain	0.0320
Veronica serpyllifolia L. subsp. ***langei*** (Lacaita) Laínz	Plantaginaceae	Iberian Peninsula	Iberian Peninsula	*H*	Mountain	0.0007

Table S2. Spatially-explicit generalized model (GLS) compared with non-spatial GLSs for the habitat and disturbance variables explored to explain the abundance of *Erysimum penyalarense.*

FACTOR	**SPATIAL CORRELATION**	**AIC**	**ANOVA**	
			L-ratio	**p-value**
Human disturbance (HD)	gls	725.67		
	Exponential	727.03	0.64	0.43
	Gaussian	727.45	0.22	0.64
	Linear	727.29	0.38	0.54
	Rational quadratic	727.41	0.26	0.61
	Spherical	727.06	0.61	0.43
Human use (HU)	gls	737.69		
	Exponential	736.39	3.29	0.07
	Gaussian	738.12	1.58	0.21
	Linear	NA	NA	NA
	Rational quadratic	737.12	2.56	0.11
	Spherical	736.22	3.47	0.06
Animal disturbance (AD)	gls	737.82		
	Exponential	737.99	1.83	0.18
	Gaus	738.22	1.60	0.21
	Linear	NA	NA	NA
	Rational quadratic	738.09	1.74	0.19
	Spherical	737.23	2.59	0.11
Grazing (GR)	gls	737.52		
	Exponential	737.48	2.05	0.15
	Gaussian	738.19	1.34	0.25
	Linear	736.66	2.86	0.09
	Rational quadratic	737.68	1.84	0.18
	Spherical	736.92	2.59	0.11

FACTOR	SPATIAL CORRELATION	AIC	ANOVA L-ratio	 p-value
Herbaceous plant cover (HC)	gls	746.88		
	Exponential	747.26	1.62	0.20
	Gaussian	747.99	0.88	0.35
	Linear	746.24	2.64	0.10
	Rational quadratic	747.49	1.38	0.24
	Spherical	746.62	2.26	0.13
Shrub cover (SH)	gls	746.93		
	Exponential	747.22	1.71	0.19
	Gaussian	748.02	0.91	0.34
	Linear	746.67	2.26	0.13
	Rational quadratic	747.55	1.38	0.24
	Spherical	746.85	2.84	0.15
Bare soil cover (SO)	gls	742.84		
	Exponential	743.42	1.42	0.23
	Gaussian	743.87	0.98	0.32
	Linear	743.28	1.56	0.21
	Rational quadratic	743.59	1.24	0.26
	Spherical	743.25	1.59	0.21
Moss and liquens cover (ML)	gls	742.46		
	Exponential	742.81	1.66	0.19
	Gaussian	743.28	1.18	0.28
	Linear	NA	NA	NA
	Rational quadratic	742.98	1.48	0.22
	Spherical	742.28	2.18	0.14
Rock cover (RO)	gls	747.39		
	Exponential	746.76	2.63	0.10
	Gaussian	746.97	2.42	0.12
	Linear	745.26	4.13	0.04
	Rational quadratic	746.64	2.76	0.09
	Spherical	745.75	3.64	0.06

FACTOR	SPATIAL CORRELATION	AIC	ANOVA	
			L-ratio	p-value
Altitude (ALT)	gls	749.25		
	Exponential	748.79	2.46	0.12
	Gaussian	749.62	1.63	0.20
	Linear	747.93	3.32	0.07
	Rational quadratic	749.03	2.23	0.14
	Spherical	748.21	3.05	0.08
Slope (SL)	gls	746.00		
	Exponential	745.56	2.44	0.12
	Gaussian	746.41	1.59	0.21
	Linear	NA	NA	NA
	Rational quadratic	745.79	2.21	0.14
	Spherical	744.91	3.09	0.08
Aspect (ASP)	gls	750.30		
	Exponential	750.25	2.05	0.15
	Gaussian	750.86	1.44	0.23
	Linear	NA	NA	NA
	Rational quadratic	750.43	1.87	0.17
	Spherical	749.60	2.69	0.10
Richness of accompanying species (RI)	gls	742.89		
	Exponential	742.65	2.25	0.13
	Gaussian	743.36	1.54	0.22
	Linear	741.71	3.19	0.07
	Rational quadratic	742.84	2.06	0.15
	Spherical	741.98	2.91	0.09
Cover of accompanying species (CO)	gls	745.85		
	Exponential	746.41	1.44	0.23
	Gaussian	747.03	0.81	0.37
	Linear	NA	NA	NA
	Rational quadratic	746.62	1.19	0.27
	Spherical	746.10	1.74	0.19

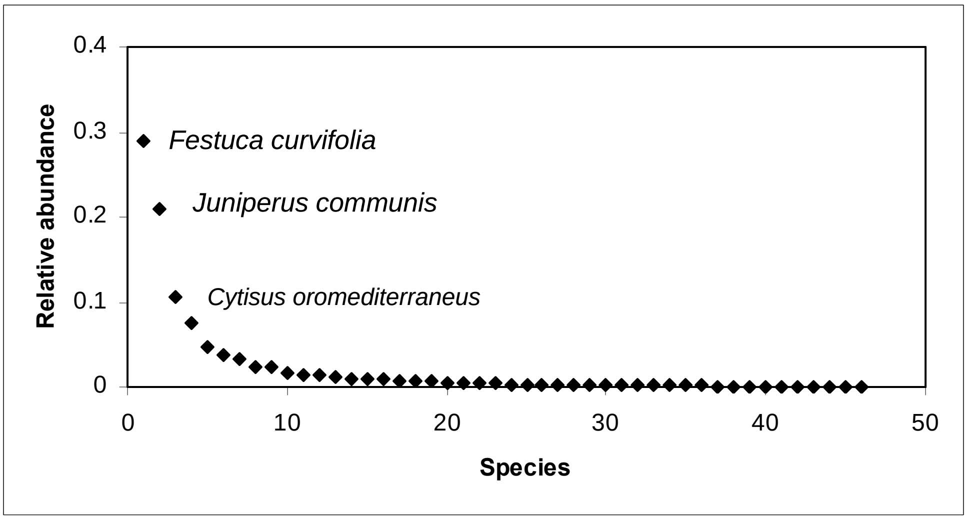
Table S3. Model selection on microsite disturbance and environmental variables.

MODEL	**VARIABLES**	**k**	**AICc**	**ΔAIC**	**L(gi|x)**	**AICc wi**
1	HD+HU+AD+SH+SL	5	1022.368	0	1.0000	**0.4215**
2	HD+HU+SH+AD	4	1023.184	0.816	0.6650	**0.2803**
3	HD+HU+A+SH+ASP	5	1024.928	2.56	0.2780	**0.1172**
4	HD+HU+A+SH+ML	5	1025.405	3.037	0.2190	0.0923
5	HD+HU+A+SL	4	1027.542	5.174	0.0752	0.0317
6	HD+HU+A	3	1027.625	5.257	0.0722	0.0304
7	HD+HU+A+ASP	4	1027.955	5.587	0.0612	0.0258
8	HD+HU+CO	3	1035.454	13.086	0.0014	0.0006
9	HD+HU+sh	3	1040.49	18.122	0.0001	0.0000
10	HD+HU	2	1041.507	19.139	0.0001	0.0000
11	HD+HU+SL	3	1043.131	20.763	0.0000	0.0000
12	HD+A+SH+ASP	4	1049.337	26.969	0.0000	0.0000
13	HD+ASP	2	1056.9	34.532	0.0000	0.0000
14	HD+A+CO	3	1059.007	36.639	0.0000	0.0000
15	HD+A+SH	3	1059.759	37.391	0.0000	0.0000
16	HD+A	2	1063.997	41.629	0.0000	0.0000
17	HD+GR	2	1069.89	47.522	0.0000	0.0000
18	HD+CO	2	1074.44	52.072	0.0000	0.0000
19	HD+SH	2	1078.315	55.947	0.0000	0.0000
20	HD	1	1078.96	56.592	0.0000	0.0000
21	HD+SL	2	1081.155	58.787	0.0000	0.0000
22	CO	1	1233.015	210.647	0.0000	0.0000
23	HU	1	1242.92	220.552	0.0000	0.0000
24	AN	1	1248.892	226.524	0.0000	0.0000
25	ML	1	1270.926	248.558	0.0000	0.0000
26	SOIL	1	1273.759	251.391	0.0000	0.0000
27	SH	1	1275.988	253.62	0.0000	0.0000
28	ASP	1	1281.583	259.215	0.0000	0.0000
29	ALT	1	1284.161	261.793	0.0000	0.0000
30	VC	1	1286.945	264.577	0.0000	0.0000
31	SL	1	1297.577	275.209	0.0000	0.0000
32	GR	1	1307.439	285.071	0.0000	0.0000
33	ROCKS	1	1311.162	288.794	0.0000	0.0000

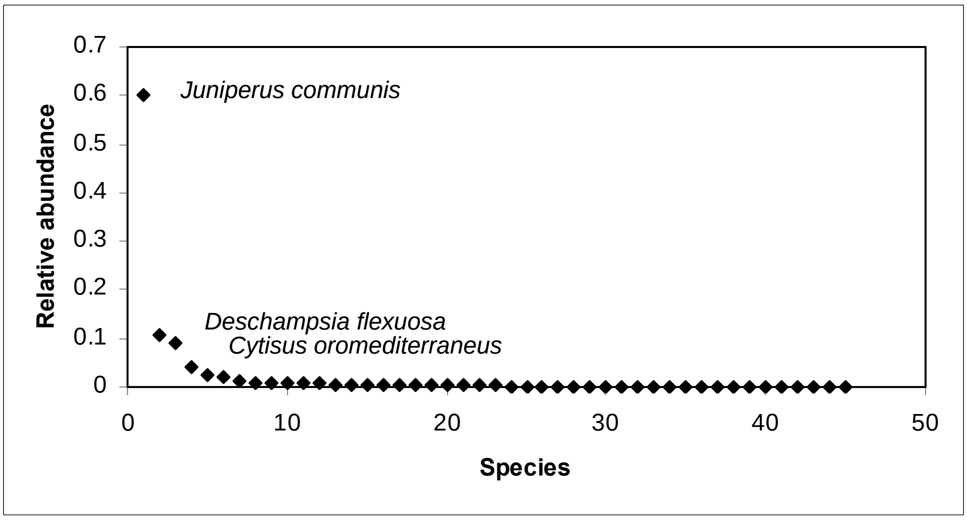
Variables: HD: human disturbance; HU: human use; AD: animal disturbance; SL: slope; CO: accompanying species cover; SH: shrub cover; AS: aspect; SO: soil cover; VC: vegetation cover; GR: presence of grazing.

Figure S1.

Relative abundance plot of species accompanying *Erysimum penyalarense* in quadrats **with animal disturbance**.



Relative abundance plot of species accompanying *Erysimum penyalarense* in quadrats **without animal disturbance**.



```
GLM: shrub ~ animal
Coefficients:
                Estimate    Std. Error   t value      Pr(>|t|)
(Intercept)     28.281        4.793      5.900        8.88e-08
animal         -18.865        6.188     -3.048        0.00314
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Capítulo 5

Seed germination and seedling establishment of the endangered high-mountain plant *Erysimum penyalarense* (Brassicaceae): species-specific facilitation

Manuscrito inédito

Seed germination and seedling establishment of the endangered high-mountain plant *Erysimum penyalarense* (Brassicaceae): species-specific facilitation

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Key words: *Erysimum penyalarense*, facilitation, Mediterranean, seed germination, seedling establishment.

