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Biosistemática del género *Hypochaeris* sect. *Hypochaeris*: implicaciones filogeográficas y evolutivas

Memoria presentada por la Licenciada María Ángeles Ortiz Herrera para optar al grado de Doctor en Biología (Doctorado Europeo) por la Universidad de Sevilla

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1 Introducción general

La familia de las Compuestas

La familia Compositae

La familia de las compuestas (Compositae o Asteraceae) es una familia natural y como tal ha sido reconocida por todos los botánicos desde la antigüedad, sin apenas variación en su contexto taxonómico. Linneo (1753) la incluyó en la Clase XIX "Syngenesia" de su sistema sexual, y dentro de ella en la "Syngenesia polygamia" atendiendo a dos cualidades que tienen los miembros de esta familia, como la fusión de los cinco estambres que tienen las flores formando un tubo por donde ha de pasar el estilo (Syngenesia) y la reunión de flores en una unidad de inflorescencia que es el capítulo o cabezuela (polygamia). Todas las flores de las Compuestas son pentámeras, epiginas y proterandras. La dehiscencia de las anteras es introrsa y cuando el estilo pasa por el tubo de las anteras éste arrastra el polen del tubo quedando depositado a lo largo del estilo. Esto es lo que se conoce como exposición secundaria del polen y lo presentan también, dentro de las dicotiledóneas, la familia Campanulaceae (incluida Lobeliaceae) y también la subfamilia Ixoroideae de la familia Rubiaceae (Bremekamp 1966). Las flores de las Compuestas tienen un disco nectarífero en la parte superior del ovario que rodea al estilo y este tipo de nectario es parecido al que presenta también la familia Umbeliferae, con la que algunos autores la habían relacionado por la presencia de aceites volátiles en cavidades esquizógenas (Cronquist 1977). Muchas especies de esta familia presentan autoincompatibilidad genética mediante la cual una planta no produce semillas con polen de la misma planta. La reacción de la incompatibilidad se establece entre las proteínas de la exina (de origen tapetal, es decir del esporofito) y las papilas estigmáticas, la cual origina un acumulo de calosa que impide la germinación del polen. Este tipo de autoincompatibilidad se ha denominado autoincompatibilidad esporofítica, y está gobernada por la acción de un gen con varias formas alélicas que exhiben desde una dominancia completa hasta una codominancia presentando típicamente relaciones de dominancia distinta en el grano de polen y en el estigma (Nettancourt 1977). Este tipo de autoincompatibili-

dad fue descubierta a mediados del siglo XX en dos géneros simultáneamente *Crepis* (Hughes & Babcock 1950) y *Parthenium* (Gerstel 1950), pero desde entonces se ha encontrado en otros muchos géneros de las Compuestas y en otras familias como Betulaceae, Brassicaceae, Caryophyllaceae, Convolvulaceae y Polemoniaceae (Hiscock & Kües 1999).

Los numerosos trabajos de filogenia molecular han situado las compuestas en el orden Asterales (Fig. 1.1), hermano filogenético de Apiales y Dipsacales (Moore *et al.* 2007). El orden Asterales está formado por seis familias: Asteraceae, Stylidiaceae, Calyceraceae, Menyanthaceae, Campanulaceae y Goodeniaceae. La familia Calyceraceae es hermana filogenética de la familia Asteraceae y Goodeniaceae hermana de ambas

Diversidad y clasificación de las Compuestas

En total la familia Compuestas contiene 1.546 géneros y 22.800 especies (sin contar los microtaxones de *Hieracium* y *Taraxacum*), es decir, casi el 10% de las especies de Angiospermas. Jeffrey (1978) dividió la familia Asteraceae en dos subfamilias: Cichorioideae (Lactucoideae), con ocho tribus y Asteroideae con nueve tribus. Esta clasificación es seguida básicamente por Judd *et al.* (1999) y por Hind (2007), pero estos autores consideran también la subfamilia Barnasioideae, que en el sistema de Jeffrey (l.c.) estaba incluida dentro de la tribu Mutisieae de la subfamilia Cichorioideae. Esto obedece a los últimos trabajos de filogenia molecular donde Panero & Funk (2002) y Funk *et al.* (2005) desmembran la familia Asteraceae en 11 subfamilias (Fig. 1.2), muchas de ellas con una sola tribu.

La subfamilia Cichorioideae

La subfamilia Cichorioideae s.s. (según Funk *et al.*, 2005) comprende cuatro tribus: Gundelieae, Cichorieae, Arctoteae, Liabeae y Vernonieae. Gundelieae, una pequeña tribu con dos géneros monotípicos del NW de África y el Mediterráneo oriental, es la hermana filogenética de Cichorieae, y estas dos tribus hermanas de

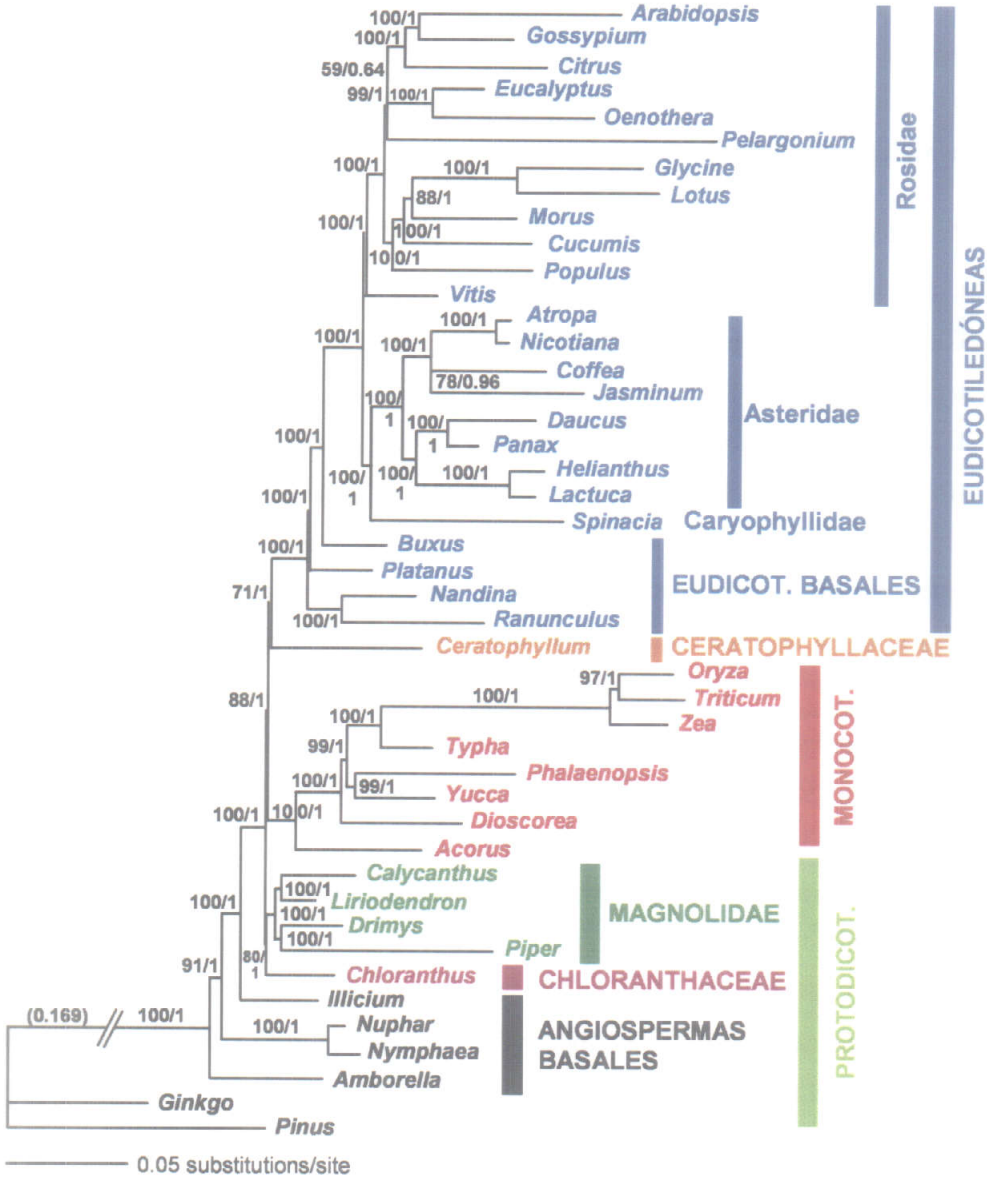

Fig.1.1. Filograma del mejor árbol (método de máxima verosimilitud) de una combinación de secuencias de 61 genes codificadores de proteínas plastídicas. Los números asociados a cada rama se corresponden con el soporte del método de máxima verosimilitud (si es mayor del 50%) / probabilidad por métodos bayesianos (si es mayor de 0,5). El número en paréntesis se corresponde con la longitud de la rama que separa a las Angiospermas de la Gimnospermas, usadas como grupo externo. Modificado de Moore *et al.* (2007).

Arctoteae, Liabeae y Vernonieae (Fig. 1.2). Las tribus Vernonieae y Cichorieae son las más diversas de la subfamilia con casi 100 géneros y unas 1.500 especies cada una (sin contar las microespecies agámicas de *Hieracium* y *Taraxacum* pertenecientes a la tribu Cichorieae).

La tribu Cichorieae

Como la tribu Gundelieae, con *Gundelia* y *Warionia*, es hermana filogenética de Cichorieae y más primitiva que ésta última (Funk *et al.* 2004), se supone que los taxones basales de Cichorieae también se originaron en el mismo marco geográfico que el de la tribu Gundeliaea, es decir en el N de África y E del Mediterráneo (Funk *et al.* 2005). En un trabajo muy reciente de secuenciación de genes nucleares (ITS) (Kilian *et al.* en revisión) encontraron que *Gundelia tournefortii*, especie endémica del Mediterráneo oriental, queda dentro claramente de la subtribu Scolyminae, mientras que el género *Warionia*, con una sola especie endémica del NW de África (*W. saharae*), es hermana filogenética de dicha subtribu. Por lo tanto la tribu Gundelieae (sensu Panero y Funk 2002 y Funk *et. al.* 2005) debe ser incluida en la tribu Cichorieae, con *Gundelia* dentro de la subtribu Scolyminae y *Warionia* formando una subtribu propia: Warioniinae.

Las mayores sinapomorfías de la tribu Cichorieae son: las flores liguladas (limbo de la corola unilateral, con 5 dientes) y la abundancia de latex blanco en los tallos y hojas tiernas. Otra sinapomorfía que afecta a todos los miembros de la tribu es la nastia de las flores. Todas las flores del capítulo en antesis se abren con la llegada del sol a la población y un poco después del mediodía todas las flores se cierran, volviéndose a abrir durante todos los días que el capítulo permanece en antesis. (Cassini 1821) dice al respecto de este fenómeno:

> *"Les corolles sont ordinairement jaunes, quelquefois orangées, rouges, violettes ou bleues; elles sont, en général, d'une substance très-délicate, et sujettes à éprouver les alternatives de la veille et du sommeil, suivant les heures du jour, ou suivant l'état de l'atmosphère. "*

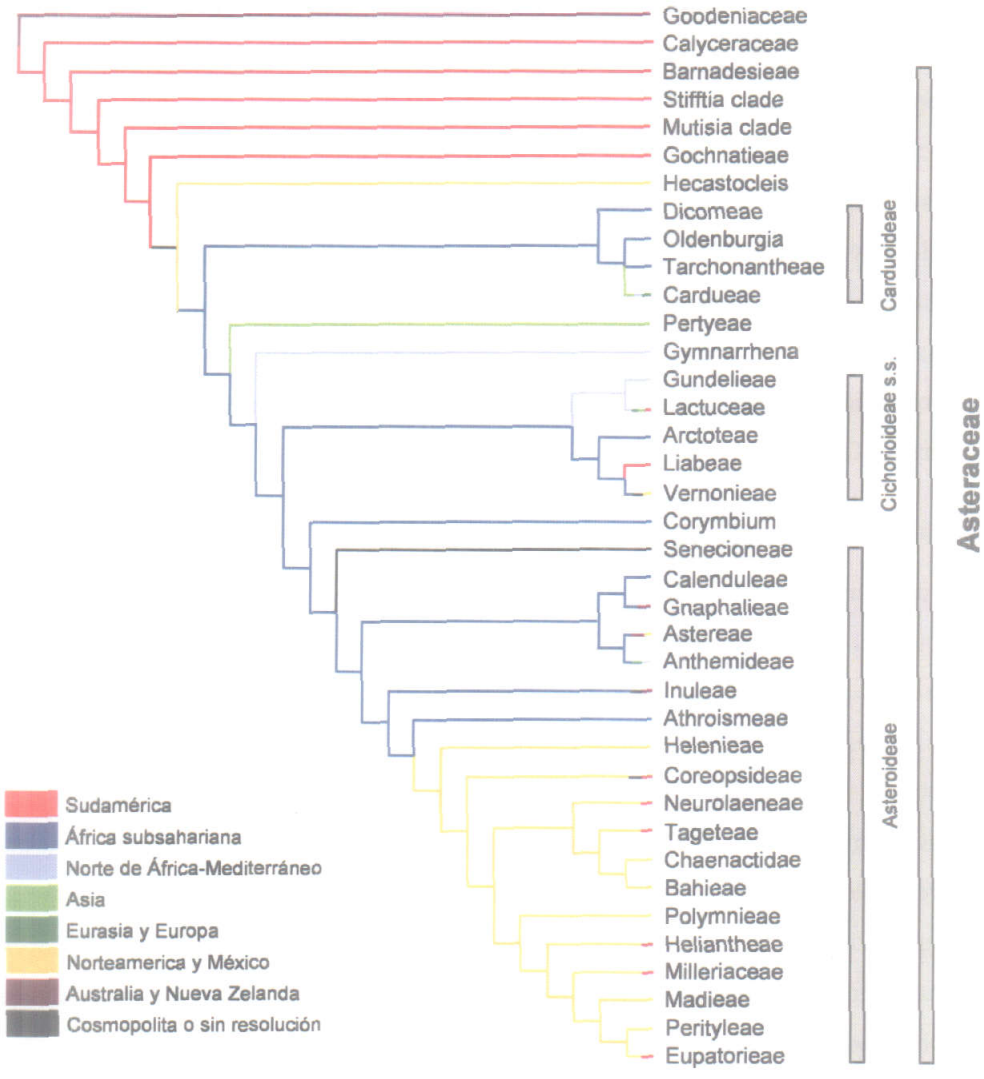

Fig.1.2. Superárbol construido con superposiciones de árboles parciales de las diferentes tribus. Modificado de Funk *et al.* (2005).

La tribu Cichorieae se divide actualmente en 11 subtribus: Warioniinae, Scorzonerinae, Scolyminae, Lactucinae, Hyoseridinae, Crepidinae, Chondrillinae, Hypochaeridinae, Hieraciinae, Microseridinae y Cichoriinae.

Todas estas subtribus tienen una distribución predominantemente mediterráneo-macaronésica o asiática, excepto la subtribu Microseridinae (incluida Stephanomeriinae y Malacothricinae) con 20 géneros todos americanos, salvo *Microseris* que tiene una especie en Australia y Nueva Zelanda (Tabla 1.1). También en la tribu Cichorieae encontramos disyunciones muy notables entre el Viejo y el Nuevo Mundo. Por ejemplo el género *Dendroseris* (hoy incluido en el género *Sonchus*) de la subtribu Hyoseridinae, con 11 especies, es endémico de las Islas de Juan Fernández (Chile) en el Pacífico oriental y el género *Hypochaeris*, con una distribución amplia en el Viejo Mundo, tiene la mayoría de las especies en Sudamérica (Tabla 1.1).

Evolución de la tribu Cichorieae

Al parecer las Cichorieae se separaron de las Arctoteae hace cerca de 35 millones de años en el continente africano (Tremetsberger *et al.* en revisión). La diversificación de la tribu se produjo pronto, hace unos 30 millones de años. El grupo actual más antiguo es el de las subtribus Warioniinae y Scolyminae (c. 20 millones de años) seguido de la subtribu Scorzonerinae (c. 19 millones de años). Estas tres subtribus tienen actualmente una distribución circunmediterránea, habitando las zonas esteparias secas y frecuentemente frías, condiciones compatibles con el clima del Eoceno Medio y del Oligoceno Inferior. Efectivamente, en el Oligoceno Inferior aparecen macrofósiles de varios géneros de Cichorieae en el sur de Europa (Small 1919). El resto de las Cichorieae divergieron más tarde durante el Terciario, y la mayoría de la subtribus sufrieron una rápida diversificación en el Mioceno Superior (Fig. 1.3). En el Mioceno Superior se cerró el mar Mediterráneo por el occidente y el clima de la cuenca mediterránea se tornó más seco y estepario, produciéndose la desecación parcial de este mar (Bocquet *et al.* 1978). Esto permitió que se establecieran puentes continentales entre Europa, el norte de África y Asia que posibilitaron la migración de especies vegetales y animales Norte-Sur y Este-Oeste (Bocquet *et al.* 1978), como debió ocurrir con las distintas ramas evolutivas de las Cichorieae.

Tabla 1.1. Sinopsis de las subtribus de Cichorieae (extraído de Kilian *et al.* en revisión)

Subtribus	**Nº de géneros**	**Nº de especies**	**Distribución**
Warioniinae	1	1	NW África
Scorzonerinae	10	c. 300	Región Mediterránea
Scolyminae	4	12	Región Mediterránea
Lactucinae	4	c. 250	Región Mediterránea
Hyoseridinae	5	c. 150	Región Mediterránea, Macaronesia, Pacífico Oriental, Nueva Zelanda y Australia
Crepidinae	22	c. 360 (+1.600 spp apomícticas de *Taraxacum*)	Hemisferio Norte
Chondrillinae	3	28	Región Mediterránea y Asia
Hypochaeridinae	7	c. 150	Región Mediterránea, Macaronesia, Australia (*Picris*) y Sudamérica (*Hypochaeris*)
Hieraciinae	5	c. 20 (+770-5.200 *Hieracium*; +110-700 *Pilosella*)	Subcosmopolita
Microseridinae (incluida Stephanomeriinae y Malacothricinae)	22	c. 120	Nuevo Mundo, una especie en Australia y Nueva Zelanda
Cichoriinae	6	25	Región Mediterránea y Macaronesia

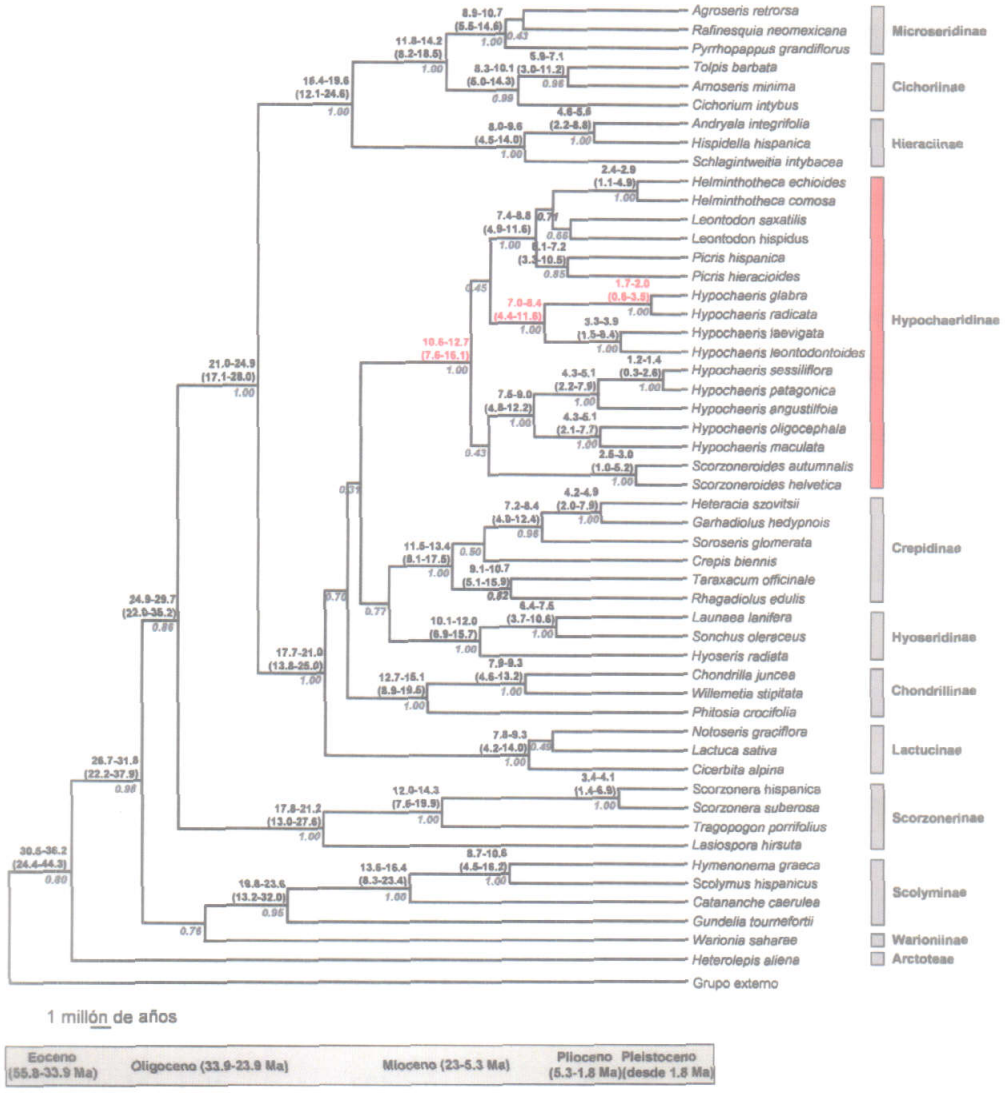

Fig.1.3. Cronograma de Cichorieae (basado en secuencias nucleares, ITS1 e ITS2 y construido con el programa BEAST). La media de la edad mínima y máxima se muestra en la parte superior de las ramas, en paréntesis para nodos con una probabilidad posterior ≥ 0.80. La probabilidad posterior bayesiana se muestra en la parte inferior de las ramas. Modificado de Tremetsberger *et al.* (en revisión).

La subtribu Hypochaeridinae

La subtribu Hypochaeridinae está formada por 7 géneros, con c. 150 especies (Tabla 1.2). Se caracteriza por ser hierbas anuales o perennes, a veces subfrutescentes, con hojas caulinares, indumento formado muy frecuentemente por pelos o setas ramificados, tubo de la corola muy peloso cerca del limbo, aquenios con pico más o menos desarrollado, al menos los centrales del capítulo, y vilano plumoso, solo en *Hedypnois* son subplumosos. Los límites genéricos son difusos, sólo el género *Hypochaeris* se diferencia de todos los de su subtribu por la posesión de brácteas interflorales, siendo ésta la única sinapomorfia. La posesión de pelos no ramificados en *Hypochaeris* también es constante en los representantes del género *Scorzoneroides*. Los análisis moleculares (Kilian *et al.* en revisión) han mostrado que el género *Urospermum* es el hermano del resto de los géneros de la subtribu;

Tabla 1.2. Los géneros de la subtribu Hypochaeridinae.

Subtribu Hypochaeridinae

	Nº cromosómico (2*n*)	**Nº de especies**	**Distribución**
Hedypnois Mill.	6, 8, 14 **	3	Región Mediterránea
Helminthotheca Vaill.	10 *	4	Región Mediterránea
Hypochaeris L.	6, 8, 10, 12 *	c. 50	Región Mediterránea, Eurasia y Sudamérica
Leontodon L.	8, 14	c. 30	Región Mediterránea, SW Asia
Picris L.	10	c. 50	Región Mediterránea
Scorzoneroides Vaill.	10, 12 *	10	Región Mediterránea
Urospermum Scop.	10, 14	2	Región Mediterránea

* poliploidía poco frecuente
** poliploidía frecuente

Picris y *Helminthotheca* son hermanos, y también lo son *Hedypnois* y *Leontodon*; estos últimos cuatro géneros son hermanos de *Hypochaeris* y los cinco géneros (*Hypochaeris*, *Picris*, *Helminthotheca*, *Hedypnois* y *Leontodon*) son hermanos de *Scorzoneroides*. De todos los géneros de la subtribu, el mejor estudiado es, sin duda, *Hypochaeris*, en el cual se centrará nuestro estudio.