Abstract

Factors correlating with seedling establishment were examined for the Mediterranean endangered herb *Erysimum penyalarense*. The harsh environmental conditions met in high mountain ecosystems make microhabitat characteristics and interactions crucial for success in germination and early seedling establishment. In addition, plant species in Mediterranean mountains, which face greater abiotic stress due to water shortage during the growing season, may have greater dependence on positive interactions with their close environment. The transition from seed to seedling is highly sensitive to environmental stochasticity and the study of factors affecting seedling establishment plants is of paramount importance to predict population viability and assess suitable conservation strategies. We investigated factors affecting early seedling emergence based on a germination experiment in the field. We hypothesized that early seedling emergence in *E. penyalarense* depends on different intraspecific interactions (with seeds and adults), interspecific interactions (with herbaceous species, shrubs and animals) and microhabitat characteristics (rock cover and bare soil). Our results show that the positive interaction with *Cytisus* shrubs was the main factor enhancing early seedling

establishment. Nevertheless, species-specific interactions vary among different *E. penyalarense* life stages.

Introduction

Mediterranean mountains are among the most diverse and singular ecosystems on Earth, holding a high number of endemic species (Quézel 1985; Körner & Spehn 2002; Vargas 2003; Körner 2004; Molau 2004). However, this type of ecosystems are harsh environments for plant establishment, and are specially vulnerable to environmental degradation, especially due to their low resilience to disturbance (Spiegelberger 2006) and climate change (Pauli *et al.* 2007). Under these circumstances reproduction and recruitment of Mediterranean plants meet many limitations (Bliss 1958; Körner 2005; Gómez 2005; Giménez-Benavides *et al.* 2007; Ramírez et al. 2006; García-Camacho & Escudero 2009).

The transition from seed to seedling is one of the most critical stages in the life history of plants (Chambers & MacMahon 1994). Information on germination ecology and seedling establishment is crucial to predict the viability of endangered plant populations and to assess suitable conservation strategies (Kaye 1997; Navarro & Guitián 2003; Herranz *et al.* 2010). These two processes are limited in montane habitats by many biotic and abiotic factors (Gómez 2005; Giménez-Benavides *et al.* 2007; Lorite *et al.* 2007; Bueno *et al.* 2011). Seed germination is highly increased in many alpine plants after a cold period (Marchand & Roach 1980; Kaye 1997; Giménez-Benavides *et al.* 2005; Lorite *et al.* 2007; Herranz *et al.* 2010). Once seeds have germinated, several factors may influence seedling survival and establishment. Animal activity and summer drought are two important causes of seedling mortality in Mediterranean mountains. For instance, in high mountains of the SE Iberian peninsula, 50% and 49% of *Erysimum mediohispanicum* and *E. baeticum* seedlings die due to ungulate trampling and desiccation, respectively (Gómez 2005). In addition, seedling mortality may occurr in alpine habitats as a consequence of competition with other seedlings, adult plants or the surrounding vegetation (Goldberg 2001, 1990). Understorey vegetation has repeatedly been proved to reduce tree seedling establishment and survival in forests (e.g. Lorimer *et al.* 1994; George & Bazzaz 1999). And even in grasslands competition from the existing vegetation has been identified as a major limiting factor for the establishment of seedlings of other species (Harrington 1991; Adams *et al.* 1992; Gordon & Rice 1993; Tilman 1997).

In montane ecosystems, however, plants interact not only negatively, but also positively (Callaway 1995; Callaway *et al.* 2002). In fact, in this type of ecosystem the ocurrence of facilitation is frequent and the presence of a plant species may favour the establishment and recruitment of other species (Arroyo *et al.* 2003; Kikvigze *et al.* 2005; Wirth *et al.* 2011). In the harsh Mediterranean mountain ecosystems the accumulation of above-ground biomass atenuates the climatic impact. Therefore, the removal of neighbours results disadvantageous for some alpine species (Callaway *et al.* 2002) and seedling mortality is reduced by shrubs and cushion plants that act as nursing plants (e.g. Gómez *et al.* 2002; Gómez-Aparicio *et al.* 2005). Indeed, at high elevations where abiotic stress is high, the interactions among plants are predominantly positive (Callaway *et al.* 2002).

Facilitation is often species-specific (Callaway 1998), since the likelihood of a seedling being eaten can be influenced by the identity of its nearest neighbours (Baraza *et al.* 2004, 2006). For instance, unpalatable species can protect surrounding seedlings, e.g. *Senecio jacobaea* seedlings were attacked more frequently when surrounded by palatable *Taraxacum officinale* seedlings than when surrounded by seedlings of their own species (Hanley *et al.* 1995), *Quercus pubescens* seedlings are protected within unpalatable *Buxus sempervirens* and *Juniperus communis* shrubs (Kunstler *et al.* 2006). In severely dry environments some species may protect others from drought, e.g. adult individuals of the grass *Stipa tenacissima* facilite saplings of *Medicago arborea*, *Quercus coccifera* and *Pistacea lentiscus* by dimishing drought (Maestre *et al.* 2001, 2003).

The aim of this study was to experimentally investigate the factors affecting seed germination and early seedling emergence of the endangered high-mountain herb *Erysimum penyalarense* (Brassicaceae) in natural conditions. We hypothesize that early seedling emergence in *E. penyalarense* depends on different sorts of interactions: (1) intraspecific interactions between different life stages (seeds and adults), (2) interspecific interactions with surrounding herbaceous and subalpine shrub species, as well as that caused by animal, and (3) interactions with microhabitat abiotic factors rock cover and bare soil.

Materials and Methods

Study system

Erysimum penyalarense (Pau) Polatscheck (Brassicaceae) is an endangered Mediterranean alpine species endemic to the Guadarrama Mountain Range in central Spain (Martínez *et al.* 2004). It is a 15-25 cm tall, perennial polycarpic herb inhabiting *Festuca curvifolia* grasslands. Adults have a basal rosette or group of rosettes with lanceolate leaves that produce a few to several hundred flowers on a variable number of reproductive stalks. Flowers are hermaphroditic, slightly protandrous, with four bright yellow petals and a tetradynamous androecium (four long and two short stamens). No seed bank is expected to occur in this species, as in other Brassicaceae species, including *Erysimum spp.* (Gómez 2005).

The species has historically been known from two populations. However, currently there is only one remaining population, where plants are locally abundant. This population occurs in a protected area in central Iberia (*Guadarrama* National Park), which has traditionally been managed for livestock grazing and leisure activities, with part of the land being occupied by ski resorts. The classical location found in *Peñalara* seems to have become extinct, or it must be composed of very few individuals that have not been found again. It was for this reason that the species was catalogued as Endangered (EN) following the IUCN criteria in the Atlas of Endangered Flora (Martínez *et al*. 2004). The current study has been carried out in the only existing population of the species located in *Cuerda Larga* (*Guadarrama* Mountain Range). At this site, the vegetation is a mosaic of grassland were the most representative herbaceous species are *Festuca curvifolia, Deschampsia flexuosa, Silene ciliata* and *Jasione crispa*, and a shrubland composed of *Cytisus oromediterraneus*, *Juniperus communis* and *Thymus praecox* (Chapter 4).

Sampling design

The effective occupancy area (Pisanu *et al.* 2009) of the species in *Cuerda Larga* was delimited by means of aerial photos first and field surveys second, in order to further exclude unsuitable sites and potential sites where the plant was not present (Chapter 4). Eighty 1 m^2 permanent quadrats were established in the delimited area (Figure 1).

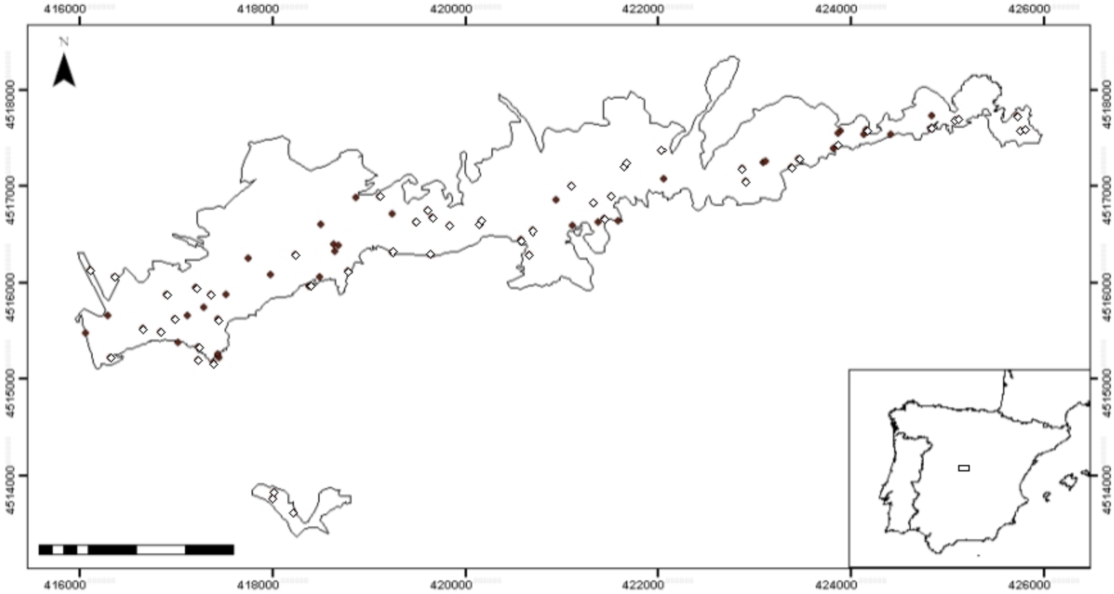

Figure 1. Map showing quadrat allocation in the study are. Quadrats with *Erysimum penyalarense* plants, were germination experiments were performed are shown in dark.

For each quadrat we estimated the percentage cover of the following set of variables to the nearest 1% per quadrat, using a 10 x 10 grid: (1) herbs, (2) *C. oromediterraneus*, (3) *J. communis*, (4) *T. praecox*, (5) bare soil and (6) rocks. In addition, we also recorded in each quadrat the presence-absence of animal disturbance *i.e.* evidence of trampling, grazing or droppings caused by cattle, sheep, horses and the Spanish ibex (*Capra pyrenaica*) (see Chapter 4 for details).

In 2006 we mapped all *E. penyalarense* plants within each quadrat, and recorded their life stage (seedling, non reproductive adult, reproductive adult). These individuals were monitored during three years (from 2006 to 2008). The number of adults considered for analysis is the addition of non reproductive and reproductive adults. In order to estimate the number of seeds produced per quadrat, three reproductive adults were selected per quadrat when available, systematically choosing individuals that kept the longest distance between them, within the quadrat. This procedure was repeated for the three years of monitoring and the fruit set of these plants was counted. In the end of 2008 three to five fruits, depending on availability, were systematically collected from the selected plants. These fruits were taken to the lab and seeds were counted. The mean number of seeds per fruit was calculated in order to estimate the total number of seeds produced per quadrat and year (Table S1). The production of seeds per plot considered for analysis was calculated as the mean of the three years monitored, since no significant differences were found among the three years (ANOVA, F = 0.09, p = 0.91).

Sowing experiment

In September 2007 we collected seeds from each quadrat. Seeds were stored in paper bags at room temperature for five months. After this length of time they were wet-cold stratified at 4 ºC for three months (Raghu & Post 2008). In May 2008 twenty seeds were sown in each quadrat using a small grid, which was located onto a place as similar as possible to that were plants were growing naturally in the field. This meant that, for instance, in quadrats were plants were growing under shrubs, seeds were also sown under shrubs. Seeds were placed 1 cm and gently covered with soil, in order to promote germinability (Bliss 1958; Herranz *et al.* 2003) and to avoid postdispersal seed predation by rodents, insects and birds (Pavlik *et al.* 1993; Gómez 2005). These seeds were monitored during the summers of 2008 and 2009.

Subsequently, seeds were collected again in September 2008 and twenty of them were sown per quadrat straight away, so that they would be exposed to natural conditions in the field. These seeds were checked for seedling emergence in the summer 2009 and surviving individuals were recorded.

Data analysis

Difference in seedling emergence between seeds sown in spring and autumn was tested using the non-parametric Mann-Whitney U test. Since no significant differences were found between the two groups (see below), data from both years were merged together in order to obtain a more robust dataset.

We checked for spatial autocorrelation patterns in seedling emergence by means of Moran’s I coefficient with 300 permutations (Rangel *et al.* 2010) at eight distance classes using SAM v.2.0. Upper limits for these distance classes were 360, 980, 1687, 2581, 3617, 4841, 6119 and 7967 m. Since Moran’s I was statistically significant for this variable, as well as for some of the microhabitat variables measured, further analysis were performed using an spatially explicit Lagged Predictor Model using SAM v.2.0. Essentially, a spatial lagged model consists of an autoregression model which explicitly incorporates the spatial relationship between pairs of sites *i.e.* the autocorrelation in the data (Rangel *et al.* 2010). This model allowed us to evaluate the effects of independent variables of microhabitat characteristics *i.e.* herbaceous, shrub, bare soil and rock cover, as well as animal disturbance on the dependent variable early seedling establishment of *E. penyalarense* taking into account spatial autocorrelation.

Results

Seedling emergence was observed on 90% of the experimental plots (Table 1). Sowing of seeds at two different times, June 2008 and September 2008, with seeds produced at two different seasons, 2007 and 2008 respectively, yielded no significant differences in emergence level in the summer of 2009 for both groups of seeds (Mann-Whitney test, U = 229.00, z = -1.13, p = 0.26). Therefore, these sets of data were merged together and the resulting seedling emergence observed was 23.79% ± 3.14.

Quadrat	2007	2008	Mean
1	-	0.50	0.50
2	0.75	-	0.75
3	-	0.20	0.20
4	0.25	0.25	0.25
6	0.00	0.20	0.10
9	0.30	0.20	0.25
10	0.05	-	0.05
11	-	0.25	0.25
17	0.05	0.50	0.28
19	0.20	0.20	0.20
20	-	-	-
26	0.00	0.20	0.10
27	0.30	0.30	0.30
31	-	0.15	0.15
40	-	0.00	0.00
45	-	0.40	0.40
46	0.40	0.55	0.48
47	0.05	0.35	0.20
51	0.40	0.40	0.40
52	0.10	0.25	0.18
53	0.25	0.25	0.25
56	0.20	0.40	0.30
58	0.15	0.15	0.15
63	0.20	0.25	0.23
71	0.20	0.20	0.20
78	0.05	0.05	0.05
79	-	0.00	0.00
82	-	0.00	0.00
83	0.20	0.40	0.30
93	0.45	0.40	0.43
Mean (SE)	0.22 (0.04)	0.26 (0.03)	0.24 (0.03)

Table 1. Seedling establishment in 2009 in quadrats sowed with seeds collected in 2007 and 2008 (*n*=20).

Seedling emergence of *E. penyalarense* was spatially autocorrelated at the highest distance class, over a distance of 7.9 Km (Figure 2). Other variables that showed some level of spatial correlation at distant classes were *C. oromediterraneus* cover and animal disturbance; whereas at closer distance classes we found rocks, *J. communis* and *T. praecox* (Table S2).

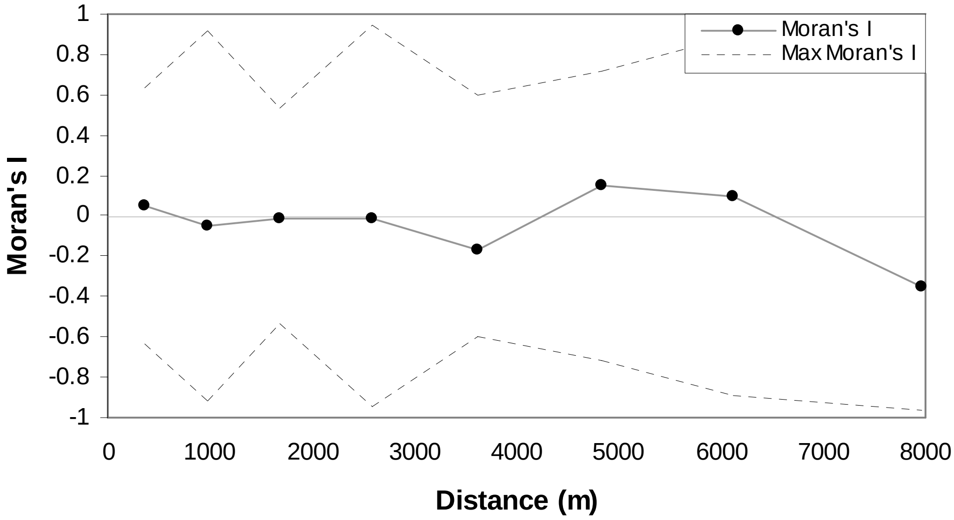

Figure 2. Correlogram corresponding to the dependent variable seedling establishment.

The presence of the *C. oromediterraneus* shrub within quadrats was the main factor correlating with early seedling establishment in *E. penyalarense* (Table 2). No other shrub or herbaceous species seemed to have an effect. Variables related to species density, *i.e.* seeds and number of adults per quadrat, showed no effect either (Table 2). Finally, animal disturbance was not correlated to seedling establishment (Table 2).

Variable	Coeff.	SE coeff	t	p value
Seeds per plot	0.205	0.197	1.04	0.311
Adults	0.097	0.203	0.476	0.639
Herbaceous cover	-0.217	0.162	-1.341	0.195
Animal disturbance	-0.155	0.178	-0.872	0.394
Cytisus cover	0.473	0.153	3.102	**0.006**
Juniperus cover	0.151	0.205	0.736	0.471
Thymus cover	-0.214	0.179	-1.197	0.245

Table 2. Results for the Lagged Predictor Model relating the response variable germination of *E. penyalarense* from sowing experiments to the predictor microhabitat variables (cover of herbaceous species and cover of *Cytisus*, *Juniperus* and *Thymus* shrubs), population density variables (seeds and adults per quadrat) and animal disturbance. Statistically significant variables in bold. Akaike's Information Criterion (AICc): 49.04.

Discussion

Seed germinability

Germination in *Erysimum penyalarense* remained the same after a year, *i.e*. seeds sowed the first year maintained the same level of germination than seeds sowed the second year. Thus, data of both years could be analyzed together. This result is congruent with that observed for other Brassicaceae species, including some *Erysimum* species, which kept almost the same germinability ability after 40 years of storage (Pérez-García *et al.* 2007). However, germination ability often increases with time of storage in many Brassicaceae species (Copete *et al.* 2005, 2009; Herranz *et al.* 2003, 2010). In particular, seed germination in *Erysimum arenicola* var. *torulosum* in the alpine of Olympic Mountains (Washington, USA) increased from 35.1% to 62.7% after a 9-month storage (Kaye 1997).

Seedling emergence in the field was higher in *E. penyalarense* (0.24 ± 0.03) than in other high-mountain *Erysimum* species, such as *E. baeticum* (0.19 ± 0.01) and *E. mediohispanicum* (0.09 ± 0.02) (Gómez 2005), although it was much lower than observed in some lowland or weedy wallflowers, like *Erysimum cheiranthoides* (0.627) (Roberts & Boddrell 1983) or *Erysimum capitatum* ssp. *angustatum* (seedling emergence ranging from 0.476 - 0.517 under optimal substrate conditions) (Pavlik & Manning 1993).

Intraspecific competition

Crowding affects individual plants by limiting their survival and growth, with intraspecific competition being an important source of density dependence. Indeed, seedlings can compete with other seedlings, and this population regulation finally leads to the same number of seed production the next reproductive season, regardless of the initial sowing density (Palmblad 1968). But seedlings may also compete with adult plants for soil resources (Reichenberger & Pyke 1990; Aguiar *et al.* 1992). This asymmetric competition, arises as a consequence of difference in size and differential variation in emergence times within a population, with plants emerging first gaining an advantage over the rest (Ross & Harper 1972). Indeed, adult neighbours can negatively affect the emergence, survival and performance of seedlings (Aguiar *et al.* 1992; Aguilera 1993; Liu 2008). However, *E. penyalarense* did not show a density dependent interaction with seeds or adults. In this sense, the species would show the characteristic behaviour of colonizing or weedy species, which often grow poorly under intense competition (Baker 1965). Under favourable conditions, weeds can produce a large amount of seeds, but have only a few germinate at a time, to decrease the probability of dense populations and strong competition, and consequently keeping a seed reservoir (Linhart 1976).

Effects of animals

Sowing seeds reduces postdispersal seed predation, especially that of animals that rely on their sight for foraging, such as birds. Therefore, early seedling establishment under completely natural conditions is probably lower than 24% in this population. Nevertheless, plots that had been previously disturbed by animals showed no statistical differences in seedling establishment with those that had not been affected by animal activity. The fact that these events were prior to sowing indicate that there are certain differences in the requirements of seeds and adults. Indeed, animal disturbance negatively affects adult establishment (Chapter4), which will in turn affect population dynamics and future recruitment. However, the early seedling establishment in these plots indicates that the effects of ungulates on the soil or other species do not affect seedling emergence, as observed in other *Erysimum* species (Gómez 2005).

Interspecific interactions

Because all plants use essentially the same resources, all individuals are potential competitors. The presence of neighbours is an important factor influencing plants at all levels of organization, from the individual to the community. At the emergence and survival stages, however, the most relevant form of competition is given by interference competition, rather than by exploitation competition (Goldberg 1990). Surprisingly, herbaceous cover did not interfere with early seedling establishment, despite *E. penyalarense* not being a good competitor (Chapter 4). This result contradicts that observed for other *Erysimum* species in which the abundance of seedlings increases when abundance of other species is reduced due to ungulate herbivory (e.g. *E. mediohipanicum* and *E. baeticum* in Gómez 2005, and *E. crepidifolium* in Dostálek & Frantík 2008). Therefore, competition with other species must occur at later life stages, during the establishment and survival of seedlings.

Positive interactions between plants is widespread in environments with strong abiotic and biotic stress, such as Mediterranean-type ecosystems (e.g. Bertness & Callaway 1994; Maestre *et al.* 2003; Gómez-Aparicio *et al.* 2005). Nurse plants facilitate the establishment of other species beneath their canopy, by offering microhabitats that are more favorable for seed germination and seedling establishment than their surrounding environment (e.g. Callaway 1992; Pugnaire *et al.* 1996; Wied & Galen 1998; Gómez *et al.* 2002).

Shrubs had a facilitative effect on *E. penyalarense* early seedling establishment. Furthermore, this effect was species-specific, as in many other species (Callaway 1998). Indeed, *Cytisus* shrubs enhanced early establishment of seeds beneath their canopy, whereas thymes and junipers did not show this effect. *Thymus praecox* shrubs are low prostrate and dense plants, which probably limit the establishment of seedlings physically. Furthermore, oils of thyme species are known to inhibit germination of some weed species (Angelini *et al.* 2003). On the other hand, *Juniperus* shrubs, which are known to contain allelopathic compounds, such as phenolics and terpenoids (Inderjit 1996), did not show such an inhibitory effect on seed germination. But surprisingly, juniper did not facilitate early seedling establishment either. This would have been expected result, since *Erysimum* plants, of this and other *Erysimum* mountain species, are often seen growing beneath juniper shrubs (Gómez 2005). This result, which contrasts with adult behaviour, could be explained because juniper shrubs may initially reduce germination of *Erysimum* due to secondary metabolites, but can afterwards act as good nursing species for those individuals that eventually proliferate. Indeed, mature juniper plants are less susceptible of being grazed by cattle and can be a good refuge for other herbaceous species (Padilla & Pugnaire 2006; Gómez 2005).