El género Hypochaeris

El género *Hypochaeris* contiene c. 55 especies, 15 en el Viejo Mundo [13 en la Región Mediterránea, uno en Tenerife (*H. oligocephala*) y otro en los montes Dahuricos y Altaicos de Asia central (*H. grandiflora*)] y c. 40 en Sudamérica, principalmente en la Región Andina y en la Patagónica. Todas las especies son perennes, la mayoría rizomatosas y muy pocas (*H. oligocephala*, *H. rutea* e *H. laevigata*) sufruticosas, excepto *H. achyrophorus*, *H. glabra*, *H. arachnoidea* e *H. salzmanniana*, que son anuales. Todas las especies tienen las flores y las anteras amarillas, pero en Sudamérica existen especies con flores blancas y anteras amarillas, como *H. albiflora*, o flores blancas con anteras purpúreas, como en *H. incana*. Los aquenios de los capítulos son todos con un pico más o menos bien desarrollado, pero en algunas especies mediterráneas, como las de la sección *Hypochaeris* e *H. achyrophorus*, de la sección *Seriola*, los aquenios más externos del capítulo carecen de pico. El vilano está presente en todos los frutos del capítulo excepto en *H. achyrophorus*, en el cual los aquenios más externos y sin pico carecen completamente de vilano, o en *H. cretensis*, cuyos aquenios más externos tienen una pequeña corona de setas; este vilano puede ser doble o simple; el vilano doble tiene los pelos de la fila externa escábridos y los pelos de la fila interna más largos y plumosos; el vilano simple sólo tiene una fila de pelos internos que son siempre plumosos.

En el género *Hypochaeris* se han encontrado los siguientes números básicos: x = 3, 4, 5 y 6 y rara vez se han encontrado poblaciones tetraploides en algunas especies sudamericanas (Weiss-Schneeweiss *et al.* en prensa). Todos estos núme-

ros básicos están presentes en las especies del Viejo Mundo. El número básico x = 3 se ha encontrado en: *H. oligocephala* (Lack 1978) e *H. cretensis* (Barghi *et al.* 1989); x = 4 en *H. radicata* (Parker 1975), *H. salzmanniana* (Talavera 1981), *H. arachnoidea* (Oberprieler & Vogt 2002) e *H. angustifolia* (Oberprieler & Vogt 2002) y en todas las especies sudamericanas (Weiss-Schneeweiss *et al.* 2003; Weiss *et al.* 2003); x = 5 en *H. glabra* (Parker 1975), *H. grandiflora*, *H. maculata* (Parker 1976) e *H. uniflora* (van Loon 1980) y x = 6 en *H. achyrophorus* (Talavera *et al.* 1984), *H. rutea* (Talavera 1987), *H. laevigata* (Oberprieler & Vogt 1993) e *H. leontodontoides* (Vogt & Oberprieler 1993). Todas las especies analizadas del Nuevo Mundo tienen x = 4, con un cariotipo bimodal, con dos pares de cromosomas grandes y los otros dos pequeños. Esta cualidad que presentan todas las especies americanas la tiene también *H. angustifolia*, una especie endémica de la región biogeográfica Atlásica (Marruecos). Por el contrario las demás especies del Viejo Mundo, con independencia del número básico, tienen cariotipos muy simétricos (Ruas *et al.* 1995, en prensa; Cerbah *et al.* 1998a, 1998b, 1999; Weiss-Schneeweiss *et al.* 2003, en prensa; Weiss *et al.* 2003).

Aunque los trabajos de filogenia molecular del género *Hypochaeris* de finales de los años 90 y comienzos del 2000 (Cerbah *et al.* 1998b; Samuel *et al.* 2003) delimitaron con una buena aproximación los distintos grupos taxonómicos dentro del género, con la inclusión en el estudio del equipo de Sevilla, se consiguió mejorar la resolución de los filogramas, gracias a la inclusión de varias especies que resultaron ser claves para el sistema. Se analizaron por primera vez *H. angustifolia*, *H. salzmanniana*, *H. arachnoidea*, *H. leontodontoides*, *H. rutea* e *H. oligocephala*, todos del oeste del Mediterráneo y de la Macaronesia. Esto permitió clarificar la filogenia del género *Hypochaeris* y establecer hipótesis de la evolución del cariotipo (Tremetsberger *et al.* 2005). En este trabajo de Tremetsberger *et al.* (2005) se puso de manifiesto por primera vez (1) que el hermano filogenético de todas las especies americanas es *H. angustifolia*, una especie endémica del Atlas de Marruecos; (2) que *H. salzmanniana* e *H. arachnoidea*,

basándose en secuencias cloroplásticas (intron *rps16*), resultaron ser hermanos de *H. radicata*; (3) que *H. leontodontoides* es el hermano de *H. achyrophorus*, *H. laevigata* e *H. rutea* y también que *H. rutea* está muy próximo a *H. laevigata*; y (4) que *H. oligocephala*, endémico de Tenerife (Islas Canarias) es hermano de *H. cretensis*, una especie del centro y este del Mediterráneo (Fig. 1.4).

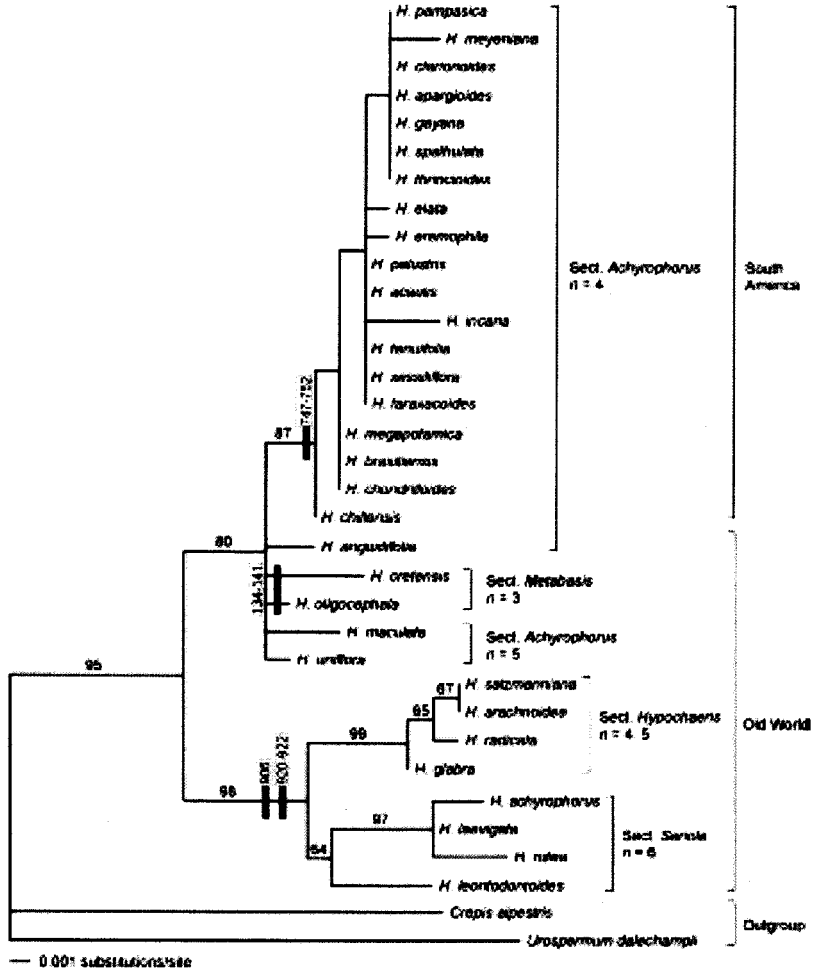

Fig.1.4. Filograma construido por el método de maxima verosimilitud con la secuencia del gen cloroplástico *rps16* intron. Modelo de sustitución nucleotídica: TVM+ I. Valores en la parte superior de las ramas indican el soporte (500 replicas). Las inserciones informativas (barras) estan indicadas con su posición en el alineamiento. Las secciones del género *Hypochaeris* están definidas en la figura según Hoffmann (1893). Modificado de Tremetsberger *et al.* (2005).

En su conjunto, el género *Hypochaeris* queda definido en el árbol como monofilético formado por cinco líneas filéticas que se corresponden con las cinco secciones en las que se puede dividir el género: sect. *Seriola* con *H. leontodontoides* como especie basal de la sección; sect. *Hypochaeris,* con *H. glabra* como especie basal, hermana de la sección *Seriola*; sect. *Achyrophorus*, hermana de la sect. *Metabasis* y estas dos últimas secciones hermanas de *H. angustifolia* y de todas las especies de Sudamérica, considerada por muchos autores pertenecientes también a la sect. *Achyrophorus.* Esto obliga a realizar algunos reajustes de la nomenclatura de las secciones por dos razones (1) la sect. *Achyrophorus* resultaría difilética y (2) que la sect. *Seriola* contiene la especie que es el tipo del género *Achyrophorus*, *H. achyrophorus*. Por ello los nombres correctos de las secciones deberían ser los siguientes:

Secciones del género Hypochaeris

1._ ***Hypochaeris* sect. *Phanoderis*** (DC.) Ortiz et Talavera, **comb. nova**

≡*Achyrophorus* sect. *Phanoderis* DC., *Prodr.* 7: 92 (1838), basión.

Lectotipo: *Achyrophorus andinus* DC., *Prodr.* 7: 92 (1838), tipificado aquí [≡*Hypochaeris arenaria* var. *andina* (DC.) Cabrera, *Fl. Patagonica* 8: 409 (1971)]

=*Hypochaeris* sect. *Oreophila* (D. Don) Benthan et Hooker, *Genera Plantarun* 2(1): 519 (1873). ≡*Oreophila* D. Don in *Trans. Linn. Soc. London* 16: 178 (1830), basión.

Tipo: *Oreophila sessiliflora* (Kunth) D. Don in *Trans. Linn. Soc. London* 16: 178 (1830) [≡*Hypochaeris sessiliflora* Kunth, *Nova Genera et Species Plantarum* 4: 2 (1820)]

–*Hypochaeris* sect. *Achyrophorus* sensu auctores p.p.

A esta sección pertenecen: todas las especies sudamericanas del género *Hypochaeris* y también *H. angustifolia* (Litard. & Maire) Maire, un endemismo del N de Marruecos (región Atlásica).

2._ ***Hypochaeris* sect. *Amblachaenium*** Benthan et Hooker, *Genera Plantarum* 2(1): 519 (1873)

Tipo: *Hypochaeris grandiflora* Ledeb., *Fl. Altaica* 4: 164 (1833)

–*Hypochaeris* sect. *Achyrophorus* sensu auctores p.p.

A esta sección pertenecen las especies Euro-asiáticas del género *Hypochaeris*: *H. maculata* L., *H. uniflora* Vill. e *H. grandiflora* Ledeb.

3._ ***Hypochaeris* sect. *Metabasis*** (DC.) Bentham et Hooker *Genera Plantarum* 2(1): 520 (1873)

≡*Metabasis* DC., *Prodr.* 7: 97 (1838), basión.

Tipo: *Metabasis hymettia* DC., *Prodr.* 7: 97 (1838) [≡*M. cretensis* var. *hymettia* (DC.) DC., *Prodr.* 7: 307 (1838)

=sect. *Serioloides* Benthan et Hooker, *Genera Plantarum* 2(1): 520 (1873)

Tipo: *Hypochaeris pinnatifida* Ten., *Fl. Napol.* 1: 323 (1811-1815) [≡*Hypochaeris cretensis* subsp. *pinnatifida* (Ten.) P. Fourn., *Quatre Fl. France:* 1030. 1940]

=*Fabera* Sch. Bip. in *Nov. Act. Nat. Cur.* 21: 129 (1845). Tipo: Fabera hispida (Willd.) Sch. Bip. in *Nov. Act. Nat. Cur.* 21: 131 (1845). ≡*Hypochaeris hispida* Willd., *Hort. Berol.* tab. 46 (1805), nom. illeg. non Roth in Usteri, *Ann. Bot.* 14: 30 (1795). =*H. cretensis* Bory et Chaub. in Bory, *Expéd. Sci. Morée* 3(2): 237 (1832)

=*Heywoodiella* Svent. et Bramwell in *Acta Phytotax. Barcinon.* 7: 5 (1971)

Tipo: *Heywoodiella oligocephala* Svent. et Bramwell in *Acta Phytotax. Barcinon.* 7: 5 (1971) [≡*Hypochaeris oligocephala* (Svent. et Bramwell) Lack, *Willdenowia* 8: 331 (1978)]

A esta sección pertenecen: *H. cretensis* (L.) Bory et Chaub. del C y E de la región mediterránea y también *H. oligocephala* (Svent. et Bramwell) Lack endémico de Tenerife (Islas Canarias).

4._ ***Hypochaeris* sect. Seriola** (L. ex Murray) Bentham et Hooker, *Genera Plantarun* 2(1): 520 (1873)

≡*Seriola* L., *Sp. Pl.* ed. 2, 2: 1139 (1763) basión.; nom. illeg., sin descrip.

≡L. ex Murray, Syst. Veg.: 601 (1774)

Tipo: *Seriola laevigata* L., *Sp. Pl.* ed. 2, 2: 1139 (1763) [tipificado por Steudel, *Nomencl.* ed. 2, 2:568 (1841)]

=*Achyrophorus* Vaill. in *Königl. Akad. Wiss. Paris Phys. Abh.* 5: 738 (1754)

Tipo: *Hypochaeris achyrophorus* L., *Sp. Pl.:* 810 (1753) [tipificado por Greuter *et al.* in *Taxon* 54:167 (2005)]

=*Agenora* D. Don in *Edinb. N. Phil. Journ.* 6: 310. (1829)

=*Piptopogon* Cass. in Cuvier, *Dict. Sci. Nat.* 48: 434, 507 (1827).

Tipo: *Piptopogon decipiens* Cass. in Cuvier *Dict. Sci. Nat.* 48: 434, 507 (1827). [≡*Seriola laevigata* sensu Desf., *Fl. Atl.* 2: 237 (1800), non L.]

A esta sección pertenecen: *H. leontodontoides* Ball., *H. achyrophorus* L., *H. rutea* Talavera, *H. laevigata* (L.) Ces. *et al.* y también *H. saldensis* Batt., todos de la región mediterránea.

5._ ***Hypochaeris* sect. *Hypochaeris***

Tipo: *H. glabra* L., *Sp. Pl.*: 811 (1753) [tipificado por N.L. Britton et A. Brown, *Ill. Fl. N. U.S.* ed. 2, 3: 309 (1913)]

=*Hypochaeris* sect. *Arachnites* DC., *Prodr.* 7: 90 (1838)

Tipo: *Hypochaeris arachnoidea* Poir., in Lam., *Encycl.* 5: 572 (1804)

≡*Porcellites* Cass. in Cuvier, *Dict. Sci. Nat.* 43: 42-43 (1826), basión.

=*Hypochaeris* sect. *Porcellites* (Cass.) DC., *Prodr.* 7: 91 (1838)

Tipo: *Porcellites radicata* (L.) Cass. in Cuvier, *Dict. Sci. Nat.* 43: 42-43 (1826) [≡*Hypochaeris radicata* L., *Sp. Pl.* 811 (1753)]

A esta sección pertenecen: *H. glabra* L., *H. radicata* L., *H. salzmanniana* DC. e *H. arachnoidea* Poir., todos de la región mediterránea; *H. glabra* y sobre todo *H. radicata* son plantas invasoras en todos los continentes, menos en la Antártida.

Clave de las secciones del género Hypochaeris

1. Vilano con dos filas de pelos, la externa casi la mitad del tamaño de la interna; aquenios con 10 costillas longitudinales; plantas anuales o perennes; número básico de cromosomas x = 4, 5; cariotipo simétrico, con todos los cromosomas de tamaño semejante; especies mediterráneas, dos de ellas cosmopolitas o subcosmopolitas..... **sect. *Hypochaeris***

– Vilano con una sola fila de pelos, o con dos filas de pelos y la externa muy pequeña, casi imperceptible; aquenios con 5 costillas longitudinales; plantas perennes, rara vez anuales; número básico de cromosomas x = 3, 4, 5, 6; cariotipo simétrico, con todos los cromosomas de tamaños semejantes, o bimodal y con la mitad de los cromosomas mucho más pequeños que los otros; especies mediterráneas, euro-asiáticas, macaronésicas o sudamericanas **2**

2. Capítulo con 2(3) filas de brácteas involucrales, la externa mucho más pequeña que la interna; vilano (al menos los internos del capítulo) con dos filas de pelos,

la externa generalmente muy pequeña, casi imperceptible, caduca; fila interna del vilano con 10 pelos escamiformes y plumosos en la mitad superior; plantas perennes y sufruticosas, o anuales; número básico de cromosomas x = 3, 6; cariotipo simétrico, con todos los cromosomas de tamaños semejantes; especies mediterráneas y macaronésicas.....**3**

– Capítulo con numerosas filas de brácteas involucrales; vilano con una sola fila de pelos; fila del vilano con c. 20 pelos ± cilíndricos y plumosos en la mitad superior; plantas perennes rizomatosas; número básico de cromosomas x = 4, 5; cariotipo simétrico (x = 5), con todos los cromosomas de tamaños semejantes, o bimodal (x = 4) con la mitad de los cromosomas mucho más pequeños que los otros; especies mediterráneas, euroasiáticas y sudamericanas.....**4**

3. Plantas anuales o perennes y sufruticosas; número básico de cromosomas x = 6; especies mediterráneas.....**sect. *Seriola***

– Plantas perennes, sufruticosas; número básico de cromosomas x = 3; especies del centro y este del Mediterráneo (*H. cretensis*) y de Tenerife (*H. oligocephala*).....**sect. *Metabasis***

4. Número básico de cromosomas x = 5; cariotipo simétrico, con todos los cromosomas de tamaños semejantes; especies del centro y sur de Europa y de Asia.....**sect. *Amblachaenium***

– Número básico de cromosomas x = 4; cariotipo asimétrico, bimodal, con la mitad de los cromosomas de mucho menor tamaño que los otros; especies del W del Mediterráneo (Atlas, Marruecos) y de Sudamérica ..... **sect. *Phanoderis***

***La sección* Hypochaeris**

La sección *Hypochaeris* es hermana de la sección *Seriola* y ambas se separaron hace 7–8,4 millones de años (Tremetsberger *et al.* en revisión), pero las dos secciones se diversificaron en el Plioceno (sect. *Seriola*) o Pleistoceno (sect. *Hypochaeris*). Estas dos secciones son estrictamente mediterráneas, y las especies

más primitivas de las secciones son *H. glabra*, en la sección *Hypochaeris*, e *H. leontodontoides*, en la sección *Seriola*, que viven actualmente en Marruecos, la primera también con una amplia distribución en el Mediterráneo y en otras partes del mundo y la segunda endémica de la región biogeográfica Atlásica (Marruecos).

Hypochaeris* sect. *Hypochaeris

Clave de las especies

1. Hierbas perennes, cortamente rizomatosas, rara vez estoloníferas; tallos de 30–100 cm, ramificados o no, casi afilos; lígulas mucho más largas que el involucro; número somático de cromosomas $2n = 8$ ..... **2. *H. radicata***

– Hierbas anuales; tallos hasta de 40 cm, poco ramificados, frecuentemente con hojas; lígulas un poco más largas o mucho más largas que el involucro; número somático de cromosomas $2n = 8, 10$ **2**

2. Lígulas sobrepasando muy poco el involucro; número somático de cromosomas $2n = 10$ ..... **1. *H. glabra***

– Lígulas sobrepasando mucho el involucro; número somático de cromosomas $2n = 8$ **3**

3. Pedúnculo generalmente ensanchado cerca del capítulo; tallos frecuentemente foliosos en la mitad superior; aquenios sin pico c. 5 mm .... **3. *H. salzmanniana***

– Pedúnculo cilíndrico en toda su longitud; tallos frecuentemente afilos; aquenios sin pico c. 3 mm ..... **4. *H. arachnoidea***

Sinopsis taxonómica

1. ***H. glabra*** L., *Sp. Pl.* 811 (1753)

Tipo: LINN 959.4 "5 glabra" [ms. Linneo] Reverso: "Hieracium minus dentis leonis folio oblongo glabro CB. Seq. Burserum sed vix est." [ms. Linneo]. El doble signo "5 glabra" escrito por el propio Linneo es indicativo

de que dicho pliego fue utilizado en la edición del *Species Plantarum*, por ello no hay la menor duda de que este material es el tipo de la especie. También la indicación del reverso hace referencia al nombre frase usado por Bauhin y que el propio Linneo menciona en su *Species Plantarum*. El material tipo consiste en un único individuo de unos 20 cm, con hojas lobuladas y con dos capítulos en flor y uno en fruto.

=*H. dimorpha* Brot., *Fl. Lusit.* 1: 332 (1804)
=*H. minima* Cirillo, *Pl. Rar. Neap.* 1, 29: 10 (1788)
≡*H. glabra* subs.. *minima* (Cirillo) Arcang., *Comp. Fl. Ital.:* 414 (1882)
=*H. simplex* Merát *Nouv. Fl. Env. Paris*: 310 (1812)
-*H. hispida* sensu Brot., *Fl. Lusit.* 1: 332 (1804), non Roth

Habita en pastizales, en suelos arenosos. Frecuente en gran parte de Europa y W de Asia, raro en el N de África, Islas Canarias y América.
Observaciones: *H. glabra* se hibrida espontáneamente en el N de Francia y S de Inglaterra, y el híbrido de primera generación, con $2n = 9$, es semejante a *H. radicata*, con el que convive, pero se diferencian porque son estériles o producen muy pocos frutos (Parker 1975).

2. ***H. radicata*** L. *Sp. Pl.* 811 (1753)
≡*Porcellites radicata* (L.) Cass. in Cuvier, *Dict. Sci. Nat.* 43: 43 (1826)
=*H. atlantica* Sennen et Mauricio in Sennen, *Diagn. Nouv.* 294 (1936)
=*H. platylepis* Boiss., Voy. Bot. Espagne 2: 376 (1839)
≡*H. radicata* subsp. *platylepis* (Boiss.) Maire in Jahand. et Maire, *Cat. Pl. Maroc* 3: 831 (1934)
=*H. radicata* var. *heterocarpa* Moris, *Fl. Sardoa* 2: 487 (1840-1843)
=*H. neapolitana* DC., *Prodr.* 7: 91 (1838)

≡*H. radicata* subsp. *neapolitana* (Dc.) Nyman, *Consp. Fl. Eur.* 470 (1879)

=*Seriola caespitosa* Porta in *Nuovo Giorn. Bot. Ital.* 19: 310 (1887)

=*Hypochaeris radicata* subsp. *ericetorum* Soest in *Ned. Kruidk. Arch.* 57: 242 (1950)

Mediterráneo, introducido en el C y N de Europa, Asia, América y Australia. Hemicriptófito. Sotobosque de alcornocales y márgenes de humedales. 0–2.050 m. Fl. III-VI (VII).

3. ***H. salzmanniana*** DC. *Prodr.* 7: 91 (1838)

≡*H. glabra* subsp. *salzmanniana* (DC.) Maire in Jahandiez et Maire, *Cat. Pl. Maroc.* 3: 831 (1934)

≡*H. glabra* var. *salzmanniana* (DC.) Amo, *Fl. Fanerog. Ibér.* 4: 497 (1872)

≡*H. glabra* var. *dimorpha* Salzm. ex Maire, in Jahandiez et Maire, *Cat. Pl. Maroc.* 3: 831 (1934), nom illeg.

≡*H. dimorpha* Salzm., *Pl. Exsicc.*, nom. nudum, non. *H. dimorpha* Brot., *Fl. Lusit.* 1: 332 (1804).

Atlántico-Iberomarroquí (S de Cádiz y NW de Marruecos). Terófito. Pastizales de dunas y arenas litorales. 0–100 m. Fl. (II) III-V.