Finally, *Cytisus* was the only shrub species which showed a facilitative effect on *Erysimum*. Again, this result contrasts with that observed for adults (Chapter 4), in which *Erysimum* plants do not grow in the *Cytisus* shrublands. However, early seedling establishment appears to be facilitated beneath low density *Cytisus* shrubs which enter the occupancy area of the species. At this early stage seedling emergence appears to be facilitated, and this could be the result of fertilization effects on the soil by this nitrogen-fixing species. However, at a latter stage plants growing underneath these shrubs are probably exposed to harsher damage than they would be beneath junipers, since *Cytisus* are more frequented by herbivores.

Although seedling establishment is likely to be affected by a wide variety of factors, our results have shown that *Cytisus* shrubs facilitate early establishment of *Erysimum penyalarense*, thereby showing the relevance that positive interactions play for the conservation of this species. Therefore, as in other Mediterranean scenarios where drought and animal trampling constitute some of the main factors limiting plant establishment (Gómez 2005), shrubs stand as some of the safer sites for herbaceous species recruitment due to their ability to modify both above and below-ground characteristics (e.g. Brooker *et al.* 2008; Nuñez *et al.* 1999). Nevertheless, excess shrub cover would displace the species in the long run (Chapter 4). Therefore, long-term studies, as well as experimental manipulations are needed to establish causal relationships between seedling establishment and the nursing effect of *Cytisus* shrubs.

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Appendix

Table S1. Estimated number of seeds per plot for the three years of monitoring.

Quadrat	Seeds 2006	Seeds 2007	Seeds 2008	Mean number of seeds
1	688	62	0	250
2	63	62	0	42
3	5	3	2	4
4	296	510	0	269
6	246	849	112	402
9	633	0	491	375
10	0	0	0	0
11	323	1288	-	537
17	171	200	21	131
19	0	85	0	28
26	0	43	29	24
27	14	63	0	25
31	37	44	73	51
40	0	0	21	7
45	173	375	770	440
46	118	0	66	61
47	0	3	83	28
51	0	0	0	0
52	174	0	48	74
53	171	0	308	160
56	143	39	247	143
58	0	33	132	55
63	0	62	0	21
71	0	0	0	0
78	88	28	517	211
79	0	21	0	7
82	0	0	478	159
83	137	226	0	121
93	105	348	510	321

Table S2. Spatial autocorrelation of all variables tested.

Variable	D.Class	DistCntr	Moran's I	Std Err	P	I (max)	I/I(max)
Seedling emergence	1	360.36	0.053	0.117	0.448	0.631	0.084
	2	980.428	-0.046	0.114	0.931	0.914	-0.05
	3	1687.903	-0.018	0.114	0.875	0.536	-0.033
	4	2581.127	-0.016	0.113	0.863	0.941	-0.017
	5	3617.607	-0.167	0.115	0.256	0.595	-0.28
	6	4841.593	0.153	0.111	0.09	0.714	0.214
	7	6119.911	0.099	0.107	0.205	0.892	0.112
	8	7967.309	-0.355	0.094	**<.001**	0.963	-0.368

Variable	D.Class	DistCntr	Moran's I	Std Err	P	I (max)	I/I(max)
Seeds per plot	1	360.360	-0.077	0.119	0.726	0.823	-0.094
	2	980.428	0.125	0.115	0.164	0.781	0.16
	3	1687.903	0.113	0.115	0.199	0.569	0.198
	4	2581.127	0.074	0.115	0.339	0.464	0.159
	5	3617.607	-0.131	0.117	0.415	0.565	-0.232
	6	4841.593	-0.171	0.113	0.229	0.83	-0.206
	7	6119.911	-0.176	0.108	0.194	0.605	-0.29
	8	7967.309	-0.092	0.094	0.551	0.47	-0.195

Variable	D.Class	DistCntr	Moran's I	Std Err	P	I (max)	I/I(max)
Adults	1	360.360	0.033	0.09	0.443	0.829	0.04
	2	980.428	0.097	0.091	0.145	0.899	0.107
	3	1687.903	-0.137	0.091	0.264	0.656	-0.209
	4	2581.127	0.052	0.091	0.336	0.536	0.096
	5	3617.607	-0.195	0.092	0.082	0.559	-0.349
	6	4841.593	-0.121	0.091	0.353	0.576	-0.209
	7	6119.911	-0.108	0.093	0.433	0.409	-0.265
	8	7967.309	0.087	0.096	0.199	0.194	0.447

Variable	D.Class	DistCntr	Moran's I	Std Err	P	I (max)	I/I(max)
Herbaceous species	1	360.360	-0.163	0.121	0.294	0.876	-0.186
	2	980.428	0.003	0.117	0.738	0.463	0.007
	3	1687.903	-0.034	0.117	0.986	0.468	-0.072
	4	2581.127	-0.105	0.116	0.548	0.619	-0.17
	5	3617.607	-0.028	0.118	0.95	0.744	-0.038
	6	4841.593	0.018	0.114	0.635	0.642	0.029
	7	6119.911	-0.046	0.109	0.923	0.833	-0.056
	8	7967.309	0.006	0.094	0.661	0.965	0.006

Variable	D.Class	DistCntr	Moran's I	Std Err	P	I (max)	I/I(max)
Soil cover	1	360.36	-0.04	0.088	0.963	0.961	-0.041
	2	980.428	0.023	0.089	0.512	0.608	0.037
	3	1687.903	-0.104	0.089	0.443	0.397	-0.262
	4	2581.127	-0.048	0.089	0.892	0.316	-0.151
	5	3617.607	0.093	0.09	0.152	0.491	0.19
	6	4841.593	0.014	0.09	0.584	0.469	0.029
	7	6119.911	-0.023	0.092	0.888	0.537	-0.042
	8	7967.309	-0.193	0.096	0.101	0.605	-0.319

Variable	D.Class	DistCntr	Moran's I	Std Err	P	I (max)	I/I(max)
Rock cover	1	360.36	0.233	0.121	**0.026**	0.941	0.248
	2	980.428	0.202	0.117	**0.042**	1.018	0.198
	3	1687.903	0.081	0.117	0.32	0.807	0.1
	4	2581.127	-0.02	0.116	0.893	0.719	-0.028
	5	3617.607	-0.019	0.119	0.885	0.731	-0.025
	6	4841.593	-0.354	0.114	**0.005**	1.079	-0.328
	7	6119.911	-0.45	0.109	**<.001**	1.267	-0.355
	8	7967.309	0.112	0.094	0.117	1.354	0.082

Variable	D.Class	DistCntr	Moran's I	Std Err	P	I (max)	I/I(max)
Animal disturbance	1	360.36	0.111	0.122	0.23	0.919	0.121
	2	980.428	-0.261	0.118	0.057	0.777	-0.336
	3	1687.903	-0.161	0.118	0.291	0.614	-0.262
	4	2581.127	-0.148	0.117	0.337	0.718	-0.206
	5	3617.607	0.128	0.12	0.173	0.67	0.19
	6	4841.593	0.224	0.115	**0.024**	0.812	0.276
	7	6119.911	-0.31	0.11	**0.012**	0.686	-0.452
	8	7967.309	0.056	0.094	0.327	0.579	0.097

Variable	D.Class	DistCntr	Moran's I	Std Err	P	I (max)	I/I(max)
Cytisus cover	1	360.36	-0.007	0.104	0.786	0.649	-0.012
	2	980.428	-0.039	0.103	0.972	0.523	-0.075
	3	1687.903	-0.106	0.103	0.494	0.586	-0.181
	4	2581.127	-0.207	0.102	0.094	0.966	-0.215
	5	3617.607	-0.135	0.104	0.341	0.963	-0.14
	6	4841.593	0.291	0.102	**0.001**	0.762	0.382
	7	6119.911	-0.01	0.1	0.797	0.565	-0.018
	8	7967.309	-0.058	0.095	0.813	0.437	-0.133

Variable	D.Class	DistCntr	Moran's I	Std Err	P	I (max)	I/I(max)
Juniperus cover	1	360.36	0.089	0.114	0.273	0.481	0.186
	2	980.428	0.421	0.111	**<.001**	0.827	0.509
	3	1687.903	0.214	0.111	**0.025**	0.694	0.308
	4	2581.127	-0.014	0.11	0.846	0.821	-0.017
	5	3617.607	-0.056	0.112	0.853	0.691	-0.082
	6	4841.593	-0.353	0.109	**0.004**	1.485	-0.237
	7	6119.911	-0.361	0.105	**0.002**	1.399	-0.258
	8	7967.309	-0.235	0.094	**0.035**	1.221	-0.192

Variable	D.Class	DistCntr	Moran's I	Std Err	P	I (max)	I/I(max)
Thymus cover	1	360.36	0.035	0.098	0.471	0.627	0.055
	2	980.428	0.245	0.097	**0.004**	0.707	0.346
	3	1687.903	0.083	0.097	0.221	0.551	0.151
	4	2581.127	-0.017	0.097	0.848	0.621	-0.028
	5	3617.607	-0.079	0.098	0.663	1.112	-0.071
	6	4841.593	0.04	0.097	0.432	0.42	0.096
	7	6119.911	-0.214	0.096	0.064	1.634	-0.131
	8	7967.309	-0.397	0.095	**<.001**	1.977	-0.201

Discusión

Los ecosistemas de montaña mediterránea son medios con condiciones ambientales extremas y cortos períodos vegetativos, ya que al largo periodo que transcurren bajo la nieve le sigue una severa sequía estival. Esto limita el período de crecimiento y reproducción de las plantas a una ventana efímera. Bajo estas condiciones la probabilidad de que se produzcan daños en la vegetación es mayor que en otros ecosistemas (Rundel *et al.* 1998). La riqueza y diversidad de especies en cotas elevadas está controlada por la intensidad del estrés ambiental en primer lugar, y la perturbación y la competición en segundo lugar (Kammer & Möhl 2002). De hecho, las actividades antrópicas en montañas mediterráneas han tenido tradicionalmente una profunda influencia en la conservación de las especies (Vogiatzakis 2012). Desgraciadamente, durante mucho tiempo el desconocimiento sobre la ecología de las plantas de montaña mediterránea era tal, que su gestión se inspiraba en ocasiones en la biología de especies de montaña de otras regiones, con muy diferente clima, y que no tenían en consideración las características especiales de los ecosistemas mediterráneos.

Las regiones de clima mediterráneo se encuentran entre las zonas más amenazadas del planeta debido a los usos del territorio (Rundel *et al.* 1998) y el cambio climático a nivel global (Giorgi & Lionello 2008). Las características de su clima las hace especialmente sensibles al cambio climático, la desertificación, la contaminación, la sobreexplotación y la exposición a fuegos devastadores. Una de las causas más importantes de perturbación en los ecosistemas naturales es la actividad humana, a través de la gestión y uso del territorio. Nuestras actividades alteran las características del suelo desplazando y eliminando especies autóctonas, promoviendo así el establecimiento de especies invasoras (Vilà *et al.* 2011; Gaertner *et al.* 2009). A pesar de ello, la generación de paisajes heterogéneos ha favorecido la diversidad de muy diferentes grupos de organismos, desde pájaros, a mamíferos, artrópodos o plantas (p. ej. Pulido & Díaz 1992; Benton *et al.* 2003; Tews *et al.* 2004). Es más, puesto que la perturbación es un fenómeno que ocurre de manera natural en todos los ecosistemas, muchas especies están adaptadas a ella, como es el caso de las plantas ruderales mediterráneas (Naveh 1967), y muchas comunidades vegetales dependen de distintos tipos de perturbación, especialmente para la regeneración (Pickett & White 1985). Aunque en un pasado la conservación se ha afanado en proteger los ecosistemas de la perturbación, la idea de que ésta constituye un fenómeno raro que altera el funcionamiento de los ecosistemas está cambiando. Este cambio de mentalidad ha contribuido al mejor entendiendo de la dinámica de los ecosistemas y está influyendo profundamente en las políticas de gestión a nivel mundial (Gurevitch *et al.* 2006).