4. ***H. arachnoidea*** Poir. in Lam., *Encycl.* 5: 572 (1804)

≡*H. glabra* subsp. *arachnoidea* (Poir.) Nyman, *Consp. Fl. Eur.* 471 (1879)

≡*H. minima* Desf., *Fl. Atlant.* 2: 238 (1799), non Cirillo (1788)

=*H. grandiflora* Sennen et Mauricio in Sennen, *Diagn. Nouv.* 236 (1936)

=*H. multicaulis* Sennen et Mauricio in Sennen, *Diagn. Nouv.* 236 (1936)

≡*H. glabra* f. *multicaulis* (Sennen et Mauricio) Maire, in Emb. et Maire, *Cat. Pl. Maroc.* 4: 1164 (1941)

=*H. radicata* var. *setulosa* Maire et Sennen, in *Bull. Soc. Hist. Nat. Afrique* N. 27 :241 (1936)

≡*H. radicata* f. *setulosa* (Maire et Sennen) Maire, in Emb. et Maire *Cat. Pl. Maroc.* 4: 1164 (1941)

=*H. salzmanniana* subsp. *marocanna* Förter et Podlech, in *Sendtnera* 8: 41 (2002)

C y N de Marruecos (desde el Anti Atlas hasta el Atlas Medio y Rif oriental)) y Argelia [Wilaya Tizi Ouzou (Mazizo de Djurdjura) y Wilaya Saïda (Atlas Sahariano)]. Terófito. Pastizales secos. 100–1.800 m. Fl. IV-VI.

Objetivos de la tesis

Los objetivos de esta tesis se centran en probar, mediante análisis moleculares, si el N de África y con ello también el occidente de la región mediterránea es un área primigenia de diversificación de la tribu Cichorieae, como se había postulado por la biogeografía de la tribu, tomando como modelo un grupo monofilético completo del género *Hypochaeris*, la sección *Hypochaeris*, y usando para ello los marcadores moleculares conocidos como AFLP ("Amplified Fragment Lenght Polymorphism").

La diversidad y singularidad genética de las poblaciones depende de varios factores, todos ellos ligados a la Historia Natural de las mismas ("live history"), tales como (1) cuellos de botella poblacionales, bien por fragmentación (vicarianza), bien por procesos migratorios (efecto fundador), y (2) estocasticidad medioambiental, que además de incidir en los procesos anteriores, modula y optimiza los sistemas reproductores de las plantas.

Por ello nos planteamos de forma paralela al estudio de genética molecular de las poblaciones, otro sobre los sistemas reproductores de todas las especies y sobre las mismas poblaciones. Esto nos permitiría obtener una información muy valiosa para explicar los procesos evolutivos.

Objetivos concretos

1. Filogeografía y características genéticas de las poblaciones en las cuatro especies que comprenden la sect. *Hypochaeris*: *H. glabra*, *H. radicata*, *H. salzmanniana* e *H. arachnoidea.*

2. Biología de la reproducción de las poblaciones en las cuatro especies de la sect. *Hypochaeris*: *H. glabra*, *H. radicata*, *H. salzmanniana* e *H. arachnoidea.*

Estructura de la tesis

La tesis se ha estructurado en forma de capítulos científicos, la mayoría publicados durante el desarrollo de esta tesis y los demás enviados a revistas especializadas, las cuales se indican en cada capítulo. Como los artículos se han redactado en lengua inglesa para su publicación se ha preferido presentarlos en esa lengua, sin modificación alguna, sólo las necesarias para la homogenización de todos los artículos y la inclusión de algunas fotografías que ilustren y embellezcan la memoria doctoral.

Capítulo 2: Relaciones de *Hypochaeris salzmanniana* (Asteraceae, Cichorieae), una especie en peligro en la Península Ibérica, con *H. radicata* e *H. glabra*, e implicaciones biogeográficas.
("Relationship of *Hypochaeris salzmanniana* (Asteraceae, Lactuceae), an endangered species of the Iberian Peninsula, with *H. radicata* and *H. glabra* and biogeographic implications").
Capítulo 3: Autoincompatibilidad y parámetros florales de *Hypochaeris* sect. *Hypochaeris* (Asteraceae).
("Self incompatibility and floral parameters in *Hypochaeris* sect. *Hypochaeris* (Asteraceae)".
Capítulo 4: Estructura poblacional de *Hypochaeris salzmanniana* DC. (Asteraceae), una especie endémica de la costa atlántica en ambos lados del Estrecho de Gibraltar, en relación con los cambios en el nivel del mar del Cuaternario.
("Population structure of *Hypochaeris salzmanniana* DC. (Asteraceae), an endemic species to the Atlantic coast on both sides of the Strait of Gibraltar, in relation to Quaternary sea level changes")
Capítulo 5: Filogeografía de la planta invasora *Hypochaeris radicata* (Asteraceae): desde su origen marroquí a las introducciones por todo el mundo.

("Phylogeography of the invasive weed *Hypochaeris radicata* (Asteraceae): from Moroccan origin to world-wide introduced populations")

Capítulo 6: Patrones biogeográficos en *Hypochaeris* sect. *Hypochaeris* (Asteraceae, Lactuceae) del oeste del Mediterráneo.

("Biogeographic patterns in *Hypochaeris* sect. *Hypochaeris* (Asteraceae, Lactuceae) of the western Mediterranean")

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2 Relationship of *Hypochaeris salzmanniana* (Asteraceae, Lactuceae), an endangered species of the Iberian Peninsula, with *H. radicata* and *H. glabra* and biogeographic implications

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Abstract

Hypochaeris salzmanniana DC. (Asteraceae, Lactuceae) is an endangered species on the Iberian Peninsula, being known from only eight coastal populations. Most authors have treated it as a variety, subspecies, or simply as a synonym of *H. glabra* L. On the basis of morphological and cytological characters, Talavera recently separated *H. salzmanniana* ($2n = 8$) from *H. glabra* ($2n = 10$). To assess taxonomic status and populational relationships, material of *H. salzmanniana*, *H. glabra*, and *H. radicata* has been collected from Spain, Italy, Sicily and Tunisia and analyzed by Amplified Fragment Length Polymorphism (AFLP). The use of six selective primer combinations allowed recognition of three well differentiated species (supported by 100 % BS). A close relationship of *H. salzmanniana* and *H. radicata* is also confirmed by AFLP analysis and chromosome number ($2n = 8$), morphology, and rDNA localization (FISH; fluorescence *in situ* hybridization). *H. salzmanniana* and *H. radicata* share three fixed diagnostic AFLP fragments out of 348 fragments scored. *H. salzmanniana* has nine fixed diagnostic fragments and *H. radicata* one. The populational structure of *H. salzmanniana* reveals distinct groups in southern Spain that are separated geographically. High differentiation among a western (Conil to Zahara), an intermediate (Punta Paloma and Los Algarbes), and an eastern (Algeciras and La Línea) group may reflect ancient separation. Population sizes and genetic compatibility differ greatly among populations and can be used to explain levels of within-populational genetic diversity together with recent documented loss of habitats through tourist developments. Populational structures of *H. radicata* and *H. glabra* show a similar geographic patterning: a strong differentiation between populations from the Betic Cordillera and those from the Iberian Massif, which are separated at present by the Guadalquivir river. Geological events at the end of the Tertiary (Tortonian-Messinian Miocene) might help explain patterns of differentiation in these three species of sect. *Hypochaeris*.

Keywords: AFLP, biogeography, conservation, *Hypochaeris*, Spain

Introduction

Hypochaeris salzmanniana DC. was described as a distinct species by A. P. de Candolle (1838). Subsequent authors working on the flora of the western Mediterranean have included *H. salzmanniana* with *H. glabra* L. as a variety (Amo, 1872; Willkomm, 1893), subspecies (Jahandiez & Maire, 1934; Emberger & Maire 1941), or simply as a mere synonym of *H. glabra* (De Fillips, 1976). Only recently did Talavera (1980, 1987) resuscitate *H. salzmanniana* out of *H. glabra* as a good taxon based on morphological and karyological characters, but for the first time as a species related not to *H. glabra* but instead to *H. radicata* L. Förther and Podlech (2003) recently characterized populations from SW Spain and NW Morocco (coastal areas) as subsp. *salzmanniana*, being differentiated by conspicuously inflated peduncles from subsp. *maroccana* Förther and Podlech in mountains of Morocco and Algeria to 1700 m [= *H. arachnoidea* sensu Oberprieler (2002) and Oberprieler and Vogt (2002)]. Morphological, cytological, compatibility and ecological differences exist among *Hypochaeris salzmanniana*, *H. radicata* and *H. glabra*. *H. salzmanniana* has hirsute, rarely glabrous, peduncles, strongly inflated under the capitula during anthesis, ovate outer involucral bracts with wide scarious margins and setose hairs at the end of the central nerve, and with ligules conspicuously exerted from the involucre (Talavera, 1987). *H. glabra* has glabrous, cylindrical peduncles, lanceolate and glabrous involucral bracts, rarely with a few setose hairs, and with ligules barely exserted from the involucre (Talavera, 1987). Field studies in 2002 by Talavera and Stuessy *et al.* have provided new morphological data to suggest recognition of *H. salzmanniana* as a good species. In comparison to its presumptive closest relative, *H. radicata*, the leaves of the former are more undulating at the margin, the leaf rosettes are usually very full, the involucral bracts often have long dark hairs, and the branches are ascending (Fig. 2.1A). *H. glabra* and *H. salzmanniana* are annuals, whereas *H. radicata* is perennial. *H. glabra* has $2n = 10$ chromosomes (Adame & Talavera, 1980) and *H. salzmanniana* $2n = 8$ (Talavera, 1981) as does *H. radicata* (Adame & Talavera,

1980). *H. radicata* is normally self-incompatible, *H. glabra* self-compatible, and *H. salzmanniana* shows variation in its compatibility system with plants being self-compatible or self-incompatible (Ortiz *et al.*, 2006).

Hypochaeris glabra and *H. radicata* are widespread in the Mediterranean region and are worldwide weeds. *H. glabra* seldom occurs in the same localities as *H. salzmanniana* (e.g., in Barbate and Punta Paloma, see Table 2.1), and *H. salzmanniana* does not occur with *H. radicata*. *H. salzmanniana* is restricted to coastal areas on both sides of the Straits of Gibraltar. The species is reported from Morocco between Tanger and Rabat (Oberprieler, 2002; Förther & Podlech, 2003). In Spain, only eight populations are known along the coast of Cádiz, from Conil to La Línea (Fig. 2.2; Table 2.1). Six of the eight populations survive among beach developments. These are the western populations (Conil to Zahara, pops. 1–4) and the population of Algeciras (pop. 7), which all live in sand dunes near the coast. The threatened population of La Línea (pop. 8) lives at the base of the Rock of Gibraltar. The only populations not disturbed by the tourism industry (Los Algarbes and Punta Paloma, pops. 5 and 6) reside in the ecotone of the Quaternary dunes with flysch materials belonging to the rocky spur of the Sierra de San Bartolomé (1 km inland).

The reported pattern and size of populations of *H. salzmanniana* suggests questions regarding their origins and biogeographical relationships. Because the populations occur in a linear fashion, it would be of interest to test if genetic relationships among these eight populations conform to this linear model. That is, did the populations originate in one location and then spread serially along the coast, or is a more complex explanation required? An attractive hypothesis is that those populations growing on fossil dunes may represent relictual populations, perhaps now genetically isolated from the others.

To assess relationships among *H. salzmanniana* and its relatives, *H. glabra* and *H. radicata*, and to determine infraspecific genetic variation within the three species, the DNA fingerprinting technique Amplified Fragment Length Polymorphism

(AFLP) has been selected for use (Vos *et al.*, 1995). AFLPs have already been shown efficacious at the populational as well as interspecific level for revealing

Table 2.1. Localities, collectors and number of individuals of *Hypochaeris salzmanniana*, *H. radicata* and *H. glabra* sampled for the AFLP study.

Taxa and localities	Collectors and number	No.
H. salzmanniana DC.		
1. Spain, Cádiz, Conil: Playa El Palmar	**ST**, **TS** et al. 5-1 to 5-4	*38*
2. Spain, Cádiz, Los Caños de Meca	*ST, TS et al. 24*	5
3. Spain, Cádiz, Barbate	*ST, TS et al. 14*	5
4. Spain, Cádiz, Zahara de los Atunes	*ST & MAO 1/03*	5
5. Spain, Cádiz, Punta Paloma: Los Algarbes	*ST & MAO 2/03*	5
6. Spain, Cádiz, Punta Paloma	*ST, TS et al. 32; 3/03*	19
7. Spain, Cádiz, Algeciras: Palmones	*ST, TS et al. 33*	5
8. Spain, Cádiz, La Línea de la Concepción	*ST, TS et al. 35*	5
***H. radicata* L.**		
9. Brazil, São Paulo, São Paulo	*ST et al. BRA 30*	1
10. Colombia, Bogotá, Bogotá	*TS 31*	2
11. Germay, Nordrhein-Westfalen, Bochum	*TS s.n.*	1
12. Italy, Fòggia, Gargano	*KT s.n.*	10
13. Italy, Règgio di Calàbria, Aspromonte	*KT s.n.*	6
14. Italy, Règgio di Calàbria, Barriteri	*KT s.n.*	2
15. Italy, Règgio di Calàbria, Palmi	*KT s.n.*	7
16. Italy, Sicily, Nebrodi	*F. & L. Ehrendorfer 5FE*	4
17. Italy, Sicily, Palermo	*Castroviejo 646283*	10
18. Mexico, México, Valle de Toluca	*Guevara s.n.*	1
19. Spain, Burgos, Quintanar de la Sierra	*MAO s.n.*	1
20. Spain, Cádiz, near Vejer de la Frontera	*ST, TS et al. 25; ST et al. s.n.*	6
21. Spain, Huelva, Aracena	*ST et al. s.n.*	5
22. Spain, Huelva, National Park Doñana	*ST et al. s.n.*	8
23. Spain, Huelva, Santa Ana la Real	*ST, TS et al. 48*	5
24. Spain, Huelva, Valverde del Camino	*ST, TS et al. 46*	5
25. Spain, Málaga, Gaucín	*ST, TS et al. 40*	3
26. South Africa, Western Cape, near Ceres	*F. & L. Ehrendorfer 83*	1
27. Tunisia, Aïn Draham	*F. & L. Ehrendorfer s.n.*	9

Taxa and localities	Collectors and number	No.
H. glabra **L.**		
28. Chile, Región VIII, Ñuble	*Baeza 3924*	3
29. Spain, Cádiz, Barbate de Franco	*ST, TS et al. 15*	2
30. Spain, Cádiz, Punta Paloma	*ST, TS et al. 31*	2
31. Spain, Canary Islands, Gran Canaria	*TS s.n.*	2
32. Spain, Huelva, Hinojos	*ST, TS et al. 43*	2
33. Spain, Huelva, National Park Doñana	*ST et al. s.n.*	2
34. Spain, Huelva, Valverde del Camino	*ST, TS et al. 45*	2

genetic relationships in *Hypochaeris* and other plant groups (e.g., Pfosser *et al.*, 2002; Schönswetter *et al.*, 2003; Stuessy *et al.*, 2003; Tremetsberger *et al.*, 2003). Karyotypic analysis of *H. salzmanniana*, *H. radicata* and *H. glabra* is also provided as a comparison with the AFLP data. The purposes of this paper, therefore, are to: (1) test the specific status of *H. salzmanniana*; (2) assess its relationships to *H. radicata* and *H. glabra*; (3) determine infraspecific genetic structures within the three species; and (4) interpret the observed patterns in the context of biogeographic history and conservation perspectives.

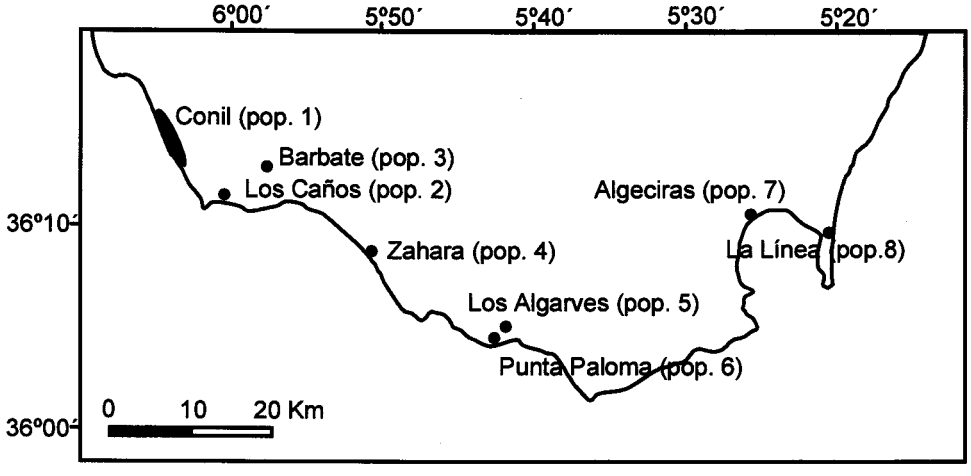

Fig. 2.2. Distribution of *Hypochaeris salzmanniana* in southern Spain, showing all known populations (all sampled; map by ArcView GIS 3.2, © Environmental Systems Research Institute, Inc.).

Materials and Methods

Sampling

Leaves were sampled in silica gel from individuals in all known populations of *Hypochaeris salzmanniana* from the Iberian Peninsula (Fig. 2.2, Table 2.1). Five individuals were analyzed in Los Caños, Barbate, Zahara, Los Algarbes, Algeciras, and La Línea (pops. 2, 3, 4, 5, 7, and 8). Nineteen individuals were analyzed in Punta Paloma (pop. *6*). This population exhibited very low genetic variation after analysis of 10 individuals sampled in 2002 (*Talavera & Stuessy et al. 32*; ind. *a* to *j*). Nine additional individuals were analyzed after the population had been resampled in 2003 (*Talavera and Ortiz 3/03*; ind. k to s). In the large population Playa El Palmar (pop. 1), 38 individuals were analyzed from four linear transects from sea to road across the dune, each approximately 1 km apart, so that genetic variation could be related to distance from the ocean. Along these transects each second m^2 was numbered sequentially from sea to road and one to two individuals, if present, were sampled in quadrats next to the ocean (low numbers: 1-3 in transects 1, 3, and 4; 4-9 in transect 2) and in quadrats far from the ocean (high numbers: 11-14 in transects 1, 2, and 4; 17-19 in transect 3). A total of 87 plants were investigated in *H. salzmanniana.*

Populations of *H. radicata* and *H. glabra* were similarly sampled (Table 2.1), with emphasis on populations of the former taxon from throughout its native range in the Mediterranean region (Spain, Italy, Sicily and Tunisia). Furthermore, a few samples were collected from introduced accessions throughout the world. Fewer populations of *H. glabra* were compared because morphological and karyological data indicated a more distant relationship with *H. salzmanniana*, despite the earlier taxonomic opinions of some authors that they were close relatives (see introduction). These samples are from Spain, where *H. glabra* is native, the Canary Islands, and Chile, where it is introduced and invasive. A total of 87 plants were investtigated in *H. radicata* and 15 plants in *H. glabra.* Vouchers of all populations sampled are on deposit at SEV and WU.

Karyotypes

The karyotypes of *H. salzmanniana* [Spain, Cádiz, Playa El Palmar (pop. *1*); see Table 1], *H. radicata* (Chile, Région VIII, Lota, *Stuessy et al. 15477*; WU), and *H. glabra* [Spain, Huelva, Valverde del Camino (pop. 34); see Table 2.1] have been determined. Surface-sterilized seeds were germinated on wet filter paper on Petri dishes. Two days after germination, seedlings were pre-treated with 0.1% colchicine for 2 h at room temperature and 2 h at 4° C, fixed in 3 ethanol: 1 acetic acid for 12 h at room temperature, and stored at -20°C until use. Feulgen staining with Schiff's reagent was done according to standard protocol (Weiss *et al.*, 2003). From each species, a preparation with at least 15 well-spread chromosome plates was chosen for analysis.

Chromosomes for fluorescence *in situ* hybridization (FISH) were prepared by enzymatic digestion/squashing as described by Weiss-Schneeweiss *et al.* (2003). The quality of spreads was checked by phase-contrast and only preparations with adequate numbers of well-spread metaphases (10-15) were selected for FISH. Slides were frozen at -80° C, and after cover slip removal, preparations were stored at -20° C. FISH was carried out according to the method of Weiss-Schneeweiss *et al.* (2003). Probes used for FISH were: 18S-25S rDNA from *Arabidopsis thaliana* in plasmid pSK+, labeled with digoxygenin (Roche; green), and 5S rDNA from *Beta vulgaris* in plasmid pBx1-2, labeled with biotin (Roche; red). Both probes were labeled by nick translation according to the manufacturer's instruction (Roche). Hybridization was carried out overnight. Stringent washes were performed as in Weiss-Schneeweiss *et al.* (2003). For digoxygenin/biotin detection anti-dig-FITC solution (Roche)/extravidin Cy3 (Sigma) has been applied to the slides at 37° C for 1 h. The preparations were mounted in antifade buffer Vectashield (Vector Laboratories, UK) containing DAPI counterstain (2 μg/ml) and stored at 4° C. Analyses of preparations were made with a ZEISS Axioscope epifluorescence microscope. Images were acquired with a CCD camera (Zeiss), and files were processed using Adobe Photoshop software with only those functions

that applied equally to all pixels in the image. For rDNA localization, a minimum of 30 well-spread metaphases and prometaphases was analyzed for each species.

AFLPs

Total DNA was extracted from dry leaf material according to a modified CTAB-protocol and quality-checked on 1 % TAE-agarose gels as in Tremetsberger *et al.* (2003). The AFLP procedure followed established protocols (Vos *et al.*, 1995; PE Applied Biosystems, 1996) with modifications. Restriction-ligation reactions were carried out as in Tremetsberger *et al.* (2003). The reaction mix contained 1.1 µl 10x T4 DNA ligase buffer (Promega), 1.1 µl 0.5 M NaCl, 0.55 µl 1 mg/ml BSA (New England Biolabs), 1 µl 50 µM *Mse*I-adaptor, 1 µl 5 µM *Eco*RI-adaptor, 1 U *Mse*I (New England Biolabs), 5 U *Eco*RI (Promega), 1 U T4 DNA ligase (Promega), and c. 0.5 µg template DNA, added up with water to a final volume of 11 µl. Ligated DNA fragments were diluted and preselective and selective amplifications performed as in Tremetsberger *et al.* (2003). The reaction mix for the preselective amplification contained 1.14 µl 10x RedTaq polymerase buffer (Sigma), 0.2 U RedTaq polymerase (Sigma), 0.22 µl 10 mM dNTPs (Fermentas), 0.58 µl preselective primers (4.8 pmol/µl each *Mse*I and *Eco*RI preselective primers), and 2 µl diluted product of restriction/ligation, added up with water to a final volume of 10 µl. As selective primers with three and four selective nucleotides were chosen (see below), separate PCRs with one and two selective nucleotides in the *Mse*I primer (*Mse*I-C and -CT; *Eco*RI-A) were performed. The preselective PCR products were checked on a 1.5 % TBE-agarose gel and diluted 10-fold with $TE_{0.1}$ buffer. An initial screening of selective primers using 39 primer combinations was performed on five individuals from three populations of *H. radicata* and *H. salzmanniana*. A second screening used 15 primer combinations with clear bands on 13 individuals from eight populations of *H. radicata* and *H. salzmanniana*. From this, we have chosen six selective primer combinations with clear bands evenly distributed over the AFLP profiles for an application to all individuals investigated. These are: *MseI*-CTCG/*EcoRI*-ATC(Fam), *MseI*-CAC/-

EcoRI-ACG(Hex), *MseI*-CTA/*EcoRI*-ACC(Ned), *MseI*-CTG/*EcoRI*-ACA(Fam), *MseI*-CTC/*EcoRI*-AGG(Hex), and *MseI*-CTGA/*EcoRI*-AAC(Ned). The reaction mix for the selective amplification contained 1 µl 10x RedTaq polymerase buffer, 0.2 U RedTaq polymerase, 0.22 10 mM dNTPs, 0.54 µl of each selective primer (*Mse*I-primer: 5 pmol/µl; *Eco*RI-primer: 1 pmol/µl), and 2 µl diluted product of the preselective amplification, added up with water to a final volume of 10 µl. The fluorescence-labeled selective amplification products were run on a 5 % denaturing polyacrylamide gel on an automated sequencer (ABI 377, Perkin Elmer). Before running, 0.8 µl NED- and HEX-labeled, and 0.4 µl FAM-labeled selective amplification products were mixed with 1.2 µl loading dye (containing 64.8 µl deionized formamide, 25.2 µl loading buffer, and 10 µl GeneScan®-500 [ROX] size standard), and denatured at 95° C for 2 min. Raw data were scored and exported as a presence/absence matrix as in Tremetsberger *et al.* (2003) using ABI Prism GeneScan® Analysis Software 2.1 (PE Applied Biosystems) and Genographer (version 1.1.0, © Montana State University 1998; http://hordeum.msu.montana.edu/genographer/).

Data analysis

To test correspondence of data gathered by each of six primer combinations, Jaccard (1908) similarity matrices on all 189 individuals analyzed were constructed for each primer combination separately using *R* package (v. 4.0; Casgrain & Legendre, 2000). The similarity matrices were used to compute pairwise correlations among them by a Mantel test (standardized Mantel statistic *r*; R package version 4.0; Casgrain & Legendre, 2000). One-tailed test statistic probabilities were obtained through 999 permutations (*R* package v. 4.0; Casgrain & Legendre, 2000).

The Jaccard (1908) distance matrix of all primer combinations combined (R package; Casgrain & Legendre, 2000) was subsequently imported into PAUP* (v. 4.0b8; Swofford, 1998) and used to construct a UPGMA dendrogram for all individuals analyzed, and for individuals of each species separately. A cophenetic

correlation coefficient, which measures the goodness-of-fit of a model to the data, was calculated with NTSYSpc (version 2.02h, © Applied Biostatistics Inc.). Support for each node was tested by 1000 bootstrap replicates with PAUP* (version 4.0b8; Swofford, 1998) using the UPGMA method in conjunction with Nei and Li's (1979) genetic distances on the original presence/absence matrix, since PAUP* does not feature the Jaccard index. The Nei-Li (1979) distance and neighbour-joining algorithm were also applied to the data and resulted in very similar dendrograms (not shown).

For each species, the total number of fragments, the number of fixed fragments (i.e., those occurring in all individuals analyzed of a species), the diagnostic fragments (i.e., those occurring in only one species), and the fixed diagnostic fragments (i.e., those occurring in all individuals analyzed of only one species) were assessed.

A Mantel test was used to correlate geographic with genetic distances in *H. salzmanniana.* A Jaccard distance matrix among all pairwise combinations of individuals was computed with the R package (version 4.0; Casgrain & Legendre, 2000) and compared to geographic distances among individuals (in kilometers; distances within populations set to 0). The standardized Mantel statistic r is the linear correlation between genetic and geographic distances and was tested for significance with 999 permutations of rows and columns in one (the genetic or geographic) distance matrix to obtain the distribution under the null hypothesis of no correlation.

Percentage of different AFLP phenotypes and percentage of polymorphic fragments were assessed as measures of within-populational genetic diversity for each population of *H. salzmanniana* (Arlequin version 2.0; Schneider *et al.*, 2000). The Shannon diversity index was calculated as $H_{Sh} = -\sum[p_i * \ln(p_i)]$, where p_i is the relative frequency of the i^{th} fragment in a population, and correlated with population size (estimated number of individuals) and self-incompatibility (percentage of self-incompatible plants; Ortiz *et al.*, 2006) with a Pearson correlation (1-tailed sig.) with SPSS (version 10, © SPSS Inc.).

The effects of geographic vicinity within a transect and proximity or distance to the ocean have been tested as factors influencing the partitioning of genetic variation in the large population Playa El Palmar (pop. *1*). Arlequin (version 2.0; Schneider *et al.*, 2000) was used to carry out an Analysis of Molecular Variance (AMOVA; Excoffier *et al.*, 1992) with 1023 permutations among three hierarchical levels: (1) among transects, (2) among quadrats next to the ocean and far from the ocean in each transect, and (3) among individuals within each of the two groups of nearby quadrats (next to the ocean and far from the ocean) in each transect. Furthermore, the differentiation between the four main groups of *H. salzmanniana*, determined by UPGMA analysis, was quantified through pairwise F_{ST} values and tested for significance by 1023 permutations of haplotypes (distance method: pairwise difference; Arlequin version 2.0; Schneider *et al.*, 2000).

Results

A total of 348 unambiguously scorable AFLP fragments was obtained from analysis of six primer combinations on 189 individuals of *Hypochaeris glabra*, *H. radicata* and *H. salzmanniana*. The primer combinations *MseI*-CAC/*EcoRI*-ACG(Hex) yielded 71 fragments, *MseI*-CTCG/*EcoRI*-ATC(Fam) 68, *MseI*-CTA/-*EcoRI*-ACC(Ned) 62, *MseI*-CTG/*EcoRI*-ACA(Fam) 57, *MseI*-CTC/*EcoRI*-AGG-(Hex) 47, and *MseI*-CTGA/*EcoRI*-AAC(Ned) 43. A correlation test performed on each pairwise combination of six Jaccard similarity matrices obtained from analysis of each primer combination separately yielded generally high values (Table 2.2). The lowest value for Mantel's r is 0.859 among *MseI*-CTG/*EcoRI*-ACA(Fam) and *MseI*-CTGA/*EcoRI*-AAC(Ned), and the highest is 0.931 among *MseI*-CAC/*EcoRI*-ACG(Hex) and *MseI*-CTA/*EcoRI*-ACC(Ned). All values are significant (1-tailed sig. = 0.001) after 999 permutations.

Differentiation of H. glabra, H. radicata and H. salzmanniana

AFLP comparisons among populations of *Hypochaeris glabra*, *H. radicata* and *H. salzmanniana* show a clear distinction among the three species (Fig. 2.3; cophe

Table 2.2. Mantel's *r* showing pairwise correlation among six Jaccard similarity matrices obtained from each of six primer combinations used (a total of 348 fragments and 189 individuals). One-tailed significance of all values is 0.001 after 999 permutations (R package; Casgrain & Legendre, 2000).

	MseI-CTCG/ *EcoRI*-ATC	*MseI*-CAC/ *EcoRI*-ACG	*MseI*-CTA/ *EcoRI*-ACC	*MseI*-CTG/ *EcoRI*-ACA	*MseI*-CTC/ *EcoRI*-AGG
MseI-CAC/ *EcoRI*-ACG	0.888				
MseI-CTA/ *EcoRI*-ACC	0.889	0.931			
MseI-CTG/ *EcoRI*-ACA	0.880	0.871	0.890		
MseI-CTC/ *EcoRI*-AGG	0.881	0.886	0.897	0.868	
MseI-CTGA/ *EcoRI*-AAC	0.874	0.902	0.927	0.859	0.891

netic correlation = 0.934). Each species consists of a highly supported (100 % BS) cluster of populations. *H. radicata* and *H. salzmanniana* are more similar to one another (level of divergence 0.327; Fig. 2.3) than to *H. glabra*, which branches off at a level of divergence of 0.351. However, a strong relationship of *H. radicata* and *H. salzmanniana* is not highly supported (51 % BS).

The total number of AFLP fragments is 142 in *Hypochaeris salzmanniana* and 269 in *H. radicata* (accessions from Spain, Italy, Tunisia, and from worldwide introduced accessions). For a direct comparison it seems more appropriate to consider the same geographical area in both species. The Spanish populations of *H. radicata* alone (a total of 33 individuals) have 210 fragments, which is still considerably more than in *H. salzmanniana*. *H. glabra* has 124 fragments, though this value might be underestimated, because only 15 individuals were investigated. The number of fixed fragments is 30 in *H. radicata* (41 when only Spanish populations are considered), 63 in *H. salzmanniana*, and 78 in *H. glabra*. Diagnostic fragments are important for species determination, especially when they are

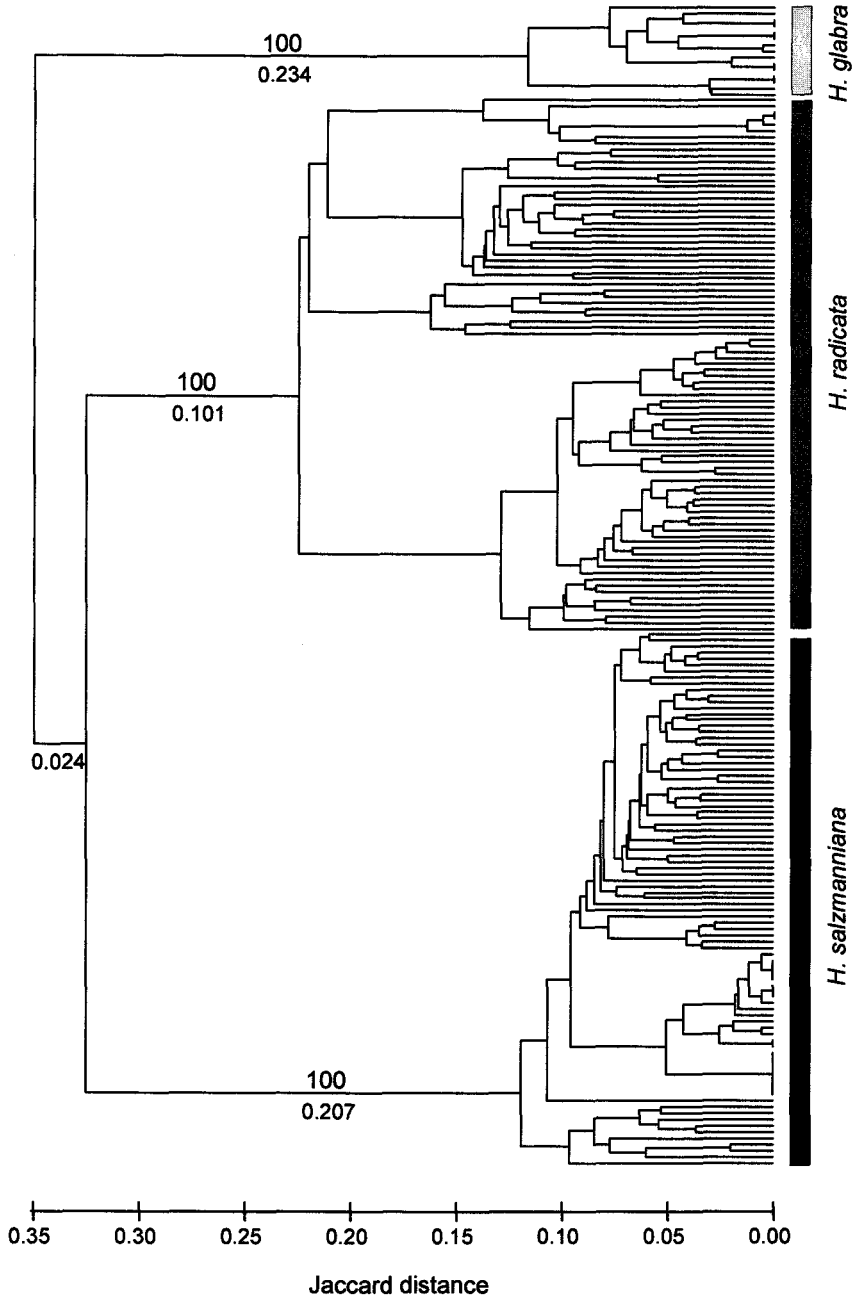

Fig. 2.3. UPGMA dendrogram of Jaccard distances (based on 348 AFLP fragments) showing general relationships among individuals of *Hypochaeris salzmanniana*, *H. radicata*, and *H. glabra*. Cophenetic correlation coefficient 0.934. Bootstrap values for the three species (1000 replicates) above branches; Jaccard distances below branches.

fixed. The number of non-fixed diagnostic fragments is 133 in *H. radicata*, 26 in *H. salzmanniana*, and 23 in *H. glabra* (Fig. 2.4A). The number of fixed diagnostic fragments is one in *H. radicata*, nine in *H. salzmanniana*, and 17 in *H. glabra* (Fig. 2.4B).

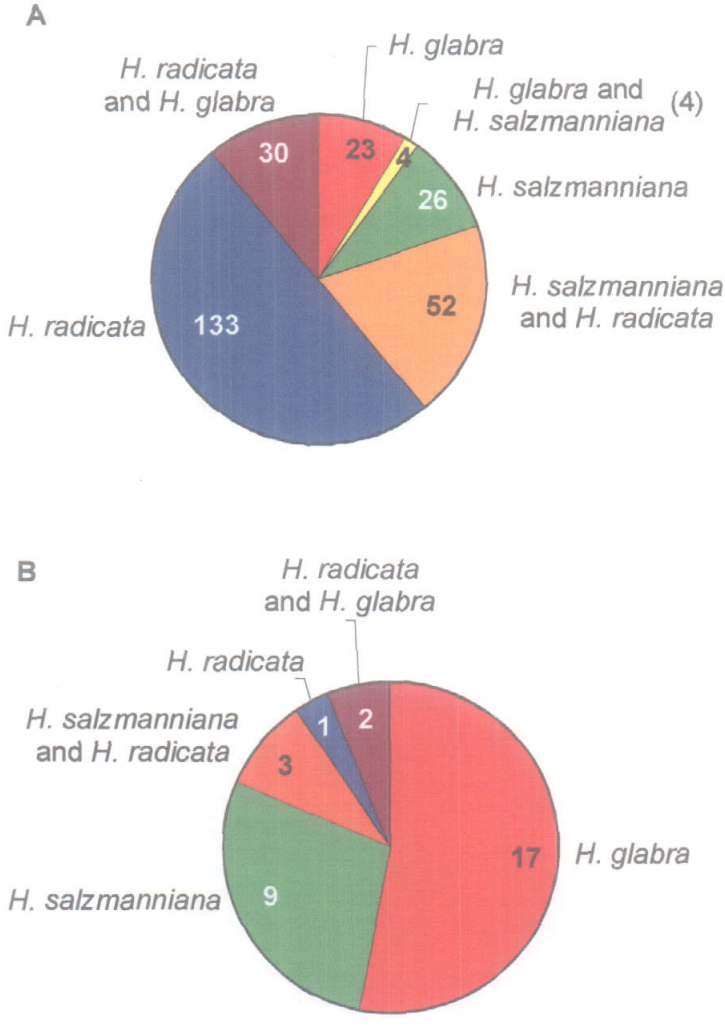

Fig. 2.4. Pie diagrams of non-fixed (A) and fixed (B) diagnostic AFLP fragments (i.e., fragments occurring in only one or in only two species of Hypochaeris salzmanniana, H. glabra, and/or H. radicata).

Shared diagnostic fragments (i.e., those occurring in two of the three species) are important for assessing evolutionary relationships among species. *Hypochaeris radicata* and *H. salzmanniana* share 52 non-fixed diagnostic fragments; *H. radicata* and *H. glabra* 30; and *H. salzmanniana* and *H. glabra* four (Fig. 2.4A). When shared diagnostic fragments are fixed in all individuals of the two species, the closer relationship of these two species becomes emphasized. *H. radicata* and *H. salzmanniana* share three fixed diagnostic fragments; *H. radicata* and *H. glabra* two; and *H. salzmanniana* and *H. glabra* none (Fig. 2.4B).

Cytological comparisons between *H. radicata* and *H. salzmanniana* (both $2n = 2x = 8$) also reveal their similarity and a clear distinction from *H. glabra* ($2n = 2x = 10$) (Fig. 2.5).

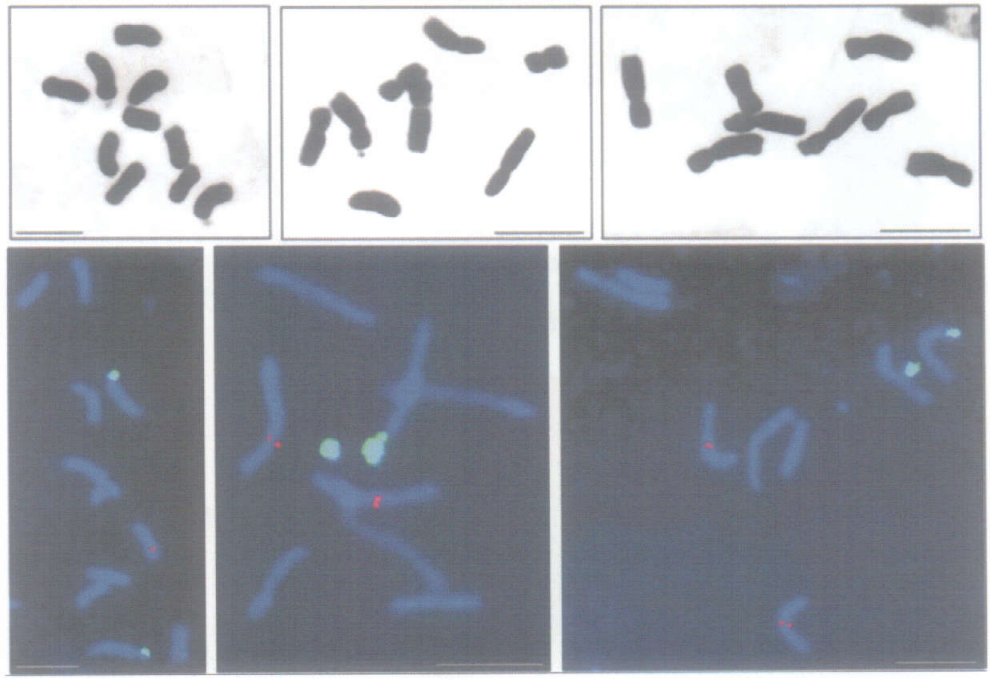

Fig. 2.5. Comparative karyotypes (A, C, E) and FISH (B, D, F) of *H. glabra* (A, B; Spain, Valverde (pop. *34*); see Table 1), *H. radicata* [C, D; Chile, *Stuessy et al. 15477*], and *H. salzmanniana* (E, F; Spain, Conil (pop. *1*); see Table 1).

Karyotypically, as analyzed by Feulgen-staining and FISH, the former two species are indistinguishable. Their karyotypes are symmetrical with metacentric and submetacentric chromosomes (Fig. 2.5C, E). Chromosome pair 2 carries a satellite on its short arm. Chromosome length is also similar in both species. Analysis of rDNA localization (5S and 18-25S rDNA genes) has shown the same localization in karyotypes of both species (Fig. 2.5D, F). The karyotype of *H. glabra* is also symmetrical, but differs in chromosome number ($2n = 2x = 10$; Fig. 2.5A). The number of 5S and 18-25S rDNA sites is the same as in the other two species (Fig. 2.5B). In *H. glabra*, 5S rDNA (red) is localized distally on the short arm of chromosome 4 (Fig. 2.5B), whereas in the other two species it is localized on the long arm of chromosome 2. 18-25S rDNA (green) is localized distally on the short arm of chromosome 1 in all three species.

Infraspecific variation within H. salzmanniana

The AFLP data also reveal similarities and distances among individuals of *Hypochaeris salzmanniana* in southern Spain (Fig. 2.6). Four main clusters were found. The first cluster includes the geographically neighboring populations Conil, Los Caños and Barbate (pops. *1-3*; with the exception of individual *2 c* clustering apart). The individual plants from the three locations are completely intermingled in the UPGMA dendrogram, suggesting a large panmictic group. The second cluster consists of population Zahara (pop. 4) and is most similar to the first cluster (pops. *1-3*). The third cluster comprises the geographically closely neighboring populations Los Algarbes and Punta Paloma (pops. *5* and *6*). In fact, two collections in Punta Paloma (pop. 6) in two subsequent years (2002: *Talavera, Stuessy et al. 32*, ind. *a* to *j*; 2003: *Talavera and Ortiz 3/03*, ind. *k* to *s*) are separated by pop. *5* with the exception of ind. *6 s* collected in 2003 clustering with the 2002 collection. The fourth cluster comprises populations Algeciras and La Línea (pops. *7* and *8*), which grow in geographic proximity on the eastern side of the Straits of Gibraltar. The highest differentiation is between pops. (5, 6) and (4) ($F_{ST} = 0.662$) and pops. (5,6) and (7,8) ($F_{ST} = 0.644$; Table 2.3). The lowest differentiation is

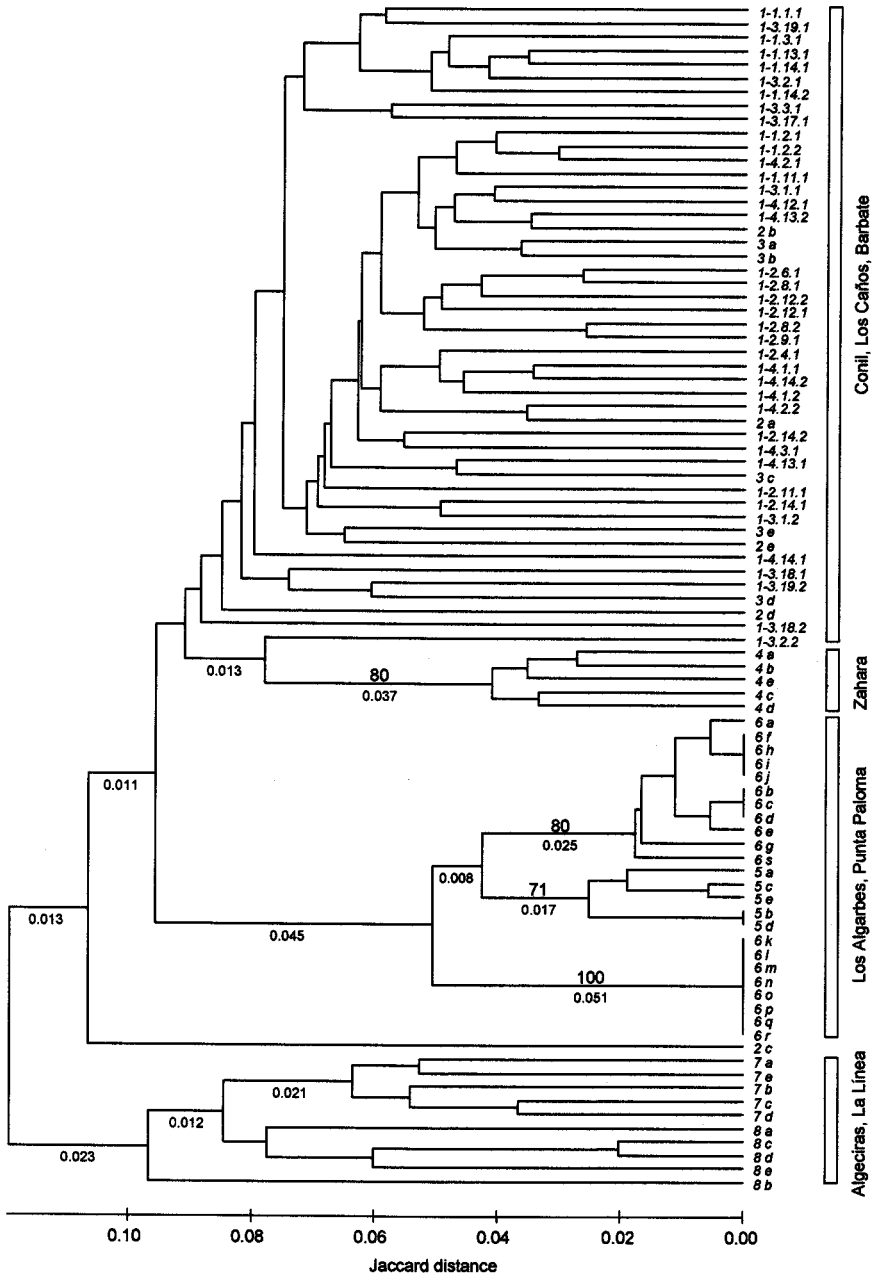

Fig. 2.6. UPGMA dendrogram of Jaccard distances (based on 142 AFLP fragments) showing relationships among individuals of *Hypochaeris salzmanniana*. Only bootstrap values > 70 % (1000 replicates) shown above branches; Jaccard distances below branches. Numbers refer to populations (see Table 1). Letters in pops. *2-8* refer to individual plants. In the large population Conil (pop. *1*) the second number refers to the transect (*1-4*), the third number to the quadrat within the transect, and the fourth number to the individual within the quadrat.

Table 2.3. Pairwise F_{ST}'s between four main groups of *Hypochaeris salzmanniana* (determined through UPGMA analysis, Fig. 6). Significance of each value is 0.000 after 1023 permutations (Arlequin version 2.0; Schneider et al., 2000).

	Pops. 1, 2, 3	Pop. 4	Pops. 5, 6
Pops. 4	0.318		
Pops. 5, 6	0.418	0.662	
Pops. 7, 8	0.364	0.510	0.644

between pops. (1-3) and (4) (F_{ST} = 0.318) and between pops. (1-3) and (7, 8) (F_{ST} = 0.364). The Mantel test revealed an overall positive, though low, correlation between geographic distances in kilometers and genetic distances measured by the Jaccard index among all individuals investigated by Mantel's r = 0.625 (1-tailed sig. = 0.001 after 999 permutations; *R* package, Casgrain, Legendre, 2000).