La presente memoria doctoral ha pretendido contribuir al entendimiento de los procesos ecológicos responsables de la rareza de táxones en las montañas mediterráneas de la Cordillera Carpetana, tomando como ejemplo dos especies amenazadas de estos ecosistemas: *Pseudomisopates rivas-martinezii* (Sierra de Gredos) y *Erysimum penyalarense* (Sierra de Guadarrama). Dichas montañas mediterráneas se caracterizan por unas extremas condiciones ambientales y una fuerte estacionalidad. Estas condiciones naturales, unidas a la explotación histórica de estas áreas han conferido un carácter especial a su flora.

La hipótesis general de la que parte la presente memoria doctoral señalaba que el grado de amenaza presentado por las dos especies de estudio se debería a ciertos factores relacionados con la estocasticidad ambiental, la perturbación de interacciones bióticas o los desequilibrios genéticos (Tabla 1). Tras los estudios realizados podemos concluir que ambas especies se ven afectadas por la perturbación de las interacciones bióticas. En cuanto a otros factores, *Pseudomisopates rivas-martinezii* se mostraría más vulnerable ante desequilibrios genéticos y *Erysimum penyalarense* ante la estocasticidad ambiental. En concreto, de entre todos los factores explorados destaca el efecto de la perturbación, que como veremos a continuación, desarrolla un importante papel en la dinámica poblacional de las especies de estudio, y por tanto deberá ser tenida muy en cuenta para su conservación.

Amenazas potenciales	***Pseudomisopates rivas-martinezii***	***Erysimum penyalarense***
Estocasticidad ambiental		
Cambio climático	✗	✓
Destrucción del hábitat	✗	✗
Alteración del suelo: erosión, urbanización, deforestación, aclaramiento de tierras, etc.	✗	✗
Cambios en usos del territorio o abandono	✓	✓
Eutrofización y fertilización	?	✓
Dinámica del fuego (supresión)	✓	✗
Prácticas agrícolas, pesticidas y herbicidas	✗	✗
Prácticas ganaderas	✓	✓
Deposición de nitrógeno	✗	✓
Actividades turísticas	✗	✓
Establecimiento de áreas protegidas	✓	✓
Sobreexplotación	✗	✗
Perturbación de interacciones bióticas		
Introducción de especies invasoras	?	✓
Declive demográfico	✓	✗
Reducción en la abundancia polinizadores	✓	✗
Reducción en la abundancia de dispersores	✗	✗
Herbivoría (ramoneo, pisoteo)	✓	✓
Sucesión de la vegetación, competencia interespecífica	✓	✓
Desequilibrios genéticos		
Endogamia	✓	?
Deriva y erosión genética	✓	?
Hibridación	✗	✗
Acumulación de mutaciones	✓	?
Pérdida de alelos S	✓	✗

Tabla 1. Factores potenciales que condicionan el estado de amenaza de las especies de estudio, extraídos de la literatura de la conservación y clasificadas según su origen (adaptado de Oostermeijer 2003).

Pseudomisopates

La clonalidad como respuesta a la perturbación

Vista de la población de la Garganta Lóbrega tras el incendio acontecido en octubre de 2005.

La reproducción vegetativa, tan común en plantas, presenta múltiples ventajas. Entre estas se encuentran: la translocación de recursos entre *ramets* en hábitats heterogéneos; beneficios demográficos como incrementar la probabilidad de supervivencia y la fecundidad; permitir un rápido incremento de tamaño y aumentar la movilidad; una diversidad genotípica superior a la de plantas no clónicas, y una variación genética oculta que favorece la adaptabilidad de los clones. Sin embargo, también puede resultar un arma de doble filo para una especie amenazada, ya que sus beneficios para la supervivencia a corto plazo pueden dar lugar a poblaciones pequeñas y endogámicas con baja fecundidad a largo. Esta situación puede condicionar futuras recolonizaciones naturales a partir de unos pocos individuos. Dado que existen todavía pocos ejemplos de esta fecundidad reducida en poblaciones de especies clónicas, los efectos de la clonalidad sobre el éxito reproductivo cobran especial relevancia en un género monotípico, amenazado, y endémico de la Sierra de Gredos, como *Pseudomisopates*.

Pseudomisopates rivas-martinezii es una planta predominantemente autoincompatible, que depende de la polinización, y que sufre limitación de polen principalmente por la calidad del polen que recibe, pero también por la cantidad del mismo (Capítulo 1). En este sentido, hemos podido constatar que poco más de la mitad de visitas que recibe corresponden a visitantes que se pueden considerar eficientes (Figura 1). La frecuencia total de visitas recibidas es de 0,002 visitas por flor y hora (Capítulo 1). Esta frecuencia resulta bastante inferior a la de otras especies mediterráneas especialistas, como sus parientes cercanos del género *Antirrhinum*, que recibe entre 0,15-0,31 visitas por flor y hora (Vargas *et al.* 2010) dependiendo de la especie, y muy inferior al del generalista *Erysimum mediohispanicum* que mostró valores de entre 33,3 y 95,5 visitas por hora y flor dependiendo de la población (Gómez *et al.* 2007). Todo esto indica que muchas flores de *P. rivas-martinezii* no son visitadas, lo que dificulta aún más las probabilidades de una reproducción sexual exitosa.

Figura 1. Bombílido visitando flores de *Pseudomisopates rivas-martinezii.* La incorporación de otros visitantes a la lista de Amat *et al.* (2011) confirma el amplio espectro de polinizadores que recibe esta planta.

En cuanto a la estructura poblacional, el estudio genético de la población de La Serrota reveló una alta diferenciación entre rodales, ya que ningún genotipo era compartido entre estos. La variación del número de genotipos por rodal resultó ser de entre uno y seis. Por otro lado, la persistencia de heterocigotos y la ausencia de gran parte de combinaciones homocigotas es un síntoma claro de la fuerte clonalidad y autoincompatiblidad de la especie (Evans *et al.* 2004; Halkett *et al.* 2005; Stoeckel *et al.* 2006), que se tradujo en un valor de 0,23 para la riqueza genotípica (Capítulo 2). Este valor es muy inferior al de otras especies clónicas cuya media se sitúa entorno a un valor de 0,44 (Honnay & Jacquemyn 2008). No obstante, se observa una alta diversidad genética (*He* = 0,42) (Capítulo 2) en comparación con los valores de otras especies herbáceas perennes (Hamrick & Godt 1989), y de otras especies autoincompatibles de *Antirrhinum* de la Península Ibérica (Jiménez *et al.* 2002; Carrió *et al.* 2010). Además, esta diversidad fue mayor en las zonas de piornal donde los *genets* presentan un patrón de ocupación de guerrilla, con mayor entrecruzamiento físico entre *ramets*. En definitiva, una diversidad colonizadora de genotipos, unida a una alta persistencia de *genets* podría estar manteniendo la población a pesar del escaso éxito reproductivo y reclutamiento, como ocurre en otras especies (Watkinson & Powell 1993).

Por su parte, la clonalidad ha dado lugar en esta población a una distribución agregada de los *ramets* y la falta de correlación espacial entre rodales muestra un escaso flujo génico (Capítulo 2). No obstante, a una escala de estudio más reducida se observa una fuerte autocorrelación espacial entre *ramets*, hasta una distancia de 115 m, pero una total ausencia de esta a nivel de *genets*. Resultado este último poco común, pero observado también en algunas especies autoincompatibles de *Quercus* (Alfonso-Corrado *et al.* 2004), en la oceánica *Zostera marina* (Reusch *et al.* 1999) o en la planta de saladares *Spartina alternifolia* (Travis *et al.* 2004). De nuevo, esta pronunciada estructura genética espacial nos indica que estamos ante una población madura, en la que ha debido transcurrir un largo tiempo desde la última perturbación, una población con una intensa clonalidad y una dispersión limitada, tanto de polen como de semillas. Es precisamente esta disposición espacial de los clones la que estaría dificultando el entrecruzamiento entre individuos compatibles y contribuyendo a la limitación de polen cualitativa detectada (Capítulo 2).

Los resultados de la cuantificación de las ventajas y desventajas indicaron que la reproducción vegetativa se ve favorecida o promovida por factores ambientales desfavorables que reducen la floración en esta especie. Estos fueron en primer lugar, el cierre de la vegetación arbustiva, con efectos negativos también documentados en otras especies como *Mercuriallis perennis* (Howe 1990) o *Virola surinamensis* (Vandepitte *et al.* 2009). Esta proliferación del piornal puede dar lugar en última instancia a la extinción de las poblaciones, como parece que está ocurriendo en la población de la Garganta de Chilla, compuesta hoy por apenas una decena de *ramets*, que ya no presentan floración y se encuentran literalmente ahogados por la densidad del piornal, habiendo sido relegados al borde de un senderito (Figura 2). En segundo lugar, la herbivoría, que es un conocido factor que reduce la reproducción sexual en plantas (Schaal & Leverich 1996), y cuya presión en el área es bastante fuerte debido a la explotación para ganado vacuno (Figura 3), así como por la proliferación en ocasiones descontrolada de la cabra montés ibérica. En consecuencia, este aumento de la clonalidad provocada por factores ambientales disminuye, como ya hemos visto, la disponibilidad de individuos compatibles para la reproducción, debido a la estructura espacial creada por los clones. Dicha estructura genética espacial afecta, a su vez, a la eficacia biológica, lo cual se ha registrado también en otras especies autoincompatibles como *Rubus saxatilis* (Eriksson & Bremer 1993), *Calystegia collina* (Wolf *et al.* 2000), *Maianthemum bifolium* (Honnay *et al.* 2006) o *Convallaria keiskei* (Araki *et al.* 2007).

B. García

Figura 2. Aspecto que presentan los *ramets* de *Pseudomisopates rivas-martinezii* localizados bajo los piornos en la población de La Serrota.

Figura 3. Individuo de *Pseudomisopates rivas-martinezii* ramoneado por ganado vacuno en la población de La Serrota.

Si tenemos en cuenta todo lo dicho anteriormente, resulta evidente que la perturbación juega un papel crucial en la renovación del material genético en esta especie. Puesto que la Sierra de Gredos ha estado tradicionalmente sometida a fuegos periódicos para la obtención de pastos y sus piornales son formaciones de naturaleza pirófila, el siguiente paso fue llevar a cabo un experimento de germinación de semillas para contrastar la hipótesis del fuego como elemento perturbador positivo en la especie de estudio. El estudio de los efectos directos e indirectos del fuego en la germinación indicó que las mejores condiciones para la germinación de las semillas se obtienen, en primer lugar, tras un período de frío (95% mayor tras la estratificación en frío) y, en segundo lugar gracias a los efectos del fuego, tanto directos (79% germinación adicional en presencia de cenizas), como indirectos (74% menor germinación en oscuridad y mayor rebrote de la especie en zonas aclaradas) (Capítulo 3). Además, las semillas procedentes de la población de La Garganta Lóbrega resultaron ser en torno a un 26% más viables que las de las otras dos poblaciones (La Serrota y Los Conventos), hecho que podría estar motivado por la incidencia del fuego sobre esta población en el año 2005, pudiendo haber dado lugar a la población más grande y vigorosa de la especie (Figura 4) (Capítulo 3). Los beneficios del fuego en esta especie debieran de influir en dos etapas de su desarrollo: las plantas adultas rebrotan incrementando su competitividad y su capacidad de reproducción sexual en ausencia de arbustos; y las semillas aumentan su germinabilidad en presencia de cenizas y espacios abiertos.