Levels of within-populational genetic variation vary among populations (Table 2.4). The large population Conil (pop. 1; c. 2,000,000 plants) has the largest variation (16.7 % polymorphic fragments, Shannon diversity 11.06), and the 38 individuals analyzed in this population harbor 125 of 142 fragments entirely found in *H. salzmanniana*. The population Los Algarbes (pop. 5) has the lowest variation (2.0 % polymorphic fragments, Shannon diversity 2.31), and the five individuals analyzed in this population harbor 96 fragments. Individuals 5 *b* and *d* share the same AFLP phenotype. Identical AFLP phenotypes are also found in Punta Paloma (pop. 6), in which three (*b-d*) and four (*f, h-j*) individuals of the 2002 collection share the same AFLP phenotype, as well as eight individuals (*k-r*) of the 2003 collection (Fig. 2.6). All individuals investigated in other populations have a unique AFLP phenotype. Considering only eight cases, we found a non-significant correlation between Shannon diversity and population size [Pearson's r = 0.533; sig.(1-tailed) = 0.087] and between Shannon diversity and self-incompatibility [Pearson's r = 0.549; sig.(1-tailed) = 0.079].

The number of private fragments in a population or cluster is a good indicator of its degree of isolation. Conil (pop. 1) has five private fragments; Punta Paloma

Table 2.4. Population size (estimated no. of individuals in population), self-incompatibility [percentage of self-incompatible plants (no. of plants analyzed in parentheses); Ortiz *et al.* 2006.], % of different AFLP phenotypes, % of polymorphic fragments, Shannon diversity and private fragments in populations of *Hypochaeris salzmanniana* based on 142 AFLP fragments (Arlequin version 2.0; Schneider *et al.*, 2000). None of the populations has fixed private fragments.

	Pop. size	% SI plants (N)	% of different AFLP phenotypes	$Frag_{poly}$ (%)	H_{SH}	$Frag_{priv}$
Pop. 1. Conil	2×10^6	33.3 (15)	100	16.7	11.06	5
Pop. 2. Los Caños	500	33.3 (15)	100	9.8	9.90	1
Pop. 3. Barbate	250	52.6 (19)	100	7.8	7.82	0
Pop. 4. Zahara	200	0.0 (12)	100	4.0	4.15	2
Pop. 5. Los Algarbes	2×10^5	0.0 (20)	80	2.0	2.31	0
Pop. 6. Punta Paloma	50	0.0 (22)	47	4.3	4.14	3
Pop. 7. Algeciras	600	100.0 (27)	100	6.3	6.97	2
Pop. 8. La Línea	250	85.7 (14)	100	8.9	9.00	2

(pop. 6) three; Zahara, Algeciras, and La Línea (pops. 4, 7, and 8) two each; and Barbate and Los Algarbes (pops. 3 and 5) none. The four main clusters derived from UPGMA analysis (Fig. 2.6) also have private fragments: eleven in the first cluster (pops. 1-3); two in the second cluster (pop. 4); six in the third cluster (pops. 5 and 6); and five in fourth cluster (pops. 7 and 8). None of the populations or clusters has fixed private fragments.

Within the large population Conil (pop. *1*), the effects of geographic vicinity within a transect and proximity or distance to the ocean have been tested as factors that might influence the partitioning of genetic variation. An analysis of molecular variance (Table 2.5) carried out on three hierarchical levels revealed 15 % genetic variation among the four transects, 0.4 % among quadrants next to the ocean and far from the ocean in each transect, and 85 % among individuals within each of the two groups of nearby quadrants (next to the ocean and far from the ocean) in each transect.

Differentiation within H. radicata and H. glabra

Cluster analyses among populations within *H. radicata* and *H. glabra* (Figs. 2.7 and 8) reveal a clear geographic patterning. In *H. radicata*, the largest divide (level of divergence 0.226) is between Spain and worldwide introduced accessions on one hand, and Tunisia, Sicily and Italy on the other hand. The level of divergence is higher in the Spanish and worldwide group than in the Tunisian and Italian-Sicilian group. Accessions from the Betic Cordillera (Cádiz and Málaga) differ to a high degree from accessions from the Iberian Massif (Huelva and Burgos) and worldwide introduced accessions (level of divergence 0.221). Within Huelva, there is also a high differentiation, namely between the accession from Doñana National Park (pop. 22) and other accessions from Sierra Morena [Aracena, Santa Ana, and Valverde (pops. 21, 23, and 24); level of divergence 0.212]. An accession from Burgos (pop. 19) clusters within the larger Sierra Morena (Huelva) group (pops. 21, 23, and 24). Worldwide introduced accessions from Brazil, Colombia, Germany, Mexico, and South Africa (pops. 9, 10, 11, 18, and 26) are grouped together and cluster with the larger Iberian Massif (Huelva and Burgos) group (pops. 19, 21, 23, and 24; level of divergence 0.149).

Table 2.5. Analysis of Molecular Variance (AMOVA; Excoffier et al., 1992) in *Hypochaeris salzmanniana* from Playa El Palmar (pop. 1) with c. 2,000,000 estimated plants; one-tailed test statistic probability obtained from 1023 permutations of the original presence/absence matrix.

Source of variation	d. f.	SS	Variance components	Percentage of variation	Test statistic probability
Among the four transects	3	50.930	1.100	14.61	0.006
Among quadrats next to the ocean and far from the ocean in each transect	4	26.225	0.033	0.44	0.419
Among individuals within groups of nearby quadrats (two in each transect)	30	191.950	6.398	84.95	0.000
Total	37	269.105	7.532		

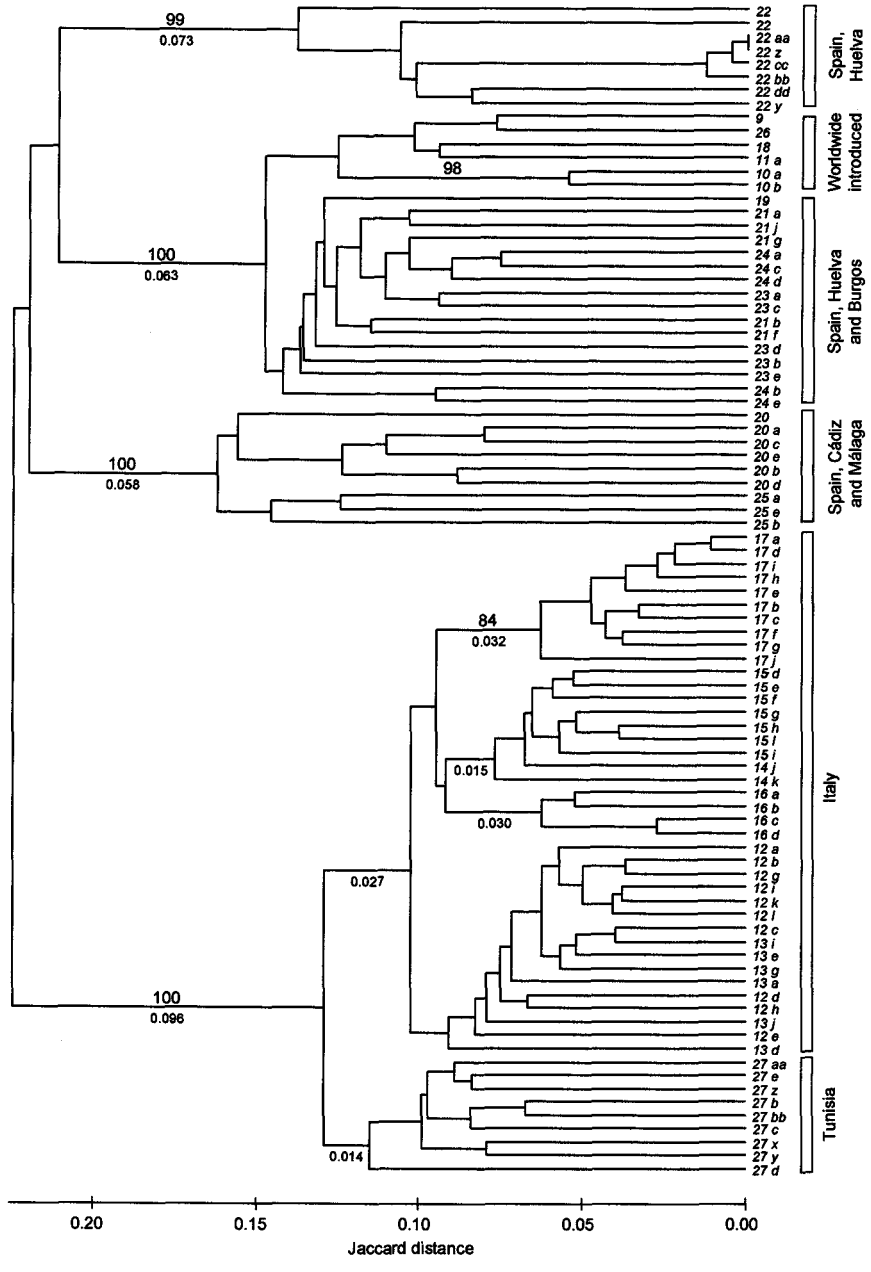

Fig. 2.7. UPGMA dendrogram of Jaccard distances (based on 269 AFLP fragments) showing relationships among individuals of *Hypochaeris radicata*. Only bootstrap values > 70 % (1000 replicates) shown above branches; Jaccard distances below branches. Numbers refer to populations (see Table 1). Letters refer to individual plants.

Hypochaeris glabra (Fig. 2.8) shows a geographic patterning similar to that of *H. radicata.* The largest divide (level of divergence 0.117) is between the Betic Cordillera (Cádiz) on one hand, and the Iberian Massif (Huelva) and worldwide introduced accessions on the other hand. Accessions from Chile and the Canary Islands (pops. 28 and 31) cluster completely within the Iberian Massif group.

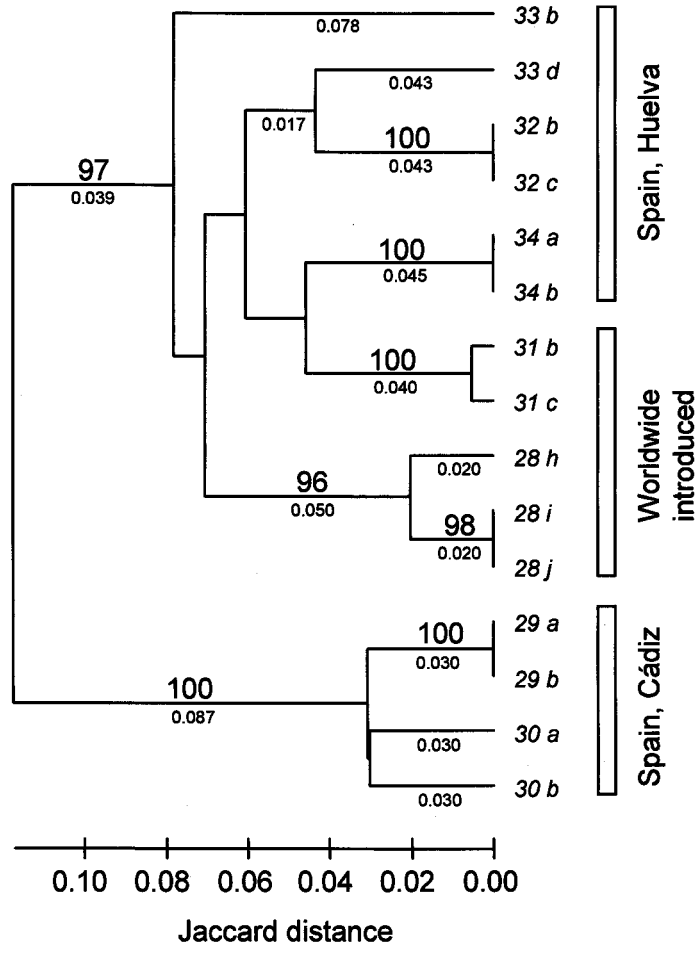

Fig. 2.8. UPGMA dendrogram of Jaccard distances (based on 124 fragments) showing relationships among individuals of *Hypochaeris glabra*. Only bootstrap values > 70 % (1000 replicates) shown above branches; Jaccard distances below branches. Numbers refer to populations (see Table 1). Letters refer to individual plants.

Discussion

Taxonomy

AFLP data have proven to be very helpful in evidencing specific limits in *Hypochaeris* sect. *Hypochaeris*. The data support recognition of *H. salzmanniana* as a good species based on 100 % BS support in the UPGMA dendrogram and the possession of nine fixed diagnostic fragments, following the hypotheses of Talavera (1980, 1987). The populational systems of *H. radicata* and *H. glabra* are also justifiably recognized as distinct species. Among the three species, *H. salzmanniana* is clearly more closely related to *H. radicata* than to *H. glabra*. This is evidenced by the greater genetic similarity between *H. salzmanniana* and *H. radicata* in the UPGMA dendrogram (though not highly supported) and the distribution of shared diagnostic fragments among the three species. *H. glabra* as a sister group to *H. radicata* and *H. salzmanniana* is consistent with DNA sequence data of the nuclear Internal Transcribed Spacer (ITS; Samuel *et al.*, 2003; Tremetsberger *et al.*, unpubl.). The similarity of karyotypes of *H. salzmanniana* and *H. radicata* also strongly supports their close relationship and distinctness from *H. glabra*. The application of molecular cytogenetic techniques further emphasizes the similarity of karyotypes between *H. salzmanniana* and *H. radicata*.

Biogeography

At the end of the Tertiary, increase in aridity and rise of mountain ranges in the course of the Alpine orogeny might have been factors promoting the biodiversity in the Mediterranean Basin (see Myers *et al.*, 2000) and particularly of the flora (Bocquet *et al.*, 1978; Blanca, 1993). The postulated evolutionary history of sect. *Hypochaeris*, in accordance with genetic and karyotypic data, is: first, differentiation of the ancestor of *H. radicata* and *H. salzmanniana* (with $2n = 8$ chromosomes) from a common ancestor with *H. glabra* (with $2n = 10$ chromosomes); and, second, differentiation of *H. salzmanniana* and *H. radicata*.

The infraspecific genetic structure of *H. salzmanniana* may be interpreted in the context of different geological ages of their habitats. Generally, isolation-by-distance, as initially presented by Wright (1943), is found in *H. salzmanniana*. However, the correlation is weak, because the entire populational system is subdivided into four main groups. The coast from Punta Paloma to Gibraltar is bordered by the Betic Cordillera (also called in this area Campo de Gibraltar or Unidad de Algeciras). The base of this unit is formed by Cretaceous to Middle Miocene basic flysch (135-22 Mya), which emerged from the sea bottom during Lower and Middle Miocene (25-16 Mya). Above the flysch layer are sandy acidic materials called "Aljibe Sandstones" or "Numidic Sandstones". These materials are allochthonous turbiditic sands that migrated from Tunisia to the Straits of Gibraltar, where they sedimented in the furrows of the older substrates (flysch) (Pendón, 1978; Gutiérrez *et al.*, 1991). When the Alpine orogeny ceased and the Straits of Gibraltar opened up, the low littoral zones were covered with Pliocene and recent Quaternary sands (Fernández-Palacios *et al.*, 1988). The populations of Conil, Los Caños, Barbate, Zahara, and Algeciras (pops. *1-4* and *7*) are in sand dunes near the coast. The population of Barbate, as an exception, is found on a fossil dune of the marine transgression of Flandriense (11,000 ya). The populations of Los Algarbes and Punta Paloma (pops. *5* and *6*) occur in the ecotone of the Quaternary dunes with the flysch materials belonging to the rocky spur of the Sierra de San Bartolomé (Cretaceous). The population of La Línea (pop. *8*) is at the base of the Rock of Gibraltar, in the loam-sandstone mica layer of the flysch (Gutiérrez *et al.*, 1991) of the Lower Miocene or Aquitanian (c. 20 Mya). The high differentiation between the three groups of populations, [(*1*, *2*, *3*), (*4*)], [*5*, *6*], and [*7*, *8*], therefore, may reflect an ancient separation of populations.

The coinciding populational structures of *H. radicata* and *H. glabra* are also most interesting. These two species independently show a clear differentiation between accessions from the Betic Cordillera (Cádiz and Málaga) and those from the Iberian Massif (Huelva and Burgos) in southern Spain. *H. radicata* in the Betic

Cordillera is also differentiated morphologically from its conspecific neighbor in the Iberian Massif and has been separated as *H. radicata* subsp. *platylepis* (Boiss.) Maire (Galán de Mera, 1995). This taxon is differentiated from subsp. *radicata* by involucral bracts with a wide scarious margin.

A possible explanation for the differentiation between populations in the Betic Cordillera and the Iberian Massif lies in the geological history of the region. The Guadalquivir river, which flows through Andalusia from east to west, separates the Precambric and Paleozoic terrains (N of the river, Iberian Massif) from the Mesozoic and Neozoic terrains (S of the river, Betic Cordillera). The south Guadalquivir river materials emerged from the sea bottom during the Alpine orogeny between the Lower Miocene (Aquitanian, 23.8-22 Mya) and Pliocene, 5 Mya (Lonergan, Johnson, 1998; Sánchez-Gómez *et al.* 2002). During Middle and the beginning of the Upper Miocene (until Lower Tortonian, 9 Mya), the Betic-Rifian bow (today's Betic Cordillera and Rif Mountains) was united and separated as a unit from the Iberian Massif (northwards) by the Betic Strait and from N Africa (southwards) by the Rifian Strait. The Mediterranean Sea was then connected with the Atlantic Ocean through two straits (Orszag-Sperber *et al.*, 1993). Between 8.5 Mya and 7.3 Mya, the eastern side of the Betic Strait, and c. 7.16 Mya, the Rifian Strait disappeared due to their silting (Guerra-Merchán, Serrano, 1993; Soria *et al.*, 1999; Meulenkamp *et al.*, 2000; Seidenkrantz *et al.*, 2000; Braga, Aguirre, 2001; Sánchez-Almazo *et al.*, 2001). These events, in addition to strong volcanic activity in the western Mediterranean, led to the Messinian salinity crisis and the subsequent desiccation of the Mediterranean Sea between 5.96 Mya and 5.33 Mya (Krijgsman et al., 1999; Riding et al. 1998; Duggen et al., 2003). Intercontinental bridges appeared between SW Europe and N Africa (Estabrook, 2001). These land bridges enabled the migration of numerous groups of animals and plants between the two continents (Bocquet et al., 1978). At the end of the Messinian (5.33 Mya), the Straits of Gibraltar opened, land bridges between Europe and Africa were flooded, and water from the Atlantic Ocean refilled the dry Mediterranean basin

(Lonergan, White, 1997; Jong, 1998). Direct land connection between both continents disappeared, therefore, and the Straits of Gibraltar definitively separated the Betic Cordillera in S Spain from those (nowadays the Rifian ones) in N Africa (Jong, 1998). The ancient separation of the two mountain chains (Iberian Massif and Betic Cordillera) is also reflected in the distribution of other animal and plant groups (Asensi, Díez-Garretas, 1987) and in their genetic structures (e.g., haplotypes of different genera of amphibians; Busack, 1986; García-París et al., 1998; García-París, Jockusch 1999). If the genetic differentiation seen in *H. glabra* and *H. radicata* indeed went back to the geological separation of the Iberian Massif and Betic Cordillera, the species might be older than 9 Mya (the end of the period, when the Betic-Rifian bow was separated from the Iberian Massif by the Betic Strait).

Conservation

Because *H. salzmanniana* is a restricted and endangered species in the vascular flora of Spain, conservation implications from analysis of genetic variation within and among populations are important to highlight. The species is now known from only eight populations in Spain, all of which have been sampled in the present study.

A conspicuous result is that the first group consisting of Conil, Los Caños, and Barbate (pops. *1-3*) actually behaves as one large panmictic population with individuals of the three populations being strongly intermingled. The population of Conil, Playa El Palmar, is nearly 10 km long and separated from Los Caños by only 6.5 km, and this from Barbate by another 6.7 km (a total of about 20 km). Two possible explanations might account for this situation: (1) that there is actually high gene flow between these populations; or, (2) that the populations were interconnected not long ago and were only recently interrupted through destruction of geographically intermediate populations by human activity. Investigations from herbarium records (Ortiz, Talavera, unpubl.) show that *H. salzmanniana* occupied a continuous territory along the Atlantic coast from Chiclana de la Frontera to Punta

Camarinal until the 1970s. The remaining populations of this formerly continuous distributional area are only four: Conil, Los Caños, Barbate, and Zahara. In contrast, historical records (back to the 1890s) reveal that *H. salzmanniana* was only locally abundant from Punta Camarinal to Gibraltar. Suitable habitats are scarce along the steep coast of the Betic Cordillera.

Population sizes in *H. salzmanniana* explain 28 % of the observed variation in Shannon diversity. The large population Conil (pop. *1*; c. 2,000,000 plants) has the highest genetic diversity. This stresses the importance of preserving not only populations of the four major groups, but also of population Conil in its entirety, because it harbors a majority of genetic diversity within the species. Within this population, there is high genetic differentiation between neighboring plants within a transect (a few meters distant), and there is also differentiation between transects (c. 1 km distant each). Proximity or distance to the ocean does not influence partitioning of genetic variation. As long as this dune system is not destroyed (shown in Fig. 2.1B), the species should continue to thrive as it does not seem bothered by relatively high levels of human disturbance.

The compatibility systems vary greatly among populations of *H. salzmanniana* (Ortiz *et al.*, 2006) and contribute 30 % to the explanation of the observed variation in Shannon diversity. 100 % self-compatible plants and possibly high levels of inbreeding in Zahara, Los Algarbes and Punta Paloma (pops. *4-6*) correlate with the low within-populational genetic diversity and many identical AFLP phenotypes in these populations.

There are also urgent needs to preserve genetic variation harbored in each of the four groups. Los Algarbes and Punta Paloma are presently under no threat, being situated along a small road away from any human development. This cannot be said for Algeciras and La Línea, however, which are on the coast completely in the middle of beach developments. Algeciras (pop. *7*) is a relatively large population broken into patches by houses and streams feeding into Bahía de Algeciras. La Línea (pop. *8*) is even more endangered, surviving along the coast in the town of

La Línea de la Concepción just adjacent to the fence that separates it from Gibraltar. This small dune area is already seriously disturbed and not likely to remain into the future.

Acknowledgements

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3 Self incompatibility and floral parameters in *Hypochaeris* sect. *Hypochaeris* (Asteraceae)

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Summary

We studied the relationships between self-incompatibility mechanisms and floral parameters in the genus *Hypochaeris* L. sect. *Hypochaeris* (consisting of *H. glabra*, *H. radicata*, *H. arachnoidea*, and *H. salzmanniana*). We assessed at intra- and interspecific levels (1) the self-incompatibility (SI) mechanism and its distribution among populations, (2) the relationship between SI and floral parameters, and (3) the relationship of SI to reproductive success. *Hypochaeris salzmanniana* is semi-incompatible, *H. glabra* is self-compatible, and *H. arachnoidea* and *H. radicata* are self-incompatible. Floral parameters differed among populations of *H. salzmanniana*: plants in self-compatible populations had fewer flowers per head, a smaller head diameter on the flower, and a shorter period of anthesis than self-incompatible populations. We also detected this pattern within a semi-compatible population of *H. salzmanniana*, and these differences were also found between species with different breeding mechanisms. Fruit to flower ratio in natural populations was generally high (>60%) in all species, regardless of breeding system. It is hypothesized that self-compatibility may have arisen through loss of allelic diversity at the *S* locus due to bottleneck events and genetic drift.

Keywords: Asteraceae; floral parameters; *Hypochaeris*; *S* alleles; sporophytic self-incompatibility; reproductive success.

Introduction

In flowering plants, three major systems of self-incompatibility (SI) are known: homomorphic gametophytic SI (GSI), homomorphic sporophytic SI (SSI), and heteromorphic SI (HetSI) (see review by Hiscock, Kües, 1999). Both homomorphic systems have a genetic control, usually of one gene with multiple alleles; however, whereas in GSI the *S* genotype of the haploid (usually two-celled gametophyte) pollen effectively determines the SI reaction, in SSI the incompatibility reaction of the (usually three-celled gametophyte) pollen is determined by both *S* alleles present in the pollen parent. Moreover, whereas the *S* alleles are codominant in GSI (see model in Talavera *et al.*, 2001), in all SSI cases analyzed thus far, the *S* alleles have a hierarchy of dominance –recessive interactions, which often differ in the stigma and pollen grain of the same plant. A consequence of such interactions is in crosses that have an *S* allele common to both parents; progeny homozygous for the incompatibility gene may be produced (Williams, 1965).

The homomorphic sporophytic self-incompatibility (SSI) mechanism was established in the Asteraceae by Gerstel (1950) for *Parthenium argentatum* (Anthemideae) and by Hughes and Babcock (1950) for *Crepis foetida* subsp. *rhoeadifolia* (Lactuceae). Since then, self-incompatibility in Asteraceae has been recorded in 40 genera (Charlesworth, 1985). Within Lactuceae, self-incompatibility has been found in several species of *Leontodon* (Izuzquiza, 1991; Ruiz de Clavijo, 2001) and in *Crepis sancta* (Cheptou *et al.*, 2000), *Reichardia picroides* (Gallego, 1983), *Hypochaeris radicata* (Parker, 1975), *H. maculata* (Wells, 1976), and *Stephanomeria exigua* subsp. *coronaria* (Brauner, Gottlieb, 1987; Gottlieb, 1973). In addition to Asteraceae, this complex self-incompatibility mechanism is known in only five dicotyledonous families: Betulaceae, Brassicaceae, Caryophyllaceae, Convolvulaceae, and Polemoniaceae (Hiscock, Kües, 1999).

The number of *S* alleles in populations of species with SSI is relatively poorly documented in the Asteraceae: six *S* alleles (Brennan *et al.*, 2002) and 7–11

(Brennan *et al.*, 2003; Hiscock, Tabah, 2003) in *Senecio squalidus*, 6–8 in *Carthamus flavescens* (Imrie, Knowles, 1971), and 16 in *Centromadia pungens* subsp. *laevis* (Friar, LaDoux, 2002). These rather low numbers of *S* alleles differ from reports for species of other SSI families: Brassicaceae—e.g., 22 in *Iberis amara* (Bateman, 1954), 20–30 in *Brassica campestris* (Nou *et al.*, 1993), 22 in *Raphanus raphanistrum* (Karron *et al.*, 1990), 52 in *Sinapis arvensis* (Stevens, Kay, 1989); Convolvulaceae—e.g., 49 in *Ipomoea trifida* (Kowyama *et al.*, 1994, 2000).

The occurrence of some self-compatible individuals within self-incompatible populations is relatively frequent and has been observed in Asteraceae species such as *Carthamus flavescens* (Imrie, Knowles, 1971), *Stephanomeria exigua* subsp. *coronaria* (Brauner, Gottlieb, 1987), *Rutidosis leptorrhynchoides* (Young (Young *et al.*, 2000), and *Senecio squalidus* (Brennan *et al.*, 2002); in Brassicaceae species such as *Leavenworthia crassa* and *L. alabamica* (Lloyd, 1968a, 1968b; Solbrig, Rollins, 1977), and in Convolvulaceae such as *Ipomoea trifida* (Kowyama *et al.*, 1994, 2000; Kakeda *et al.*, 2000).

Occasional self-compatible individuals, in the absence of apomixis, are unlikely to persist in a population with a moderate number of alleles at the *S* locus if the mutated allele (S_c) is not dominant in the allelic series. However, in the family Asteraceae, the number of *S* alleles at the *S* locus may be low (Imrie, Knowles, 1971; Brennan *et al.*, 2003; Hiscock, Tabah, 2003;), and in these circumstances, if the self-compatibility is inheritable and mate availability is limited, then self-compatible individuals would be selected and the population would become self-compatible. Pollen limitation is considered to be a condition favoring the breakdown of self-incompatibility (Baker, 1955; Charlesworth, Charlesworth, 1979).

In many SI species, some plants produce a low proportion of seeds with self-pollen. This phenomenon is known as pseudo-self-compatibility (PSC) (see Nettancourt, 1977) or pseudo-self-fertility. In the PSC plants, cross-pollen has an earlier germination and more rapid tube growth than self-pollen, whereas plants

with true self-compatibility (SC) have similar seed production following self or cross pollinations, with similar germination and growth rates of self- and cross-pollen tubes. Pseudo-self-compatibility allows some seed production following crosses between individuals that share both *S* alleles (Levin, 1996).

In the Asteraceae, the self-incompatible species have, in general, bigger heads than the congeneric self-compatible ones. Gibbs *et al.* (1975), in a study of five species of *Senecio*, found that the three incompatible species (*S. joppensis*, *S. aetnensis*, and *S. squalidus*) had a larger head diameter than the self-compatible species (*S. viscosus* and *S. vulgaris*), and Parker (1975) showed that SI *Hypochaeris radicata* has bigger heads than SC *H. glabra*. This pattern has also been observed in other self-incompatibility systems, as in *Eriotheca* (Oliveira *et al.*, 1992), with late-acting self-incompatibility, *Anagallis* (Gibbs, Talavera, 2001), with GSI, and also, in general, in allogamous species as opposed to their autogamous congenerics (see Levin, 2000). But, intraspecific information is scarce on how such changes in the size of flowers or inflorescences have accompanied the change from self-incompatibility toward self-compatibility.

The present study focuses on all the species of *Hypochaeris* sect. *Hypochaeris* (*H. glabra* L., *H. radicata* L., *H. arachnoidea* Poir., and *H. salzmanniana* DC.). We address the following objectives: (1) the occurrence of SI at species and population levels, (2) whether flower number and diameter of the head and length of period of anthesis of the head vary with the degree of self-compatibility in populations of the different species, and (3) compare the pre-emergent reproductive success (fruit to flower ratio) in natural populations.

Material and Methods

Section Hypochaeris*: species, populations and metapopulations*

Hypochaeris sect *Hypochaeris* contains four species, *H. glabra* L., *H. radicata* L., *H. arachnoidea* Poir., and *H. salzmanniana* DC. In phylogenetic molecular analyses (Tremetsberger *et al.*, 2005), this section is monophyletic, with *H. glabra* sister

to *H. radicata*, *H. arachnoidea*, and *H. salzmanniana* and with *H. radicata* sister to *H. arachnoidea* and *H. salzmanniana*.

In the Asteraceae, flowers show protandry and develop centripetally within the head. In species of *Hypochaeris* sect. *Hypochaeris*, the heads show nystinastic movements during anthesis, which lasts 3–10 days. Flowers in the head open daily late in the morning and close in the evening, allowing pollen in the anthers of the inner male-phased flowers to touch the stylar branches of the outer female-phased ones.

The differential morphological characters, ecology, and distribution of the four species of sect. *Hypochaeris* are shown in Table 3.1. Localities of the studied populations are given in Appendix. *Hypochaeris salzmanniana* is the most restricted species, with only eight known populations in Spain and eight in Morocco (Fig. 3.1). In recent molecular studies using the AFLP technique in *H. salzmanniana* from Spain (Tremetsberger *et al.*, 2004) and from Morocco (Ortiz *et al.*, 2007, see chapter 4), the populations from Conil (pop. 1, see Fig. 3.1), Barbate (pop. 2), and Los Caños (pop. 3) were shown to form a metapopulation (Barbate metapopulation) that contains more than 90% of the Spanish plants (2×10^6–3×10^6 individuals). Of the other Spanish populations, three (Zahara, pop. 6; Punta

Table 3.1. Characters, habitat, and geographic distribution of the species of *Hypochaeris* sect. *Hypochaeris*.

	H. glabra	*H. radicata*	*H. arachnoidea*	*H. salzmanniana*
Chromosome number (2*n*)	10	8	8	8
Habit	annual	perennial	annual	annual
Stem	aphyllous	aphyllous	aphyllous	foliaceous
Peduncle	cylindrical	cylindrical	cylindrical	inflated
Ligules	≥ involucre	>> involucre	>> involucre	>> involucre
Density (plants/m^2)	10–50	1–5	2–30	9–50
Habitat	shrubland	oak forest	shrubland	sand dunes
Distribution	Circum-Mediterranean (cosmopolitan)	Circum-Mediterranean (cosmopolitan)	NW Africa (Morocco and Algeria)	SW Spain (Cádiz) & NW Morocco

Paloma, pop. 7, and Los Algarbes, pop. 8) are small and isolated, and the other two (Palmones, pop. 4 and La Línea, pop. 5) form another well-differentiated metapopulation (Bahía de Algeciras metapopulation) (Fig. 3.1). The Moroccan populations are structured into three groups: two small ones near Tangiers (Tanger, pop. 9

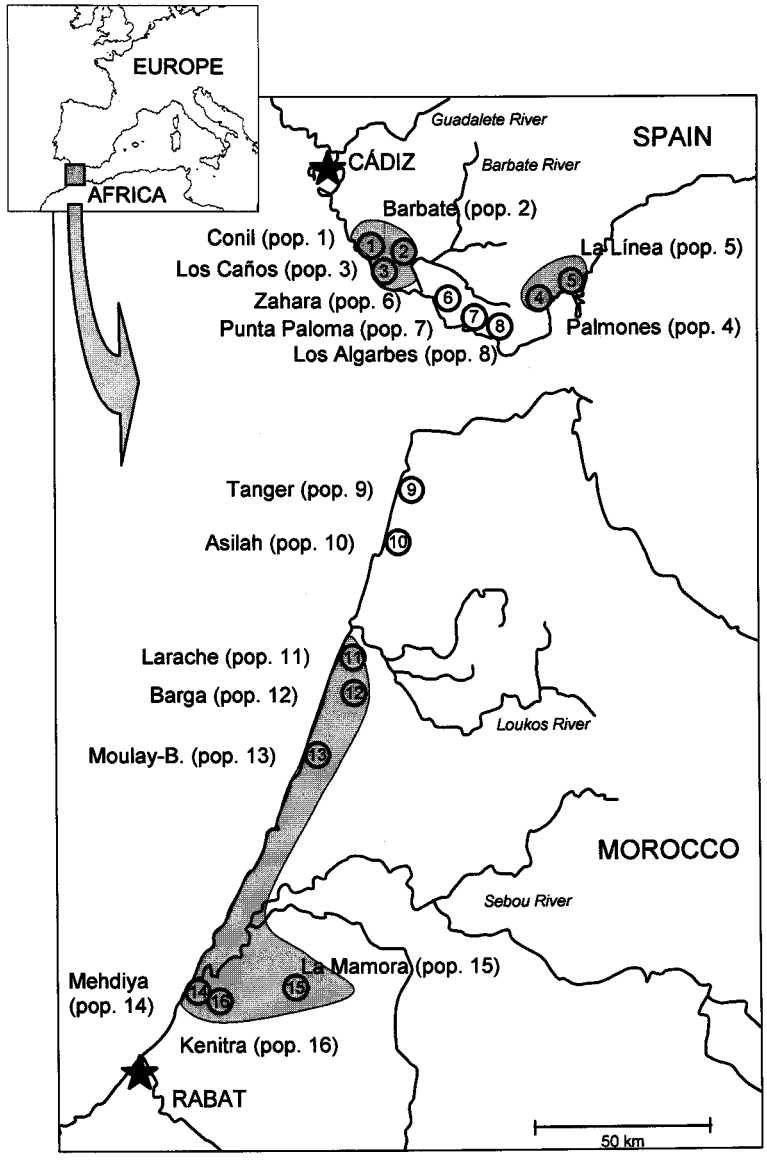

Figure 3.1. Geographical distribution of the studied populations and metapopulations of *Hypochaeris salzmanniana*. The metapopulations Barbate (pops. 1–3), Algeciras (pops. 4 and 5) and La Mamora (pops. 11–16) with a gray background.

and Asilah, pop. 10) and a third consisting of six populations (pops. 11–16) that form a metapopulation (La Mamora metapopulation) containing more than 90% of the individuals of *H. salzmanniana* in Morocco (>10^7 individuals) (Fig. 3.1). We compared the floral parameters (number of flowers per head, diameter of the head, and duration of anthesis of the head) at an individual, population and metapopulation level.

Vouchers of all the studied taxa are deposited in Sevilla, Spain (SEV), Vienna, Austria (WU), and/or Uberlândia, Brazil (HUFU).

Self-incompatibility

In all of the hand pollination experiments, we used plants that originated from field-collected seeds (each seed from a different mother plant) or seedlings transplanted from the field (mother plants or seedlings at least 2 m apart). Plants were cultivated in the glasshouse of the University of Seville in 2001, 2002, and 2004. The photoperiod (16 h light/8 h darkness), temperature (18–22°C), and watering (every 4 h) were controlled. Individual plants were grown in plastic pots (18 × 15 cm) in a substrate of peat and perlite (3 : 1 v/v).

Once the flowering period had begun in *Hypochaeris glabra*, *H. radicata*, and *H. salzmanniana*, we carried out a series of diallel crosses within populations. These comprised seven individuals of *H. glabra*, 12 of *H. radicata*, and nine of *H. salzmanniana*. To avoid pests or pollen contamination, all plants under treatment in the greenhouse were covered by a translucent white cloth. Most pollinations were effected by rubbing the two heads together or by rubbing a cotton-tipped swab against the inner pollen-bearing flowers and then against the stigmas of the outer ones of the same head (self-crosses) or the head of a different individual (outcrosses). This operation was repeated at least twice during the period of anthesis of the head; 2–4 (8) heads on each individual were not pollinated and used as a control. After the period of anthesis, all heads were individually bagged until fruit collection (after at least 25 days). We counted flowers and fruits with embryos and estimated fruit to flower ratio (number of flowers transformed into fruits).

In a preliminary experiment to determine whether automatically selfed heads (i.e., pre-anthesis bagged heads that were not hand-pollinated) had a different fruit to flower ratio to hand self-pollinated heads (geitonogamy), we applied a GLM (general linear model) considering the individual as a block effect. We did not detect any statistical difference between these treatments (hand self-crosses and automatic self-crosses, with heads covered with tea bags during anthesis) for the fruit to flower ratio (Ortiz et al., unpublished data). This lack of difference indicated that hand self-pollinations were unnecessary to determine self-compatibility and allowed us to subsequently study self-incompatibility in a larger number of populations and plants per population, by simply bagging pre-anthesis heads.

An individual is considered as SI when the fruit to flower ratio of its heads is null, PSC when the fruit to flower ratio of its heads is >0 and ≤0.12, and as SC when the ratio is >0.12. For statistical analyses, PSC individuals were considered as SI. In this study, we used 771 individuals belonging to 57 populations (13 of *H. glabra*, 21 of *H. radicata*, 7 of *H. arachnoidea*, and 16 of *H. salzmanniana*).

Parameters of head in anthesis

We obtained head measurements from the same plants used in the self-incompatibility study (under the same growing conditions). The first heads of at least four individuals were marked and left unpollinated. For these heads, we measured the head diameter at midday (1200–1500 hours) on the second or third day of the period of anthesis, and also noted the number of days the head was open. This allowed us to relate the SC or SI status of each individual with its head characteristics. These variables were measured in 269 heads of 103 individuals of 16 populations of the four species.

Fruit to flower ratio in natural populations

Reproductive success in the wild was studied in 3–56 individuals per population, selecting in each case, individuals both from the inner and the outer parts of the population area. All sampled individuals were chosen randomly, at least 2 m from

each other. We selected the head of the main stem of each plant, and, once dry in the laboratory; we counted the number of flowers and achenes with embryos. All the Moroccan populations were sampled in the spring of 2003 and the Spanish ones during 2002 and 2003. In total, 407 individuals (43 individuals in three populations of *H. glabra*, 59 individuals in nine populations of *H. radicata*, 66 individuals in five populations of *H. arachnoidea*, and 185 individuals in 14 populations of *H. salzmanniana*) were studied.

Statistical analyses

Statistical analyses were carried out with JMP, version 4.0.1 (SAS Institute, Cary, North Carolina, USA.). Fruit to flower ratios were arcsine-square root transformed prior to analyses. To examine differences among individuals, populations (or metapopulations), and species for the number of flowers per head, head diameter, and length of the period of anthesis, we applied GLM analyses, considering the individual (nested within populations and species) as a random effect and the species and population (nested within species) as fixed effects; random effects were evaluated using the method of moments (EMS, expected mean square). A post-hoc Tukey-Kramer honestly significant difference (HSD) test was applied to detect differences in these variables among populations and species (considering for each species all the individuals of a population if there was only one population and mean values of different populations if more than one). We considered significant differences at a 5% confidence level (Bonferroni correction applied). Finally, for *H. salzmanniana*, we applied a principal components analysis (PCA) on correlations among number of flowers, diameter of head, and duration of anthesis.

Results

Self-incompatibility

In the diallel of seven plants of *Hypochaeris glabra*, all the hand-pollinated heads, both selfs and crosses, as well as the non hand-pollinated controls produced viable

fruits, with an average fruit to flower ratio per plant of 0.79–0.98 and 0.52–0.91 in hand selfs and crosses, respectively (Fig. 3.2).

Every individual (N = 178) of the 13 sampled populations of *H. glabra* developed a high number of viable fruits in bagged heads (Table 3.2), with an average fruit to flower ratio >0.60 (data not shown). We conclude, therefore, that every individual in this species is self-compatible and predominantly geitonogamously self-pollinated.

In the diallel of 12 plants of *Hypochaeris radicata*, 11 were self-incompatible and one (no. 14) was self-compatible (Fig. 3.3). In plant 14 the fruit to flower ratio was 0.33 ± 0.20 and 0.50 ± 0.07 of the self-crosses and the outcrosses, respectively. In general, all 11 SI individuals of this population had moderate fertility (average fruit to flower ratio 0.20–0.45). All these SI individuals belong to a different compatibility phenotype (see Fig. 3.3).

♀ \ ♂	1	2	4	5	6	7	9	Control
1	80.7±13 N = 4	—	52.5 N = 1	22.7 N = 1	97.5 N = 1	62 N = 1	36 N = 1	74.3±10 N = 2
2	—	98.2±2 N = 2	—	—	95.3 N = 1	—	87.7 N = 1	76.9±12 N = 4
4	52.8 N = 1	—	98.9±1 N = 2	93.8 N = 1	61 N = 1	—	97.9 N = 1	97.4±2 N = 4
5	69.1 N = 1	—	93.9 N = 1	95.8±2 N = 2	—	80.9 N = 1	96.3 N = 1	87.5±6 N = 4
6	87 N = 1	98 N = 1	31.9 N = 1	78.9 N = 1	98±0 N = 2	77.2 N = 1	97.9 N = 1	82.6±11 N = 4
7	82 N = 1	—	—	—	22 N = 1	85.5±2 N = 2	—	91.3±1 N = 4
9	94.7 N = 1	64.4 N = 1	97.6 N = 1	96.4 N = 1	91.2 N = 1	—	81.5±11 N = 2	80.7±8 N = 4

Figure 3.2. Results of diallel cross in a sample of nine plants of *H. glabra*. Unpollinated heads were used as control.

Table 3.2. Percentage of self-compatible (% SC) and pseudo-self-compatible (PSC) plants in populations of *Hypochaeris glabra*, *H. radicata*, *H. arachnoidea*, and *H. salzmanniana* studied in a glasshouse. An individual is considered: as SI when the fruit to flower ratio of its heads is null; PSC when the fruit to flower ratio of its heads is >0 and ≤0.12; as SC when the fruit to flower ratio of its heads is >0.12 (see Material and Methods; for population no., see Appendix).

Species (Population no.)	SC plants (%)	PSC plants (%)	*N*
H. glabra (pops. 1, 2, 4, 5, 6, 8, 9–15)	100	0	178
H. radicata (pops. 1–11, 15–25)	4.4	13.3	205
H. arachnoidea (pops. 1–7)	0	1.3	79
H. salzmanniana			
Barbate metapopulation (pops. 1–3)	49.1	12.2	108
Zahara (pop. 6)	100	0	12
Punta Paloma (pop. 7)	100	0	29
Los Algarbes (pop. 8)	100	0	20
Bahía de Algeciras metapopulation (pops. 4–5)	0	8.3	52
Tanger (pop. 9)	0	42.9	10
Asilah (pop. 10)	0	25	10
La Mamora metapopulation (pops. 11–16)	0	19.3	68

Note: N = number of individuals analysed.

All plants of *Hypochaeris radicata* (N = 205) in 21 populations were self-incompatible except for nine individuals, which were self-compatible (Table 3.2); these nine self-compatible individuals had low fruit to flower ratio (0.15–0.55, data not shown). Of these self-compatible individuals, three had an American origin (pops. 18 and 21) and six a Spanish one (pops. 1, 4, 6, 7, and 8).

All plants (N = 79) of the seven populations of *Hypochaeris arachnoidea* behaved as self-incompatible (Table 3.2).

In *Hypochaeris salzmanniana*, of the nine individuals used in diallel crosses, four were self-compatible and five were self-incompatible (Fig. 3.4). Individual 9 had, as female and as male, the same pattern as individual 7. That is, they were

♀ \ ♂	1	2	4	5	7	8	9	10	11	12	13	14	Control
1	3.4±2 N = 3	35 N = 1	46.1 N = 1	0 N = 1	55.4 N = 1	56.2 N = 1	57.8 N = 1	68.6 N = 1	0 N = 1	38.9±10 N = 2	40 N = 1	66.7 N = 1	0 N = 1
2	6.8 N = 1	0.1±0.1 N = 7	85 N = 1	6.6±6 N = 2	36.4 N = 1	35.8 N = 1	45.6 N = 1	4.7 N = 1	0±0 N = 2	43.9 N = 1	71.2 N = 1	25±9 N = 2	0±0 N = 6
4	69.8 N = 1	89 N = 1	0±0 N = 2	25.3 N = 1	64.4 N = 1	—	15.2±15 N = 2	47.6 N = 1	33.7±26 N = 2	56.2 N = 1	33.6 N = 1	53.4 N = 1	0 N = 1
5	6.2 N = 1	14.3±4 N = 2	46 N = 1	0.2±0.2 N = 6	15.2±4 N = 2	38.4 N = 1	35.2±18 N = 2	42.8 N = 1	41.3±19 N = 2	72.2 N = 1	35.8 N = 1	58.8 N = 1	0±0 N = 6
7	76.5 N = 1	1.2 N = 1	61.8 N = 1	16±15 N = 2	0±0 N =5	36 N = 1	0 N = 1	1.1 N = 1	32.1±32 N = 2	0 N = 1	51.3 N = 1	52.5 N = 1	—
8	24.5 N = 1	16.4 N = 1	—	16.4 N = 1	44.2 N = 1	0±0 N = 2	30.8 N = 1	47.6 N = 1	0 N = 1	25.2 N = 1	0 N = 1	—	—
9	53 N = 1	29.8 N = 1	27.3±8 N = 2	6.5 N = 1	42.8 N = 1	50.7 N = 1	0±0 N = 2	58.2 N = 1	25.5 N = 1	—	61.3 N = 1	53.6 N = 1	—
10	38 N = 1	21.6 N = 1	38.4 N = 1	4.0 N = 1	0 N = 1	54.9 N = 1	58 N = 1	0.1±0.1 N = 6	24.2 N = 1	0 N = 1	42.2 N = 1	28.4 N = 1	0±0 N = 6
11	3.5 N = 1	12.3±12 N = 2	64.4±15 N = 2	57.6±42 N = 2	47.4±41 N = 2	2 N = 1	37.9 N = 1	67.3 N = 1	0.5±0.5 N = 6	12.7±12 N = 2	77.7 N = 1	72.7±21 N = 2	0±0 N = 10
12	—	58.9 N = 1	55.2 N = 1	12.9 N = 1	1.7 N = 1	51.8 N = 1	—	—	8±2 N = 2	0.4±0.2 N = 4	22.3 N = 1	30.6±14 N = 2	0±0 N = 6
13	42.8 N = 1	3.3 N = 1	46.5 N = 1	0 N = 1	0.6 N = 1	4.2 N = 1	23 N = 1	32.5 N = 1	23.6±2 N = 2	24.2 N = 1	0 N = 2	36 N = 1	0±0 N = 3
14	57.6 N = 1	15.6±4 N = 2	55.7 N = 1	7.7 N = 1	53.3 N = 1	—	68 N = 1	71.3 N = 1	50.4±32 N = 2	55.9±3 N = 2	82.6 N = 1	33.2±20 N = 4	—

Figure 3.3. Results of diallel cross in a sample of 12 plants of *Hypochaeris radicata*. Unpollinated heads were used as control.

the same compatibility phenotype, whereas the other three individuals (nos. 3, 6, and 8) had different compatibility phenotypes (see Fig. 3.4). In SC plants, the average fruit to flower ratio was 0.22–0.75 for the self-crosses, and 0.47–0.72 for outcrosses; in SI individuals the fruit to flower ratio was 0.16–0.40 for outcrosses (see Fig. 3.4).