Finalmente, los resultados mostraron que la ausencia de perturbaciones, y en concreto la ausencia de aclaramientos por fuego, limita la reproducción sexual en favor de la asexual, lo cual a su vez disminuye la disponibilidad de individuos para la reproducción sexual en esta especie autoincompatible. Optar por un sistema reproductivo mixto permite a esta especie escapar de la estocasticidad ambiental. En conclusión, paradójicamente, las actividades humanas de perturbación han podido resultar beneficiosas para algunas especies amenazadas como *P. rivas-martinezii*.

Figura 4. Extensa población de *Pseudomisopates rivas-martinezii* en la Garganta Lóbrega dos años después del incendio sufrido en 2005.

Erysimum

La perturbación beneficia a los malos competidores


E. Amat

Individuos de *Erysimum penyalarense* creciendo sobre el asfalto de la carretera de acceso a la cima de la Bola del Mundo (Sierra de Guadarrama).

Erysimum penyalarense se distribuye por los pastizales alpinos de *Festuca curvifolia* (Minuartio-Festucion curvifoliae) de Cuerda Larga (Sierra de Guadarrama). El estudio de su abundancia mostró que la especie es 43 veces más abundante de lo observado en estimaciones previas (Martínez *et al.* 2004), por lo que estaría compuesta de unos 11 millones de individuos, si bien se encontraría relegada a un área algo menor de la estimada anteriormente (Capítulo 4). En cuanto a los factores que explican su distribución dentro de su hábitat, el mapa de interpolación de la abundancia relativa indicó que el medio óptimo para la especie, donde ésta resultó ser más abundante, se encuentra en los extremos de su distribución, especialmente en el extremo oeste, que es el que presenta mayor perturbación antrópica (Capítulo 4). La especie mostró una distribución espacial agregada, debido tanto a su escasa capacidad de dispersión, como a sus requerimientos de hábitat, encontrándose distribuida por las zonas menos productivas del pastizal. Las respuestas a la perturbación varían entre especies. En nuestro caso, *Erysimum penyalarense* resultó ser más abundante en las zonas con mayor perturbación antrópica (Capítulo 4), es decir, en aquellas zonas donde se había producido remoción de suelos debido a distintas infraestructuras, como son los bordes de carreteras, pistas de esquí, escombreras o bordes de edificaciones.

No obstante, cabe destacar que el efecto global de la actividad humana en la zona no resultaría positivo, puesto que el aumento provocado por la perturbación es contrarrestado por otros efectos que conlleva dicha actividad humana, como es el impacto de los visitantes y los animales domésticos. Es más, el resultado neto del efecto de los distintos factores perturbadores implicaría una disminución estimada del 22% en la población. En este sentido, todo parece indicar que la reducción poblacional, provocada por la perturbación animal y la presencia humana en la zona, es debida a los daños indirectos ocasionados por estos dos factores. El efecto de la herbivoría de ungulados no parece ser una de las principales amenazas, puesto que sólo el 3,8% de las parcelas mostró algún daño por herbivoría en plantas de *Erysimum penyalarense* (Capítulo 4). Y esto, a pesar de que la presión de herbívoros, tanto domésticos como silvestres, es bastante fuerte en el área de estudio. De hecho el 60% de las parcelas mostraron alguna evidencia de presencia animal (heces, pisoteo o herbivoría) (Capítulo 4). Por lo tanto, el negativo efecto de los animales, pudiera estar relacionado con el pisoteo que provoca daños indirectos sobre las plantas. Igualmente, Gómez (2005) observó cómo el pisoteo de ungulados afectaba no sólo al rendimiento individual, la dinámica poblacional y estructura espacial de *Erysimum mediohispanicum* y *E. baeticum*, según el cual las plantas quedaban en muchas ocasiones relegadas al cobijo de los arbustos y el 50% de las plántulas morían, sino que además los ungulados afectaron a la distribución del hábitat y estructura de los nichos ecológicos de estas especies (Gómez 2005). En definitiva, el efecto de la perturbación animal unido a la escasa capacidad competidora de *E. penyalarense* hace que, a pesar del supuesto aporte de nitrógeno al suelo aportado por la fauna, dicha planta sólo prolifere abundantemente en lugares donde la competencia interespecífica haya sido eliminada. De la misma manera, la actividad turística en la zona tendría un efecto equivalente, que afectaría a la dinámica poblacional de *E. penyalarense*.

Figura 6. Cabra montés ibérica en los pastizales del Collado de Valdemartín (Cuerda Larga).

Por otro lado, aunque el nivel de germinación observado en el campo para *E. penyalarense* es satisfactorio y no es distinto del observado para otros *Erysimum* (Gómez 2005), cabe destacar que el comportamiento observado en las primeras etapas de su ciclo vital difiere ligeramente del observado en adultos de esta misma especie. De forma que, los factores que benefician la germinación y establecimiento temprano de plántulas difieren de los observados en individuos adultos. En primer lugar, la especie de estudio no mostró una competencia asimétrica intraespecífica con otras semillas o adultos en esta primera etapa de su ciclo vital. La especie de estudio muestra así el comportamiento característico de una planta ruderal o pionera, es decir, de una mala competidora que germina en bajas cantidades reduciendo así la competencia (Linhart 1976). En segundo lugar, la perturbación animal previa no produjo un descenso en la germinación de semillas, indicando que las alteraciones provocadas en el suelo por este tipo de perturbación no afectan a la germinación de semillas, aunque sí se produce una interferencia en etapas posteriores (Capítulo 4). En tercer lugar, en las fases tempranas del ciclo vital la competencia interespecífica suele producirse por interferencia tanto física, como química, más que por explotación de recursos (Goldberg 1990). Sorprendentemente, la comunidad de herbáceas no interfirió en el establecimiento temprano de plántulas de *E. penyalarense*, a pesar de no tratarse de un buen competidor. Este resultado contradice lo observado para otras especies de *Erysimum*, que aumentan su reclutamiento cuando disminuye la abundancia de especies acompañantes debido a la herbivoría (e.g. *E. mediohipanicum* y *E. baeticum* en Gómez 2005, y *E. crepidifolium* en Dostálek & Frantík 2008). Por tanto, la competencia con otras especies debe producirse en etapas posteriores del establecimiento y supervivencia de las plántulas.

Finalmente, destaca la facilitación especie-específica que ejerce *Cytisus oromediterraneus* en el establecimiento temprano de plántulas de *Erysimum* y que no es ejercida por el resto de especies arbustivas de los géneros *Juniperus* y *Thymus* presentes en la zona. Por su parte *Thymus praecox* además de ejercer una barrera física para el establecimiento de plántulas y desarrollo de adultos (Capítulo 5), tiene la capacidad de inhibir la germinación de otras especies por medio de la excreción de compuestos secundarios (Angelini *et al.* 2003). Por su parte, *Juniperus communis*, con su contenido de fenoles y terpenoides alelopáticos (Inderjit, 1996), si bien no inhibió la germinación tampoco la facilitó como habría cabido esperar, puesto que es común ver plantas adultas de *E. penyalarense* creciendo bajo estos arbustos.

La germinación y establecimiento temprano de *E. penyalarense* contrasta con el comportamiento observado en adultos (Capítulos 4 y 5), ya que estos no se encuentran presentes en las zonas de piornal. No obstante, las semillas ubicadas bajo individuos de *Cytisus* dispersos dentro del área de ocupación se verían favorecidas por esta especie fijadora de nitrógeno en las primeras etapas de desarrollo. En etapas posteriores de la especie sufrirán probablemente mayores daños físicos por la actividad de los ungulados, mientras que aquellas que hallan logrado germinar bajo los enebros estarán más protegidos ante este tipo de perturbación. Los fenómenos de facilitación son comunes en especies mediterráneas sometidas a fuertes condiciones de estrés, tanto abiótico como biótico (e.g. Bertness & Callaway 1994; Maestre *et al.* 2003; Gómez-Aparicio *et al.* 2005), y en muchas ocasiones son además especie-específicos (Callaway 1998). La especificidad de esta facilitación se encuentra, como hemos visto, en la interacción con las especies arbustivas debido a su fisionomía y a la producción de metabolitos secundarios.

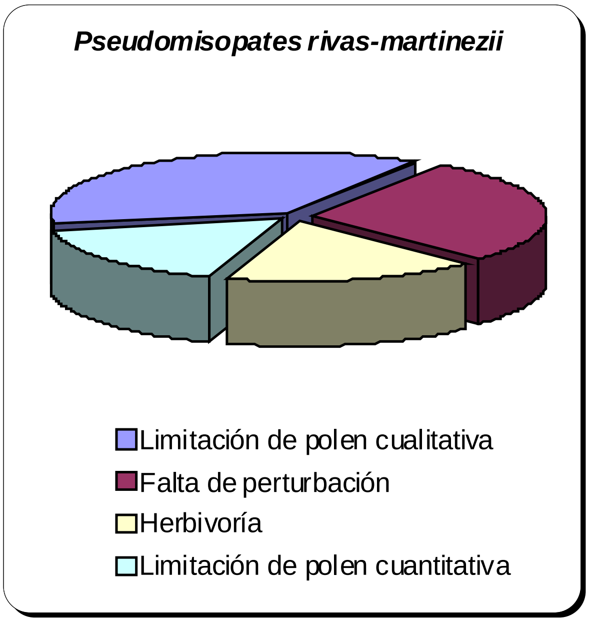
En definitiva, nos encontramos ante una mala competidora, como indica su mayor presencia en zonas altamente perturbadas y con baja presencia de competidores, así como su menor abundancia en zonas con mayor pendiente donde la heterogeneidad de hábitats aumenta la riqueza de competidores. A pesar de ser una especie poco ramoneada, la perturbación animal, así como el uso antrópico reducen su abundancia debido principalmente a los daños colaterales ocasionados. La situación observada en *Erysimum penyalarense* no es una excepción a la situación mundial de las especies ruderales y pioneras. De hecho, cada vez surgen más ejemplos de especies ruderales extintas o incluidas en listas de especies amenazadas (Kozlowski *et al.* 2009; Lososová & Simonová 1998; Kirkpatrick & Gilfedder 1998, 1995). Los regímenes de perturbación son un factor determinante en la composición y estructura vegetal, y muchas especies amenazadas que dependen de estos son en su mayoría especies ruderales de corta vida (Kirkpatrick & Gilfedder 1998, 1995).

La perturbación se ocasiona de forma natural en los ecosistemas de montaña debido a movimientos de ladera. Sin embargo, la actividad humana los ha favorecido debido a la construcción de infraestructuras en estas áreas. No obstante, existen efectos colaterales provocados por la actividad humana, por lo que aunque la especie de estudio se beneficie de la perturbación antrópica, no depende de ella para su supervivencia. Por otro lado, cabe destacar que la restauración llevada a cabo en los últimos años en el Parque Regional de Peñalara, en el que se han cerrado pistas de esquí y restaurado taludes, podría contarse entre las causas de la presunta desaparición de la especie en ésta, su localidad clásica. La especie bien podría haber sido desplazada en pastizales maduros, aquellos en los que se ha extendido la vegetación arbustiva o en los que la sobreexplotación por parte del ser humano y su actividad ganadera se ha hecho patente.