Individuals of the 16 studied populations of *H. salzmanniana* were very diverse in their levels of self-compatibility (Table 3.2). In the three populations (pops. 1–3) of the Barbate metapopulation (N = 108), the percentage of self-compatible individuals was 50, 40, and 68%, respectively (average 49%), whereas all individuals

♀ \ ♂	1	2	4	5	3	6	7	8	9	Control
1	60.1±5 N = 4	24.6 N = 1	56 N = 1	85.5 N = 1	69.3 N = 1	74.2 N = 2	55.5 N = 2	75 N = 1	72.3 N = 1	66.4±6.6 N = 2
2	63.8 N = 1	85.1±7 N = 2	—	—	94.2 N = 1	—	66.6 N = 1	80.8 N = 1	—	56.3 N = 1
4	77.8 N = 1	—	21.5±7 N = 2	70.7 N = 1	31.2 N = 1	47.2 N = 1	6.4 N = 1	48.8 N = 1	45.0 N = 1	—
5	83.2 N = 1	—	65.6 N = 1	47.1±14 N = 4	68.7 N = 1	14.3 N = 1	75.2 N = 1	54.9 N = 1	49.5 N = 1	79.4 N = 1
3	48.9 N = 1	52.4 N = 1	54.2 N = 1	37 N = 1	0.75±0.5 N = 4	17.4±17 N = 2	35.8 N = 1	20±20 N = 2	55.8 N = 1	0±0 N = 6
6	51.3±2 N = 2	—	52.8 N = 1	58.5 N = 1	12.7±12 N = 2	1.7±1.7 N = 2	42.5±14 N = 2	27.2±9 N = 2	39.8±17 N = 2	0±0 N = 3
7	12±12 N = 2	63.1 N = 1	1.2 N = 1	11.4 N = 1	34.6 N = 1	60.6±16 N = 2	3.4±0.8 N = 4	35.8±10 N = 2	2.2 N = 1	1.8 N = 1
8	50.6 N = 1	32.8 N = 1	40.8 N = 1	22.3 N = 1	20.7±20 N = 2	2.7±2.7 N = 2	82.3±0.1 N = 2	0.7±0.7 N = 4	59.6±35 N = 2	0.3±0.3 N = 8
9	47.7 N = 1	—	8.4 N = 1	0 N = 1	53.5 N = 1	1.7±0.5 N = 2	1.7 N = 1	13.4±9 N = 2	0.7±0.3 N = 6	0±0 N = 3

Figure 3.4. Results of diallel cross in a sample of nine plants of *H. salzmanniana*. Unpollinated heads were used as control.

of *H. salzmanniana* from the three populations (pops. 6–8) near Tarifa were self-compatible. In contrast, all individuals from the two populations (pops. 4 and 5) from Bahía de Algeciras metapopulation in Spain and from the eight populations (pops. 9–16) from Morocco were self-incompatible (Table 3.2).In the three species with self-incompatible individuals (*Hypochaeris radicata*, *H. arachnoidea*, and *H. salzmanniana*), a variable proportion of the individuals developed heads after selfing, bearing 1–8 fruits with embryos (Table 3.2). All these individuals have been considered as pseudo-self-compatible (PSC). In *H. arachnoidea* only one PSC individual was found in pop. 1 (7% PSC value), whereas in *H. radicata* we found 23 PSC individuals (of 205 plants): 11 from Spain (pops. 2, 33%; 4, 13%; 6, 25%; 7, 7%; and 9, 13%), one from Sicily (pop. 12, 20%), three from Morocco (pops. 14, 50%; and 17, 25%), and eight from Americas (pops. 18, 19%; 20, 29%; and 21, 40%). In *H. salzmanniana*, of 309 plants we found 26 PSC individuals: six, four, and 11 PSC individuals from Barbate (6%), Bahía de Algeciras (8%), and La Mamora (16%) metapopulations, respectively, and the rest (five individuals) from the two most northern Moroccan populations (pops. 9, 30%; and 10, 20%).

Floral parameters

Number of flowers per head

Hypochaeris arachnoidea and *H. salzmanniana* possess heads with the lowest number of flowers and *H. radicata* with the highest number (Fig. 3.5A). The GLM applied was significant ($F_{102,166} = 13.57$; $P < 0.0001$; $R^2 = 0.8271$) as were all the effects considered (individual, population, and species; Table 3.3). However, the number of flowers/head was not statistically different between *H. glabra* and *H. radicata* or between *H. glabra*, *H. salzmanniana*, and *H. arachnoidea* (Fig. 3.5A).

The number of flowers per head among the populations of *Hypochaeris radicata* did not differ statistically, but we did find significant differences among

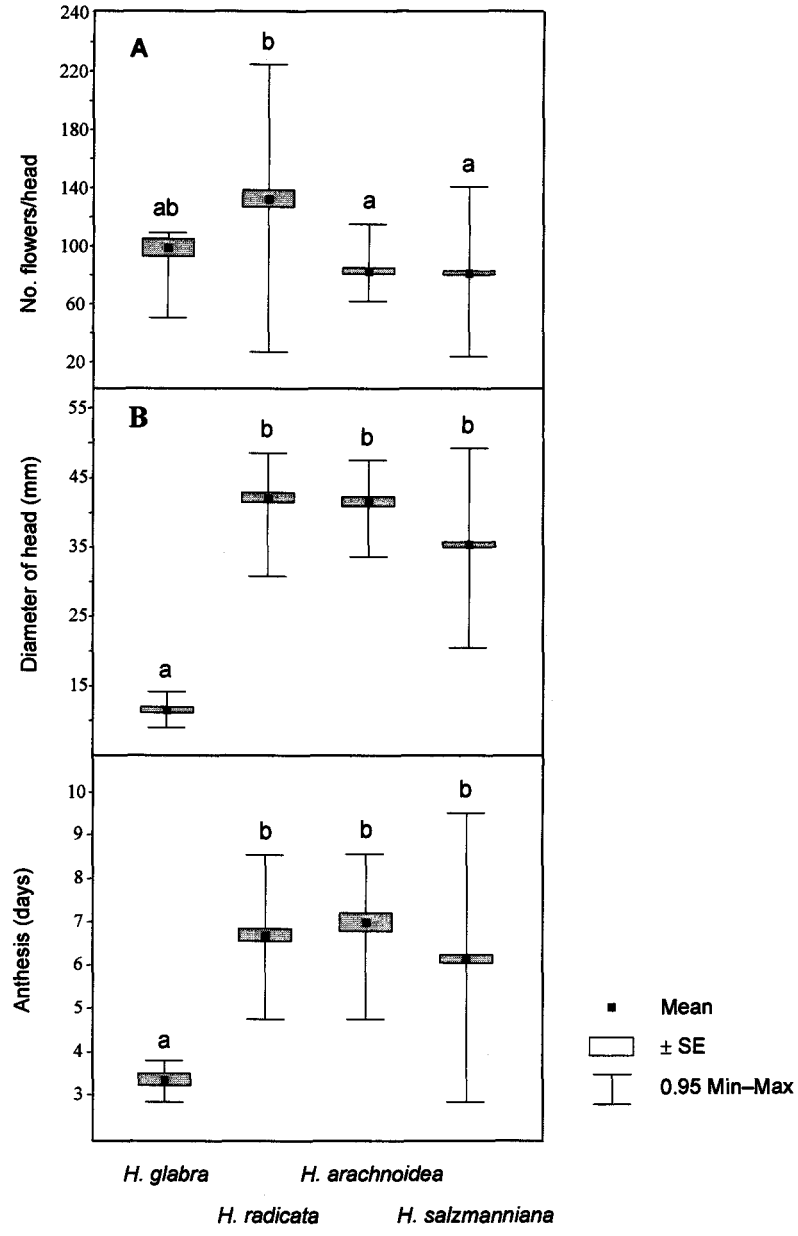

Figure 3.5. Number of flowers per head, diameter of the heads (mm) in anthesis, and duration of anthesis (days) of the heads on plants of *Hypochaeris glabra* (pop. 1), *H. radicata* (pops. 4, 6, 7, 11, 22, 23), *H. arachnoidea* (pop. 1), and *H. salzmanniana* (pops. 1–8) in a glasshouse. Plots with the same letter are not significantly different at a 5% confidence level. For population no. see Appendix.

Table 3.3. Results of the analyses of variance (general linear model) applied to number of flowers per head, diameter of head during anthesis, anthesis period of the head, and fruit to flower ratio of *Hypochaeris glabra, H. radicata, H. arachnoidea*, and *H. salzmanniana.*

Factors	SS	df	*F*	*P*
No. of flowers per head				
Individual (species, metapopulation)$_{random}$	77747.8	89	4.99	<0.0001
Metapopulation (species)	62976.9	10	7.11	<0.0001
Species	39393.2	3	17.78	<0.0001
Diameter of head during anthesis				
Individual (species, metapopulation)$_{random}$	2919.0	89	8.67	<0.0001
Metapopulation (species)	5090.5	10	15.28	<0.0001
Species	6053.9	3	74.22	<0.0001
Anthesis period of the head				
Individual (species, metapopulation)$_{random}$	134.6	89	2.78	<0.0001
Metapopulation (species)	226.1	10	14.78	<0.0001
Species	93.4	3	23.49	<0.0001
Fruit to flower ratio				
Population (species)	10.4	27	5.19	<0.0001
Species	3.8	3	17.00	<0.0001

Note: SS = sum of squares

populations of *H. salzmanniana.* In *H. salzmanniana* (Tukey-Kramer HSD test: q* = 3.34; $\alpha = 0.016$) we found that three SC populations of *H. salzmanniana* (pops. 6, 7, and 8) have a significantly lower number offlowers per head (mean ± SE = 69.30 ± 1.95) than the SI individuals from the Barbate and Bahía de Algeciras metapopulations, and of the SC populations, the individuals of the Los Algarbes population had a significantly lower number of flowers per head than the individuals from the populations at Punta Paloma and Zahara (Fig. 3.6A).

Head diameter in anthesis

Hypochaeris glabra and *H. salzmanniana* have the smallest head diameters (Fig. 3.5B). The GLM applied was significant ($F_{102,166} = 47.14$; $P < 0.0001$; $R^2 = 0.9461$) as well as all the effects considered (Table 3.3). The head diameter of *H. glabra* differed significantly from the rest of the species (Tukey-Kramer HSD test: q* = 3.36; $\alpha = 0.016$), whereas we did not find significant differences among *H. arachnoidea, H. salzmanniana,* and *H. radicata* (Fig. 3.5B).

In *Hypochaeris radicata*, mean values of head diameter of the six studied populations were not significantly different, but we did find significant differences among populations and metapopulations of *H. salzmanniana*

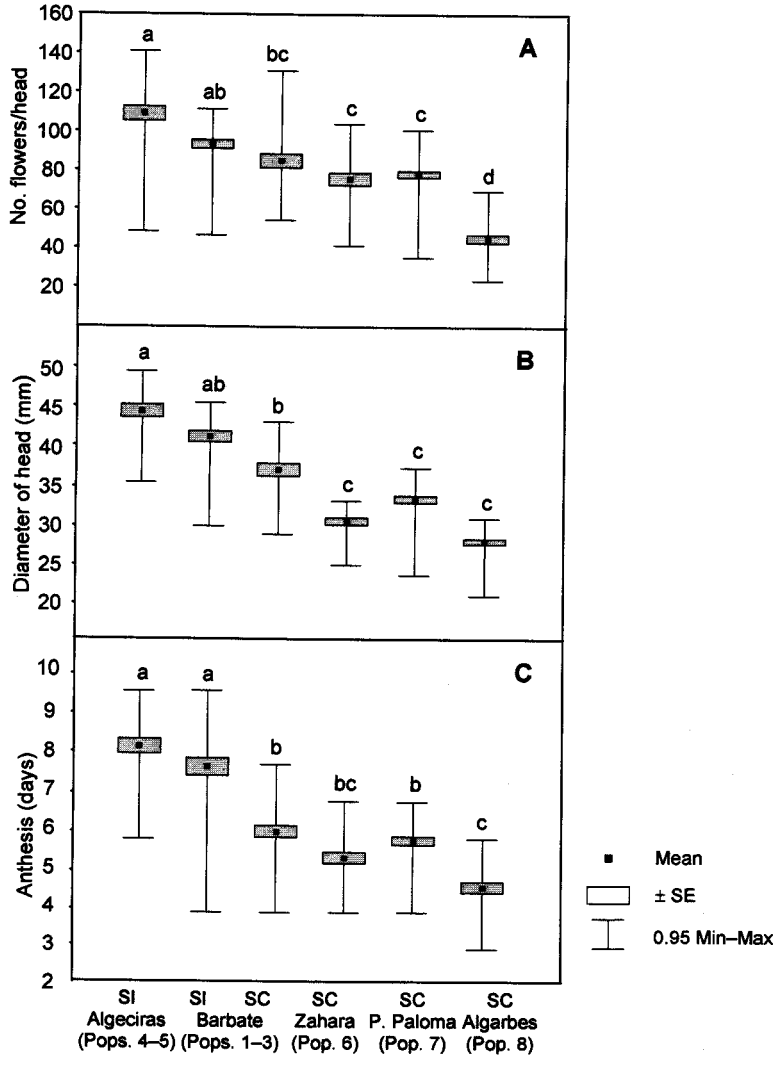

Figure 3.6. Mean ± SE of the no. of flowers/head (A), diameter of the head at anthesis (B), and duration of anthesis of the head (C) of the different populations and metapopulations, studied in glasshouse, of *Hypochaeris salzmanniana*. The Barbate metapopulation (pops. 1–3) was divided into two groups: one for SC plants and another for SI plants. Groups with the same letter cannot be considered significantly different at at a 5% confidence level.

In *Hypochaeris salzmanniana*, the means of the three SC populations (pops. 6–8; mean ± SE = 30.97 ± 0.39) were equal and significantly lower than those for individuals from the Barbate metapopulation (mean ± SE = 38.97 ± 0.63) (regardless of whether they were SC or SI) and also the Bahía de Algeciras metapopulation (Fig. 3.6B).

Length of the period of anthesis of the head

In Hypochaeris glabra, 83% of the analyzed heads were in anthesis for 3 days and the remaining 17% for 4 days. Heads of the other species, in general, last longer, up to 10 days (Fig. 3.5C). The GLM analysis applied was significant ($F_{102,166}$ = 9.84; $P < 0.0001$; $R^2 = 0.7708$), as were all the effects considered (Table 3.3). The heads of *H. glabra* had a duration of anthesis much shorter than those of *H. arachnoidea*, *H. radicata*, and *H. salzmanniana* (Tukey-Kramer HSD test: $q^* = 3.36$; $\alpha = 0.016$). In *H. radicata*, the mean duration of anthesis of the head did not differ significantly among the six populations, but we did find significant differences among the populations and metapopulations of *H. salzmanniana* (Fig. 3.6C) (Tukey-Kramer HSD test: $q^* = 4.78$; $\alpha = 0.016$).

In *Hypochaeris salzmanniana* (Tukey-Kramer HSD test: $q^* = 3.34$; $\alpha = 0.016$), the duration of anthesis of the heads in individuals from the three SC populations (pops. 6, 7, and 8) and SC individuals from the Barbate metapopulation had a significantly lower mean (mean ± SE = 5.39 ± 0.08) than the SI ones (mean ± SE = 7.79 ± 0.16) (Fig. 3.6C).

Correlation of floral parameters in Hypochaeris salzmanniana and relationship with self-compatibility

The PCA applied to the number of flowers per head, head diameter, and duration of anthesis showed that they are highly correlated (Fig. 3.7). PC1 accounted for 87.16% of the variance and showed high correlation with variation in the three features (Pearson correlation coefficients of 0.9118, 0.9691, and 0.9282, respectively); PC2 accounted for only 8.92% of the variance and was not strongly

correlated with any of the measured variables (Pearson correlation coefficients of 0.3970, –0.0619, and –0.3259, respectively).

Populations of *Hypochaeris salzmanniana* spread along the PC1 axis, with those self-compatible on the left and those self-incompatible on the right, i.e., SC populations (Zahara, Punta Paloma, and Los Algarbes) had, simultaneously, fewer flowers per head, smaller head diameter, and a briefer anthesis, whereas the SI metapopulation (Bahía de Algeciras) had the opposite pattern. Even in the Barbate

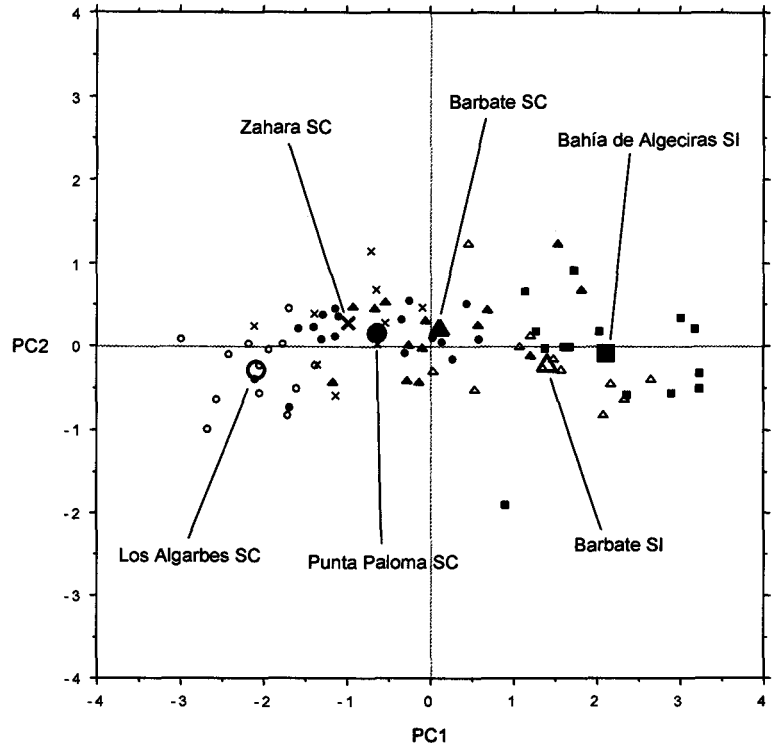

Figure 3.7. Principal component analysis applied to the number of flowers per head, head diameter, and duration of head anthesis of the individuals of *Hypochaeris salzmanniana*. PC1 explains 87.16% of the variance and is highly correlated with all three variables, whereas PC2 explains only 8.92%. Different symbols are used for a different population, metapopulation or fraction of a population (SI vs. SC in the semi-compatible Barbate metapopulation); small symbols = individuals; thick symbols = means; SI = self-incompatible; SC = self-compatible. See results of floral parameters for details.

metapopulation the SC individuals clustered closer to those SC of the other populations (Zahara, Punta Paloma and Los Algarbes), and SI individuals clustered closer to those SI of the Bahía de Algeciras metapopulation.

Fruit to flower ratio in natural populations

The reproductive success (flowers that result in fruits, or fruit to flower ratio) of all species and almost all populations is very high (generally >0.80). At the population level, the lowest fruit to flower ratios were encountered in Tiznit (pop. 6; Morocco) for *H. arachnoidea* (ratio = 0.68; Fig. 3.8A), in Doñana (pop. 7; Spain) for *H. radicata* (ratio = 0.18, Fig. 3.8B) and in the Barbate metapopulation (pops. 1–3; Spain) for *Hypochaeris salzmanniana* (ratio = 0.63 on average, Fig. 3.8C). The GLM analysis testing differences in fruit to flower ratio among species and populations was significant ($F_{22,329}$ = 7.56; $P < 0.0001$; R^2 = 0.2912; Table 3.3). The mean fruit to flower ratio in the Doñana population of *H. radicata* and that of the Tiznit population of *H. arachnoidea* were statistically different from the rest of the populations in their respective species (Tukey-Kramer HSD test: q* = 3.24, α = 0.05 for *H. radicata* and q* = 2.81, α = 0.05 for *H. arachnoidea*). In the analysis of *H. salzmanniana*, statistically significant differences (Tukey-Kramer HSD test: q* = 2.88; α = 0.05) were only found between the means of two large metapopulations at Barbate (mean ± SE = 0.66 ± 0.03) and La Mamora (mean ± SE = 0.84 ± 0.02). These two metapopulations had differences in patterns of fruit to flower ratio (Fig. 3.9): whereas in the Barbate metapopulation 39% of the plants had a ratio <0.50, in the La Mamora metapopulation only 8% of plants had a ratio <0.50.

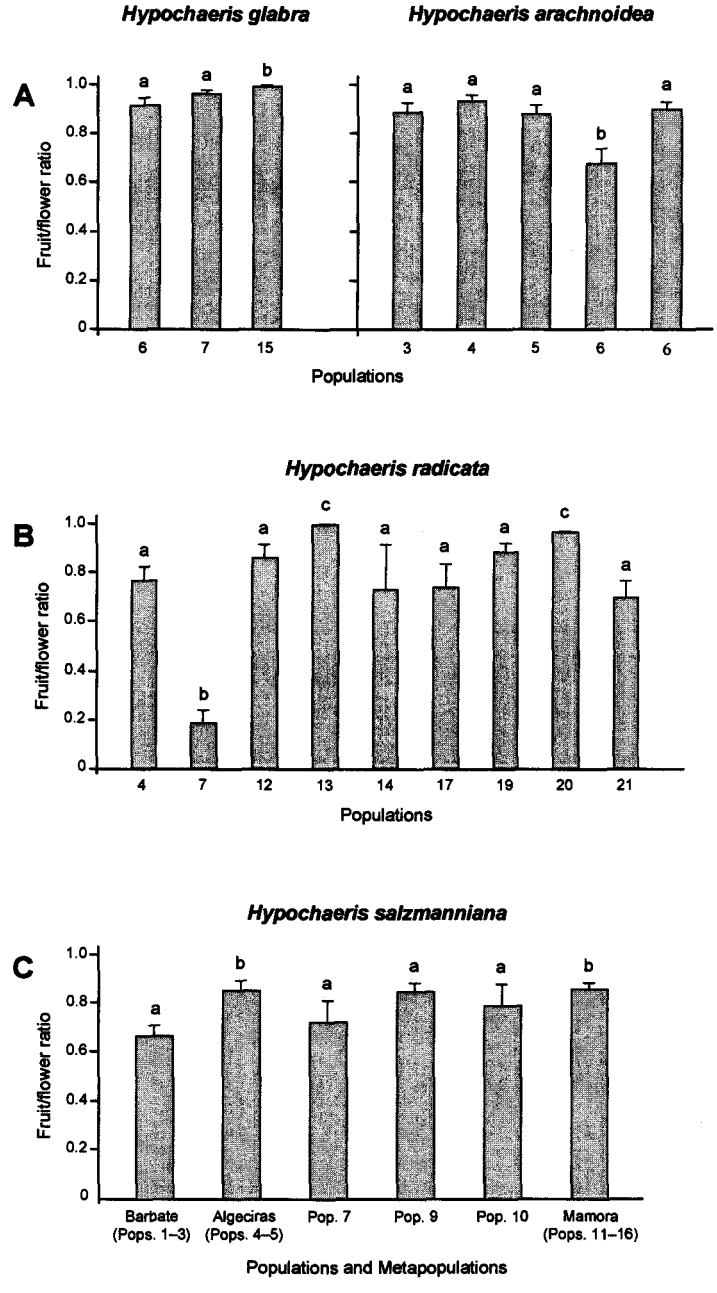

Figure 3.8. Fruit to flower ratio (fruits to flowers) of populations in the wild: (A) *Hypochaeris glabra* and *H. arachnoidea*; (B) *H. radicata*; (C) *H. salzmanniana* (mean ± SE). For population numbers, see Appendix.