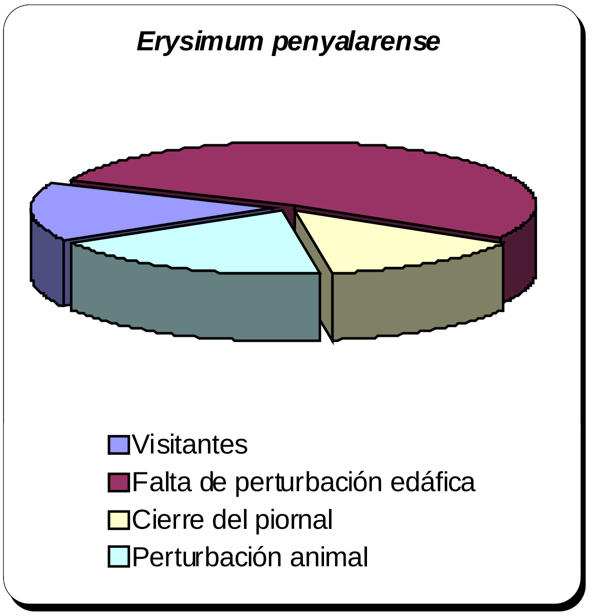
Figura 7. Individuos de *Erysimum penyalarense* creciendo en una antigua escombrera en la Bola del Mundo (Cuerda Larga).

Medidas para la conservación

Los resultados recogidos en la presente memoria doctoral tienen implicaciones para la conservación de estas dos especies de la montaña mediterránea. Además arrojan luz a la posible situación presente en otras especies amenazadas. A continuación se muestran a modo de resumen las causas de amenaza detectadas para *P. rivas-martinezii* y *E. penyalarense* en el presente estudio, atendiendo a su importancia relativa.



La falta de polen de calidad es la causa principal de amenaza de Pseudomisopates. Esta situación deriva de la falta de perturbación en su hábitat, la cual provoca el cierre de la vegetación arbustiva, favoreciendo así la clonalidad y reduciendo el número de visitantes florales. Por otro lado la notable herbivoría por ungulados, de nuevo, impulsa la clonalidad e impide la reproducción sexual.



Actualmente la falta de perturbación edáfica limita el establecimiento de nuevos individuos de Erysimum y es su principal causa de amenaza. Los daños indirectos provocados por herbívoros y visitantes interfieren en el asentamiento de la especie. Mientras que a largo plazo el cierre total de la vegetación arbustiva acabaría con el hábitat propio de la especie.

Nos encontramos ante dos especies catalogadas como amenazadas debido a su aparente rareza o escasez. Sin embargo, al igual que en el caso de muchas especies amenazadas, son precisamente el desconocimiento de su biología de poblaciones, y a la intención, en ocasiones, de mantener los ecosistemas libres de toda perturbación los que han evitado el establecimiento de medidas de conservación adecuadas. No es la primera vez que el desconocimiento de la biología de las especies, su genética y requerimientos ecológicos han dado al traste con las medidas dispuestas para su conservación, e.g. *Lysimachia minoricensis* (Rosselló & Mayol 2002). Por tanto, una vez determinada la situación actual de amenaza y sus causas, se proponen las siguientes medidas de conservación.

En primer lugar, *Pseudomisopates rivas-martinezii* ha demostrado ser una especie adaptada a un cierto grado de perturbación. En concreto, esta especie muestra una especificidad hacia el fuego en un ambiente que precisamente ha sido sometido a este tipo de perturbación desde hace años. De hecho, el registro palinológico de Gredos muestra que antes del siglo XII dominaban los bosques de *Pinus sylvestris*; mientras que el actual paisaje de Gredos está claramente antropizado según un mosaico de bosques, pastos y formaciones de piornal generado a partir del sigo XVII. Fue en este periodo cuando comenzaron las actividades de gestión que incluían fuegos y ganadería, lo que generó la deforestación y expansión del piornal pirófilo (López-Merino *et al.* 2009). Considerando este escenario, es posible que *P. rivas-martinezii* haya estado más amenazado en un pasado y que las técnicas de manejo tradicionales de los últimos años hayan favorecido su expansión. Esto supone que la aplicación de fuegos controlados podría asegurar la supervivencia de sus poblaciones, al mantener un cierto grado de perturbación que reproduzca las condiciones naturales. Estas medidas encaminadas a fomentar la apertura de la vegetación arbustiva, contribuirían a su vez a disminuir la limitación de polen cualitativa que sufre la especie, contrarrestando sus dos factores principales de amenaza.

Ahora bien, también hay que destacar que la sustitución de piornales por pastos para explotación ganadera no es la solución a largo plazo, puesto que esta presión de herbivoría obliga a la especie a mantenerse mediante un crecimiento vegetativo. Situación que no puede mantenerse de forma definitiva. Se recomienda en todo caso un control de la presión ganadera en sus poblaciones, concediendo a la especie períodos de tiempo suficientes para permitir tanto el asentamiento de nuevos individuos, como la reproducción sexual de los individuos maduros. Habida cuenta de los resultados aquí obtenidos se desaconseja el vallado de poblaciones con el fin de excluir al ganado, ya que esto supondría la expansión del piornal. Además, la experiencia que supuso el vallado de la población de la Covacha en 2003, apoya la inconsistencia de esta medida, puesto que con ella se produjo una drástica reducción de la floración (del 50%) en años sucesivos. Esta situación también se ha observado en la población de la Garganta de Chilla, en la que los poco *ramets* que perviven al borde de un sendero, en un piornal completamente cerrado que no presenta reproducción sexual alguna.

La alta heterocigosidad en la población de La Serrota hace de ella una población candidata a la conservación, si bien la erosión de alelos que regulan la autoincompatibilidad puede limitar la capacidad reproductiva de las poblaciones (Young *et al.* 1999). Además, estudios en curso, incluyendo muestras de todas las poblaciones conocidas de la especie, muestran que la ausencia de una estructura genética espacial y su alto nivel de diversidad genética son indicativos de la salud de la que goza la especie, y cuyas poblaciones han debido de fragmentarse muy recientemente. No obstante, *a priori* no se recomienda incrementar el número de individuos en sus poblaciones, ya que en el caso de plantas clónicas expuestas a perturbaciones de forma natural, el rebrote y la producción de semillas juegan un papel crucial en la regeneración de las poblaciones (Keely & Fotheringham 2000; Ojeda *et al.* 2005) y por tanto en el mantenimiento de su diversidad genética de forma natural. En este sentido, se requiere de estudios más detallados de la dinámica poblacional y su respuesta al fuego *in situ*, puesto que aunque se ha observado una vigorosa proliferación de la especie tras el fuego de 2005 en la población de la Garganta Lóbrega y una mayor viabilidad de sus semillas, no se ha podido constatar si esta se debió estrictamente a rebrotes o también al asentamiento de nuevos individuos de semilla.

Por su parte, *Erysimum penyalarense* muestra un comportamiento pionero y se beneficia, como hemos visto, de la perturbación que provoca el movimiento de tierra y la eliminación de competidores. La actividad antrópica ha promovido estas condiciones en su área de distribución, lo cual no significa que dependa de ella. En el caso de esta especie, las recomendaciones para su conservación se basan principalmente en permitir un régimen natural de perturbaciones en el espacio natural en el que está incluida.

En relación a los demás factores explicativos de su abundancia, se recomienda un control de la presión de herbívoros en la zona, que repercutirá no sólo en esta especie, sino también en el alto nivel de herbivoría registrado en el área para sus especies acompañantes y facilitadoras. En cuanto a la presencia humana en la zona, la presión registrada en el presente estudio no es excesivamente alarmante. Además dicha actividad se encuentra concentrada entorno a los caminos y la estación de esquí, por lo que en principio no se precisarían medidas inminentes. En este sentido serían necesarios estudios a largo plazo que cuantificaran el daño ocasionado tanto por animales como por humanos en esta especie y su comunidad. En definitiva, será el equilibrio entre las actividades que se desarrollan en su área de distribución el que determinará la viabilidad de la especie.

En cuanto al avance observado del piornal en Guadarrama (Sanz-Elorza *et al.* 2003) y que pudiera suponer un riesgo para la especie, este puede ser promovido tanto por los cambios en la gestión del suelo, como por el cambio climático global. En caso de una proliferación excesiva de la vegetación arbustiva, una gestión que mantenga el aclarado podría ser suficiente para esta especie, ya que presenta un rango altitudinal bastante amplio para una especie de montaña, por lo que se puede prever que presenta una tolerancia a distintas temperaturas mayor que otras especies de la zona.

Por último, se propone la reintroducción de la especie en su localidad clásica. En este sentido, el presente estudio aporta suficiente información para seleccionar aquellas zonas más adecuadas para el desarrollo de la especie.

Reflexión final

A lo largo de la presente memoria se ha intentado poner de manifiesto el importante papel que juega la perturbación en las especies de los ecosistemas de montaña mediterránea, dónde el equilibrio ecológico es si cabe más delicado que en ningún otro. La creencia de que la perturbación es un proceso natural que debe ser incorporado en los planes de gestión está revolucionando la gestión de espacios naturales en todo el mundo. Hoy sabemos que una intensidad y frecuencia moderada de perturbación genera una riqueza máxima de especies, según predice la hipótesis de perturbación intermedia (Connell 1978). Por este motivo, los espacios naturales deben ser lo suficientemente grandes para permitir un régimen natural de perturbaciones y contener zonas en distintos niveles de sucesión (Pickett & Thompson 1978). De esta manera, las medidas de gestión han de aplicarse teniendo en cuenta el régimen de perturbaciones requerido por las especies que se pretenda fomentar (Hobbs & Huenneke 1992).

Si bien las zonas protegidas juegan un papel crucial en la conservación de las especies amenazadas, especialmente de aquellas más sensibles a la perturbación antrópica, en ocasiones resulta complicado establecer un equilibrio entre las prácticas que resultan beneficiosas y las que resultan perjudiciales. De hecho, la presencia de actividad antrópica en zonas protegidas (ganadería, turismo, extracción de madera, etc.) ha visto mermada la abundancia de muchas especies (e.g. Caro 1999; Kerbiriou *et al.* 2009). Trabajos recientes sobre otra especie de *Erysimum* del sur de la Península Ibérica, *Erysimum popovii*, ponen de manifiesto el desequilibrio existente entre conservación y mantenimiento de prácticas tradicionales de manejo en la zona de distribución de la especie (Fernández & Gómez 2012). En concreto, los autores señalan que los efectos beneficiosos derivados de la protección (mayor eficacia en la polinización) son contrarrestados por los efectos negativos (proliferación de la vegetación arbustiva, en una especie que ocupa espacios aclarados, y sobre todo herbivoría excesiva sobre la especie). A la luz de los resultados observados en los estudios sobre distintas especies amenazadas del género *Erysimum*, se hace necesario un control más estricto de la permisividad de ciertas actividades humanas perjudiciales para las especies de flora en zonas protegidas y sobre todo una reducción de la presión ganadera en estas zonas.

A pesar del reconocido papel que juegan las perturbaciones en los ecosistemas, su aplicación como medida de conservación no está exenta de polémica. De entre todas las medidas de gestión quizás sea la del uso del fuego la más conflictiva, no sólo por el riesgo que entraña para la vida humana y nuestros bienes, sino por el valor sentimental de muchos espacios naturales. De hecho, aún hoy en día se reconoce que muchos fuegos son extinguidos simplemente porque resulta más sencillo apagarlos que tener que dar explicaciones de porqué no se apagaron, y eso a pesar del tremendo coste que supone para las arcas del estado y el riesgo que entraña para los equipos de extinción de fuegos. Sin embargo, no podemos olvidar que el fuego es un proceso natural en muchos ecosistemas mediterráneos y ha sido aplicado con eficacia para la gestión de especies amenazadas (Fensham & Fairfax 1996), entre las cuales destaca el caso de *Sequoiadendron giganteum* (Swetnam *et al.* 2009). En efecto, el mejor ejemplo en la gestión del fuego lo encontramos en las políticas llevadas a cabo por el Servicio Nacional de Parques de Estados Unidos.

La disminución de las tormentas, la protección contra incendios y el ganado que pastaba en los montes desde los inicios del siglo XX en Estados Unidos disminuyeron drásticamente la intensidad de los incendios en los bosques. Esto contribuyó al aumento de combustible de suelo y la proliferación de especies muy sensibles a los incendios, a la vez que perjudicaba a especies que requerían directa e indirectamente del fuego para su reproducción. Fue por ello que en 1970 el Servicio Nacional de los Parques empezó las quemas controladas de sus arboledas para corregir estos problemas. Las políticas actuales van encaminadas a permitir la presencia de fuegos naturales sin interrupciones (Bradstock *et al.* 1995). Un caso paradigmático en conservación fue precisamente el incendio del Parque Nacional de Yellowstone en 1988 (Romme & Despain 1989).

En cuanto a nuestro territorio, la presencia de fuego en el Mediterráneo ha supuesto a lo largo de la historia no sólo un agente de perturbación (que abre claros en zonas boscosas o de matorral y genera oportunidades), sino un agente selectivo (que ha influido en la evolución de algunos rasgos de la flora mediterránea). La evidencia histórica de presencia humana, que es mayor en el Mediterráneo en relación al resto de Europa, se remonta a unos 400.000 años antes del presente, con el descubrimiento del hombre de Tautavel (Francia). Los fuegos que parecen haber ocurrido de forma natural al menos desde el Mioceno (Dubar *et al.* 1995), fueron incrementando con la actividad humana, especialmente desde el Holoceno, unos 4.500 años antes del presente (Carrión *et al.* 2010; Pons & Reille 1988). Esto contribuyó al aumentando progresivo de la sequía y habría promovido la evolución de muchas especies adaptadas a la actividad antrópica en el Mediterráneo (Carrión *et al.* 2010). No obstante, estudios recientes muestran que a pesar de que la actividad humana haya contribuido a la degradación de nuestros ecosistemas desde el Holoceno medio hasta nuestros días, esta no ha resultado indispensable para el establecimiento de la vegetación esclerófila mediterránea (Collins *et al.* 2012). Estos resultados contradicen la idea previa de que la vegetación actual del Mediterráneo corresponde a una fase de degradación. Además, el paisaje del Mediterráneo habría estado compuesto en el pasado por un mosaico de ecosistemas más que por un continuo de bosques (Thompson 2005). En la actualidad, la periodicidad de los fuegos producidos de forma natural en la cuenca del Mediterráneo parece ser menos predecible que en otras zonas de clima mediterráneo (Keeley & Fotheringham 2000). La menor frecuencia de fuegos en nuestras latitudes en relación con las regiones florísticas mediterráneas de África o Australia han dado lugar a una mayor proporción de especies adaptadas a rebrotar tras el fuego y no son tantos los ejemplos de plantas que se ven directamente estimulados por elementos propios del fuego, como el humo o las cenizas (Keeley & Fotheringham 1999, 2000). En este sentido, *Pseudomisopates rivas-martinezii* constituye un claro ejemplo de planta rebrotadora, cuyos estolones sobreviven al fuego; a la vez que semilladora, cuya germinación se ve estimulada por las cenizas proporcionadas por el fuego.

A pesar del importante ejemplo aportado por Estados Unidos, las políticas agroambientales y de conservación en nuestro país van encaminadas a la prevención de incendios y supresión del fuego de nuestros ecosistemas (Suárez *et al. 1*997). Por ejemplo, en el Parque Nacional de Doñana (Huelva) se practica una supresión activa de cualquier foco de incendio. Esta eliminación del fuego del ecosistema provoca una acumulación progresiva de combustible. Simultáneamente existe un uso puntual del fuego para reducir ciertas acumulaciones de combustible resultado del aclarado de los pinares. El uso del fuego queda relegado a prácticas tradicionales para favorecer rodales de pastizal que favorezcan a las poblaciones de conejo, que a su vez puedan revertir positivamente en las poblaciones de lince (Lloret 2002). En el Parc Natural del Cap de Creus (Gerona), también se practica una supresión del fuego, pero se permite su uso tradicional para favorecer pastizales que mejoren la calidad cinegética o reduzcan el combustible (Lloret 2002).

También recientemente han surgido propuestas en nuestro país de introducir ganado caprino y bovino, como tratamiento preventivo frente al fuego en la Comunidad de Madrid (www.cam.org 2011). Y todo ello en aras de la protección de la biodiversidad, conservación de la riqueza y prevención de incendios forestales. Dicho esto, nadie duda de que la incorporación del fuego como herramienta de conservación para aumentar la abundancia de especies amenazadas debe ser estudiada con precaución, ya que el fuego podría tener un efecto negativo en la comunidad y efectos impredecibles en la especie que se desea gestionar (Pendergrass *et al.* 1999). Aún así este tipo de iniciativas nos indican que todavía estamos muy lejos de gestionar nuestros espacios naturales de acuerdo a su dinámica natural.

El presente trabajo muestra dos ejemplos claros de especies amenazadas de montaña que se ven favorecidas por la perturbación de sus hábitats. Este podría ser el caso de muchas otras especies catalogadas como amenazadas. Sin embargo, son cada vez más los casos de poblaciones de especies protegidas que se vallan y aíslan para preservarlas de la alteración de su medio. Desde nuestra experiencia, queremos resaltar la importancia del conocimiento de la dinámica poblacional de cada especie antes de tomar medidas que puedan resultar contraproducentes para su conservación. No debemos olvidar que aunque los eventos de perturbación, y en especial los provocados por el fuego, resultan muy dramáticos a escala temporal humana, pueden no serlo tanto a una escala más amplia de regeneración y evolución de la vegetación. Queda por tanto por resolver la cuestión de cuántas especies ibéricas han sobrevivido a pesar de su estado de amenaza, por ser resistentes e incluso verse favorecidas por las actividades humanas, y cuantas no han podido resistir a la rápida expansión humana a lo largo y ancho del Mediterráneo.

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Conclusiones

Sobre *Pseudomisopates rivas-martinezii:*

1) Es una especie predominantemente autoincompatible, que sufre de limitación de polen cuantitativa, debido a la baja frecuencia de visitas de polinizadores, y cualitativa, debido a la reducida disponibilidad de individuos compatibles para la reproducción.

2) La limitación de polen en su hábitat natural está motivada por la fuerte estructura genética espacial de sus clones, que dificulta el entrecruzamiento entre individuos.

3) La fuerte clonalidad de la especie se pone de manifiesto a través de una baja riqueza genotípica (*PD* = 0,23), si bien su alta diversidad genética (*He* = 0,42) apunta a una alta persistencia de *genets*, que estarían manteniendo la población a pesar del escaso reclutamiento de nuevos individuos.

4) Los factores ambientales que favorecen la reproducción vegetativa frente a la reproducción sexual, son el cierre de la vegetación arbustiva y la herbivoría. Ambos factores reducirían significativamente el nivel de floración, a la vez que promoverían el crecimiento de los clones, dificultando cada vez más el acceso a individuos para la reproducción.

5) Los requisitos para la germinación son en primer lugar un período de frío, seguido de los efectos directos e indirectos del fuego, en segundo lugar. Entre los factores directos del fuego destaca el estímulo químico que ejerce la presencia de ceniza en el medio; mientras que, entre los efectos indirectos se encuentran el aclarado de la vegetación arbustiva que favorece la germinación, y provoca a su vez el intenso rebrote de la especie, promoviendo así una vigorosa fructificación.

6) Su sistema reproductivo mixto le permite adaptarse a la estocasticidad ambiental, a la espera de una perturbación provocada preferiblemente por el fuego, en un entorno de matorrales pirófilos y que tradicionalmente han sido gestionados mediante quemas.

7) Las medidas de conservación para *Pseudomisopates rivas-martinezii* deben ir encaminadas a respetar las perturbaciones naturales de su ecosistema y fomentar el aclarado de la vegetación, así como a controlar la presión de herbívoros, con el fin de permitir la regeneración de la especie. La aplicación de quemas controladas sólo debe llevarse a cabo después de un estricto análisis de la frecuencia e intensidad requeridas.

Sobre *Erysimum penyalarense:*

8) Se distribuye por los pastizales alpinos de *Festuca curvifolia* de la Cuerda Larga (Sierra de Guadarrama). Desciende por las zonas más aclaradas de la cara norte, evitando zonas con densas formaciones de piornal, cervunales, canchales y gleras, así como los rincones más húmedos donde abundan especies como *Festuca iberica*, *Campanula herminii* o *Thymus praecox.*

9) El estudio de la distribución y abundancia de la población de la Cuerda Larga en 2006 indicó que esta se presenta un área de ocupación efectiva de 7,09 Km^2 y que estaba compuesta por al menos 11 millones de individuos, en contraposición a la estimación de trabajos previos que apuntaban a un área superior, pero con una abundancia muy inferior.

10) *Erysimum penyalarense* no es una buena competidora, por lo que se beneficia de la perturbación y es facilitada por especies arbustivas como *Cytisus oromediterraneus* en sus primeras fases de establecimiento. Si bien la actividad humana puede favorecer su expansión a través de la creación de huecos en el piornal, los daños indirectos provocados por ungulados y visitantes resultan negativos para su supervivencia. Por lo tanto, el efecto neto de la actividad humana depende del equilibrio de las distintas actividades desarrolladas en su área de distribución.

11) Las medidas de conservación en *Erysimum penyalarense* deben ir encaminadas a garantizar un nivel de perturbación intermedia, que permita la presencia de zonas en los primeros estadios de sucesión tanto en la Cuerda Larga como en la localidad clásica del macizo de Peñalara. La presión de herbívoros y visitantes en la zona deben ser controlados, y sus efectos deberían ser objeto de un estudio más detallado.

12) Se recomienda la reintroducción de la especie en el macizo de Peñalara con material representativo de la población de Cuerda Larga, siguiendo un muestreo como el realizado en el presente trabajo.

Conclusión general*:*

La perturbación es un proceso natural que forma parte de los ecosistemas mediterráneos de montaña, por lo que muchas especies están adaptadas a ella, e incluso dependen de ella para su regeneración. Predecimos que éste podría ser el caso de muchas otras especies aún no estudiadas y dependientes de un cierto grado de perturbación para su supervivencia.

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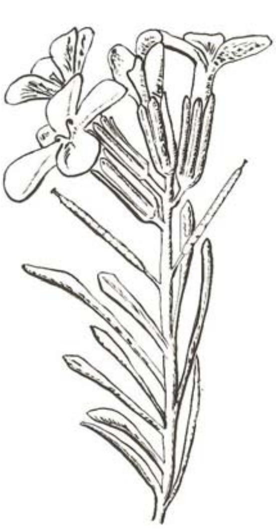

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"There are places I'll remember all my life
Though some have changed
Some forever not for better
Some have gone and some remain
All these places had their moments
With lovers and friends I still can recall
Some are dead and some are living
In my life I've loved them all"

Lennon and McCartney

¿Sabías que los ecosistemas de la montaña mediterránea son especialmente vulnerables debido a sus características climáticas? ¿Y que es probable que muchas especies de plantas mediterráneas estén catalogadas como amenazadas debido al desconocimiento de sus ciclos vitales? ¿Te has preguntado alguna vez por qué algunas especies persisten a pesar de habitar en lugares sometidos a altos grados de perturbación?

"Biología de la conservación de ***Pseudomisopates rivas-martinezii*** y ***Erysimum penyalarense***, dos táxones amenazados de la Cordillera Carpetana" da respuesta a estas preguntas a través del estudio de dos táxones emblemáticos de la montaña mediterránea. Partiendo de las supuestas causas de amenaza de estos dos táxones, el estudio de su biología y requerimientos ecológicos nos lleva a desvelar los factores que verdaderamente afectan a su supervivencia y a proponer las medidas de conservación más adecuadas para ellos. Los resultados obtenidos arrojan luz a la posible situación de otras especies amenazadas.