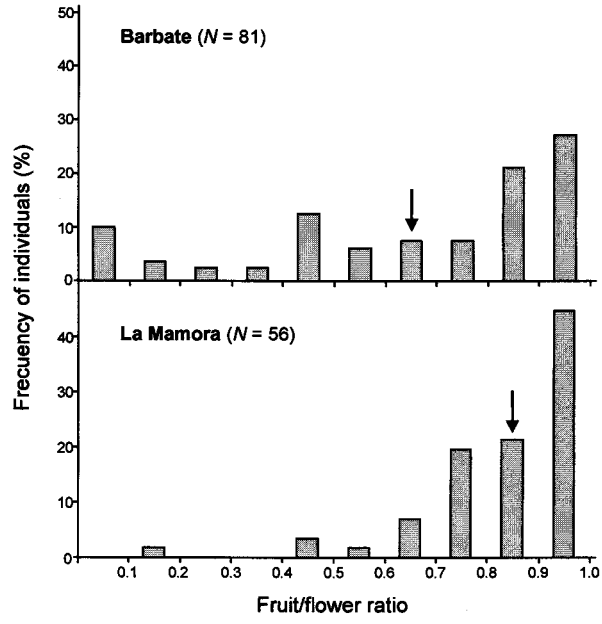

Figure 3.9. Frequency distribution (%) of the fruit to flower ratio in the wild of the plants of *Hypochaeris salzmanniana* in two metapopulations: Barbate (pops. 1–3, Spain) and La Mamora (pops. 11–16, Morocco). Arrows point at the mean values.

Discussion

Self-incompatibility vs. self-compatibility

The 21 populations of *Hypochaeris radicata* and the seven populations of *H. arachnoidea* were self-incompatible, whereas the 13 populations of *H. glabra* were self-compatible. *H. salzmanniana* had varied breeding systems.

In the diallel cross series with *H. radicata* and *H. salzmanniana*, the frequency of positive or negative results that follow the crossing direction indicates that many individuals have a common allele at the *S* locus, and these *S* alleles have a different dominance patterns in the pistil and the pollen grain. This pattern is characteristic of the sporophytic self-incompatibility mechanism (SSI) as established for other Asteraceae genera such as *Crepis* (Hughes, Babcock, 1950), *Parthenium* (Gerstel, 1950), *Senecio* (Hiscock, 2000a; Brennan *et al.*, 2002, 2003; Hiscock, Tabah,

2003), *Cosmos* (Crowe, 1954), *Carthamus* (Imrie, Knowles, 1971; Imrie *et al.*, 1972), *Rutidosis* (Young *et al.*, 2000), and *Centromadia* (Friar, LaDoux, 2002).

In *H. salzmanniana*, of the 310 individuals from 16 populations, 197 were self-incompatible and xenogamous and 113 self-compatible and potentially geitonogamous (see Fig. 3.1). All the SC individuals of *H. salzmanniana* belonged to six populations: in three of these (Zahara, pop. 6; Punta Paloma, pop. 7; and Los Algarbes, pop. 8), the samples were comprised entirely of SC individuals (12, 29, and 20 studied individuals, respectively). However, in the other three populations (Conil, pop. 1; Barbate, pop. 2; and Los Caños, pop. 3) belonging to Barbate metapopulation, we found both SC individuals (18, 22, and 13 studied individuals, respectively) and SI (included PSC) individuals (18, 31, and 6 studied individuals, respectively) with an average percentage of SC individuals of 49% for this metapopulation. These self-compatible or partially self-compatible populations are all located in the NW periphery of the distributional area of the species (Fig. 3.1), and the occurrence of autogamous populations at the edge of a distribution area is a relatively frequent situation, e.g., "Baker's Law" (Baker, 1966). However, to our knowledge, the co-occurrence of SC and SI individuals in the same population, as found in the Conil, Barbate, and Los Caños populations of *H. salzmaniana*, is a novel situation.

Diverse genetic models have been proposed to explain the loss of self-incompatibility in SSI systems. In *Ipomoea trifida* (Convolvulaceae), a species with the SSI mechanism, only one individual in a sample of 224 Central American populations was self-compatible (Kowyama *et al.*, 1994, 2000; Kakeda *et al.*, 2000). These authors concluded that self-compatibility is due to a mutation in the *S* locus (S_c), and that the S_c allele is the most recessive among all the alleles in the series (49 alleles of the *S* locus) except for S_3 ($S_c > S_3$). Obviously, a mutation at the *S* locus, which produces a weakly dominant allele in a large allelic series, as described by (Kowyama *et al.*, 1994), should not lead to the loss of self-incompatibility in more than a few individuals in the population. This might explain why SC

individuals are so infrequent in SI populations, as found here in *H. radicata* and *H. arachnoidea* or in SI populations of other species (see Introduction). Similar results were reported by Brauner and Gottlieb (1987) in *Stephanomeria exigua* subsp. *coronata* (Asteraceae, and like *Hypochaeris*, tribe Lactuceae), native from Oregon (USA). Another model for loss of SI in SSI taxa was described in *Brassica campestris* and *B. oleracea* (Brassicaceae) where some mutations at loci unlinked to the *S* locus can cause the breakdown of self-incompatibility (Hinata *et al.*, 1983; Nasrallah, 1989).

A sequence of events, can be proposed to explain the change of a population from SI to full SC: (1) a mutation of the *S* locus originating an allele (S_c), which is dominant over other wild S_i alleles in the population ($S_c > S_i$); (2) enhanced fitness of the self-compatible individuals and, particularly, the selfing-progeny (S_cS_i or S_cS_c) in comparison to the self-incompatible individuals and the outcrossed progeny; (3) a bottleneck event (founder effect or a decrease of the population size), which would reduce the *S* allele variability, with consequent diminished mate availabilty (for models, see Imrie *et al.*, 1972), and (Byers, Meagher, 1992), may favor the evolution towards self-compatibility (Baker, 1955; Charlesworth, Charlesworth, 1979). Similarly, diminished pollinator availability might permit genetic drift and selection to favor the mutated *S* allele, leading to homozygosis of this *S* allele (S_cS_c).

The results from the diallel crosses involving individuals of *H. salzmanniana* from Conil (pop. 1) provide an insight into the number of alleles at the *S* locus controlling the self-incompatibility mechanism. In the nine individuals of this diallel, we found only five different phenotypes, one represented by the four self-compatible individuals and the other four phenotypes represented by the five SI ones. Moreover, in the three semi-incompatible populations studied of Barbate metapopulation, 49% of the individuals were SC (Table 3.2). For this reason, allelic variation at the *S* locus is expected to be low, despite the fact that this metapopulation has a high number (2×10^6–3×10^6) of individuals. These

H. salzmanniana SC populations are located in one of the windiest regions in the western Mediterranean Basin, where the pollination by insects is disrupted repeatedly during the flowering period. Therefore, SI individuals that depend on insects to fructify are in a disadvantageous position compared with SC individuals of the same population, particularly because in this species nystinastic movements during anthesis can promote spontaneous geitonogamy in all individuals in the population that have lost their SI. Perhaps, as (Stebbins, 1957) stated, "flowers may resort to self-pollination when conditions become unfavorable for crossing."

Population genetic studies of *H. salzmanniana* using AFLP markers (Tremetsberger *et al.*, 2004; Ortiz *et al.*, 2007) showed that, in general, self-incompatible and semi-incompatible populations have a higher percentage of polymorphic fragments than self-compatible populations. This might indicate that self-compatible and semi-incompatible populations have suffered a bottleneck in the past that has reduced their allelic diversity, as in *Senecio squalidus* (Brennan *et al.*, 2002) or that selfing causes a loss in diversity as homozygosity increases. A low allelic diversity at the *S* locus has also been found in small populations of other Asteraceae such as *Eupatorium resinosum* (Byers, 1995).

In the self-incompatible population samples of *H. radicata*, *H. arachnoidea*, and *H. salzmanniana* some individuals developed 1–8 selfed fertile fruits (fruit to flower ratio <0.12) and were labeled as pseudo-self-compatible (PSC). Again, this is relatively frequent in species with the SSI mechanism (Byers, 1995; Hiscock, 2000a) and was also shown in *H. radicata* by Picó *et al.* (2004). Reasons for PSC may be genetic, mainly due to genes not linked to the *S* locus, or environmental, such as a temperature rise, a long photoperiod, or plant senility (Levin, 1996). Selfing may also be induced experimentally, e.g., by bud pollination or deposition of a weak saline solution on the stigma (Hiscock (Hiscock, 2000b). Some experimental studies have indicated that PSC may have been pivotal in the transition from self-incompatibility to true self-compatibility in some groups of plants, e.g., in *Phlox drummondii* and *P. cuspidata* (Bixby, Levin, 1996). In general, PSC can

play an important role in self-incompatible species because it might be the only way to generate sexual offspring when pollinator vectors are limited and/or the population allelic diversity of the *S* gene is too low (Levin, 2000).

Floral parameters associated with incompatibility

Of the four species in *Hypochaeris* section *Hypochaeris*, *H. glabra* is the only strictly self-compatible and geitonogamous species, and it has heads 3–4 times smaller in diameter and a period of anthesis half as long in comparison to the self-incompatible and semi-incompatible species. Because *H. glabra* occupies a basal position in the phylogeny of the section *Hypochaeris* (Tremetsberger *et al.*, 2005), it is possible that this species is the oldest in the section and that *H. glabra* lost its self-incompatibility a long time before the loss of self-incompatibility in some populations of *H. salzmanniana*. This could explain why all the head parameters associated to the loss of self-incompatibility are so marked in *H. glabra*.

It is widely documented that, in general, autogamous species have smaller flowers and remain in anthesis for a shorter time than allogamous congenerics (Stebbins, 1957; Grant, 1971; Levin, 2000). This has been shown in self-compatible species in *Senecio* (Gibbs *et al.*, 1975), *Anagallis* (Gibbs, Talavera, 2001), *Amsinckia* (Schoen *et al.*, 1997; Barret, 2002), *Eriotheca* (Oliveira *et al.*, 1992), *Phlox* (Bixby, Levin, 1996), and *Hypochaeris* (Parker, 1975).

Floral longevity in other species is linked to the growth of pollen tubes and ovule fertilization because these processes promote the production of growth hormones necessary for the development of fruit, but which also cause floral senescence (Crane, 1964; Biale, 1978; Stephenson, 1981). This would explain why heads of SC *Hypochaeris glabra* and heads of the self-compatible (and geitonogamous) individuals of *H. salzmanniana* do not last long as those of self-incompatible species. Effectively, this parameter is an indirect measurement of a species' capacity to self.

Of the species of *Hypochaeris* studied here, *H. salzmanniana* is the only species that has statistically significant differences in the measured parameters (num-

ber of flowers, head diameter, and length of the period of anthesis of the head) among populations and individuals within populations. In general, heads of self-incompatible individuals from all populations had more flowers, a larger diameter, and remained in anthesis longer than heads of self-compatible individuals (Fig. 3.6). Further, we found a strong correlation among these three variables (Fig. 3.7).

There is little information on variation of flower parameters within the same species that may be associated with the emergence of self-compatibility from within self-incompatible populations. In *Baldellia ranunculoides* (Alismataceae), the subspecies *ranunculoides* is self-compatible and has much smaller flowers than the self-incompatible subspecies *repens* (Vuille, 1987). Likewise, in *Euphrasia* species, French *et al.* (2005) found a negative correlation between corolla size (the area of the central lower lobe) and inbreeding coefficient (F_{IS}), which is an indirect measure of selfing. In these taxa, as in *Hypochaeris* species, high selfing rates are associated with small flower size.

Fruit to flower ratio in natural populations

In general, the fruit to flower ratio of all examined species of *Hypochaeris* is exceptionally high. This to be expected in *H. glabra* (fruit to flower ratio 0.96), a self-compatible and geitonogamous species, and is in accordance with results from other autogamous species (Gibbs, Talavera, 2001; Wiens *et al.*, 1987). However, the ratio in *H. radicata* and *H. arachnoidea*, both self-incompatible and therefore obligatorily xenogamous, is also high, in general more than 0.7 in all populations except one. Such high fruit to flower ratios in SI species are uncommon (Wiens *et al.*, 1987), but have also been found in other obligately outbreeding taxa, such as *Cistus ladanifer* (ratio = 0.95; Talavera *et al.*, 1993) and *Anagallis monelli* (ratio = 0.80; Gibbs, Talavera, 2001). In contrast, the fruit to flower ratio in the Doñana (Spain) population of *H. radicata* (ratio = 0.18) and in the Tiznit (Morocco) population of *H. arachnoidea* (ratio = 0.68) was significantly lower (see Fig. 3.7) than that of other populations of these species (futher discussed later).

Hypochaeris salzmanniana also has a fairly high fruit to flower ratio (>0.60), but if we compare the two large metapopulations, La Mamora, which is completely self-incompatible, and Barbate, which is semi-incompatible (with SI and SC plants at around 50%), whilst most plants of the former had a fairly high fruit to flower ratio (only 8% of the plants have <0.50), some 39% of the plants from Barbate metapopulation had a low ratio (<0.50) (Fig. 3.8). This last group probably comprises the self-incompatible plants of this metapopulation and the diminished fecundity of these SI plants may be due to the consequences of bottleneck events, e.g., low *S* allelic diversity and restricted mate choice or early-acting inbreeding depression due to consanguineous matings. It is possible that the low fecundity of the Tiznit population of *H. arachnoidea*, which is in the extreme SW of the distribution of this species, and the Doñana population of *H. radicata*, in which a high degree of apomictic reproduction occurs (Ortiz *et al.*, unpublished observations), is also due to a low diversity of *S* alleles in these populations.

Although the populations of *Hypochaeris* species probably originally had many *S* alleles with unequal frequencies, subsequent loss of alleles may have occurred in some populations due to genetic drift following bottleneck events (founder effects or a decrease of the population size). Once the number of *S* alleles is limited, the availability of compatible crosses will be diminished, leading to lowered fruiting success. Such a scenario has been analyzed through modeling by Byers and Meagher (1992). Indeed, populations with low seed set in SSI species of the Asteraceae seem to be due, in the majority of cases, to a low frequency of compatible phenotypes in the population as shown in *Aster furcatus* (Reinartz, Les, 1994), *Scalesia affinis* (Nielsen *et al.*, 2003), *Hymenoxis acaulis* var. *glabra* (DeMauro, 1993), and possibly also in *Hypochaeris maculata* (Wells, 1976).

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Appendix 3.1. Populations, localities, and collector numbers for analyzed populations of *Hypochaeris glabra*, *H. radicata*, *H. arachnoidea*, and *H. salzmanniana*.

***H. glabra* L.**

SPAIN—**Pop. 1**: Cáceres, Aldeanueva de la Vera; 650 m; 40°08′N/5°42′W; *Ortiz 85/01; 88/01; 89/01*. **Pop. 2**: Ciudad Real, Sierra Madrona; 700 m; 38°30′N/4°04′W; *Talavera, s.n.* **Pop. 3**: Sevilla, Cazalla; 800 m; 37°56′N/5°44′W; *Talavera et al. s.n.* **Pop. 4**: Córdoba, Zuheros; 700 m; 37°32′N/4°18′W; *Talavera, Ortiz et al. s.n.* **Pop. 5**: Sevilla, Carmona; 100 m; 37°28′N/5°37′W; *Talavera, Ortiz et al. s.n.* **Pop. 6**: Huelva, Hinojos, Pino Gordo; 70 m; 37°17′N/6°22′W; *Talavera, Ortiz et al. s.n.* **Pop. 7**: Huelva, Ayamonte, Isla Canela; 2 m; 37°11′N/7°20′W; *Talavera, Ortiz et al. s.n.* **Pop. 8**: Huelva, Punta Umbría; 5 m; 37°11′N/6°59′W; *Talavera, Ortiz et al. s.n.* **Pop. 9**: Huelva, Mazagón, Rivatehilo; 15 m; 37°10′N/6°40′W; *Talavera, Ortiz et al. s.n.* **Pop. 10**: Cádiz, Vejer-Barbate; 30 m; 36°12′N/5°56′W; *Talavera, Stuessy et al. 15.* **Pop. 11**: Cádiz, Punta Paloma; 30 m; 36°04′N/5°41′W; *Talavera, Stuessy et al. 31*. FRANCE—**Pop. 12**: Midi-Pyrénées, Toulouse; 150 m; 43°36′N/1°26′W; *Ortiz s.n.* MOROCCO—**Pop. 13**: Tanger, Cap Spartel; 10 m; 35°45′N/5°54′W; *Talavera, Stuessy et al. 13/03M.* **Pop. 14**: Tetouan, Larache; 50 m; 35°07′N/6°09′W; *Talavera, Stuessy et al. 34/03M.* **Pop. 15**: Kènitra, Forêt de La Mamora; 100 m; 34°12′N/6°16′W; *Talavera et al. 73–75; 88/03M.*

***H. radicata* L.**

SPAIN—**Pop. 1**: La Coruña, Outeiro; 140 m; 42°48′N/8°55′W; *S. Ortiz s.n.* **Pop. 2**: Huesca, Panticosa; 2500 m; 42°43′N/0°17′W; *Luceño s.n.* **Pop. 3**: Soria, Puerto de Oncala; 1500 m; 41°57′N/2°18′W; *Ortiz s.n.* **Pop. 4**: Huelva, Aracena; 700 m; 37°53′N/6°32′W; *Talavera, Ortiz et al. s.n.* **Pop. 5**: Córdoba, Sierra de Rute; 700–800 m; 37°30′N/4°15′W; *Talavera, Ortiz et al. s.n.* **Pop. 6**: Huelva, V. de los Castillejos; 200 m; 37°29′N/7°16′W; *Talavera, Ortiz et al. s.n.* **Pop. 7**: Huelva, Doñana N.P., La Algaida; 10 m; 37°03′N/6°30′W; *Talavera, Stuessy et al. s.n.* **Pop. 8**: Cádiz, Zahara-Grazalema; 800–1100 m; 36°45′N/5°23′W; *Talavera, Ortiz et al. s.n.* **Pop. 9**: Cádiz, Puerto de Gáliz; 500 m; 36°33′N/5°33′W; *Ortiz 89b/02.* **Pop. 10**: Málaga, Gaucín; 400 m; 36°31′N/5°18′W; *Talavera, Stuessy et al. 40.* **Pop. 11**: Cádiz, Vejer, Montenmedio; 190 m; 36°16′N/5°58′W; *Talavera, Ortiz et al. s.n.* GERMANY—**Pop. 12**: Lower Saxony, Hannover; 10 m; 52°23′N/9°42′E; *Ortiz s.n.* FRANCE—**Pop. 13**: Midi-Pyrénées, Toulouse; 150 m; 43°36′N/1°26′W; *Ortiz s.n.* ITALY—**Pop. 14**: Lombardia, Como, San Fermo; 280 m; 45°46′N/9°06′E; *Ortiz s.n.* SICILY—**Pop. 15**: Palermo, La Pizzuta; 1020 m; 38°07′N/13°21′W; *Castroviejo 5692.* MOROCCO—**Pop. 16**: Tanger, Cap Spartel; 26 m; 35°45′N/5°54′W; *Talavera, Stuessy et al. 1/03M.* **Pop. 17**: Tetouan, Larache; 20–30 m; 35°07′N/6°09′W; *Talavera, Stuessy et al. 38/03M.* **Pop. 18**: Tetouan, Talasemtam; 1000 m; 35°04′N/5°09′W; *Talavera, Ortiz et al. 464/03M.* **Pop. 19**: Tetouan, c. Bab-Taza; 1 km W; 730 m; 35°03′N/5°12′W; *Talavera, Ortiz et al. 454/03M.* **Pop. 20**: Tetouan, c. Bab-Berred; 12 km E; 1160 m; 34°59′N/4°52′W; *Talavera, Ortiz et al. 538/03M.* **Pop. 21**: Meknés, Col du Zad; 2100–2200 m; 32°57′N/5°08′W;

Talavera, Ortiz et al. 709/03M. COLOMBIA: **Pop. 22**: Bogotá, Autopista Norte; 2600 m; 4°38´N/74°05´W; *Fernández-Alonso 19552.* MEXICO—**Pop. 23**: México, Toluca; 2600–3000 m; 19°17´N/99°40´W; *Guevara s.n.* BRASIL—**Pop. 24**: São Paulo, Guarulhos Airport; 680 m; 23°28´S/46°31´W; *Talavera 30/01.* CHILE—**Pop. 25**: VIII Región, Talcahuano, Caleta Lenga; 10 m; 36°43´S/73°07´W; *Stuessy et al. 18093.*

***H. arachnoidea* Poir.**

MOROCCO—**Pop. 1**: Nador, Ras Kebdana, Cap de l'Eau; 35°08´N/2°35´W; *García and López NL 1267.* **Pop. 2**: Taza, Tsoul, c. Taza; 12 km N; 500 m; 34°19´N/3°57´W; *Talavera, Ortiz et al. 585/03M.* **Pop. 3**: Safi, Essaouira; 10–100 m; 31°38´N/9°39´W; *Talavera et al. 92–98/03M.* **Pop. 4**: Agadir, c. Tizi-n-Test; 25 km S; 950 m; 30°47´N/8°23´W; *Talavera, Stuessy et al. 181/03M.* **Pop. 5**: Agadir, c. Biougra; 10 km SE; 950 m; 30°06´N/9°13´W; *Talavera, Stuessy et al. 138/03M.* **Pop. 6**: Agadir, c. Tiznit; 15 km N; 160 m; 29°49´N/9°37´W; *Talavera, Stuessy et al. 180/03M.* **Pop. 7**: Agadir, c. Tafraoute; 33 km NW; 1400–1600 m; 29°48´N/9°13´W; *Talavera, Stuessy et al. 153/03M.*

***H. salzmanniana* DC.**

SPAIN—Cádiz: **Pop. 1**: Barbate, Conil-El Palmar; 10 m. 36°13´N/6°04´W; *Talavera, Stuessy et al. 5.1–5.4.* **Pop. 2**: Barbate, Vejer-Barbate; 50 m; 36°12´N/5°56´W; *Talavera, Stuessy et al. 14.* **Pop. 3**: Barbate, Los Caños de Meca; 10 m; 36°11´N/6°01´W; *Talavera, Stuessy et al. 24.* **Pop. 4**: Algeciras, Palmones; 10 m; 36°10´N/5°25´W; *Talavera, Stuessy et al. 33.* **Pop. 5**: Algeciras, La Línea; 10 m; 36°09´N/5°20´W; *Talavera, Stuessy et al. 35.* **Pop. 6**: Tarifa, Zahara de los Atunes; 10 m; 36°08´N/5°51´W; *Talavera and Ortiz 1/03.* **Pop. 7**: Tarifa, Punta Paloma; 50 m; 36°04´N/5°41´W; *Talavera and Ortiz 3/03.* **Pop. 8**: Tarifa, Los Algarbes; 80 m; 36°04´N/5°41´W; *Talavera and Ortiz 2/03.* MOROCCO—**Pop. 9**: Tanger, c. Tanger, Oued Hachef; 10 m; 35°35´N/5°59´W; *Talavera, Stuessy et al. 18/03M.* **Pop. 10**: Tanger, Asilah; 10 m; 35°29´N/6°01´W; *Talavera, Stuessy et al. 20/03M.* **Pop. 11**: Tetouan, Larache; 20 m; 35°07´N/6°09´W; *Talavera, Stuessy et al. 31/03M.* **Pop. 12**: Tetouan, c. Barga; 35°04´N/6°09´W; *Talavera, Stuessy et al. 44/03M.* **Pop. 13**: Kènitra, Moulay-Bousselham; 10 m; 34°43´N/6°15´W; *Talavera, Stuessy et al. 51/03M.* **Pop. 14**: Kènitra, Mehdiya; 16 m; 34°15´N/6°39´W; *Talavera, Stuessy et al. 57/03M.* **Pop. 15**: Kènitra, Forêt de La Mamora; 37 m; 34°15´N/6°19´W; *Talavera, Stuessy et al. 71/03M.* **Pop. 16**: Kènitra, c. Kènitra; 46 m; 34°13´N/6°35´W; *Talavera, Stuessy et al. 53/03M.*

4 Population structure of *Hypochaeris salzmanniana* DC. (Asteraceae), an endemic species to the Atlantic coast on both sides of the Strait of Gibraltar, in relation to Quaternary sea level changes

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Abstract

To detect potential Pleistocene refugia and colonization routes along the Atlantic coast, we analysed Amplified Fragment Length Polymorphism (AFLPs) in 140 individuals from 14 populations of *Hypochaeris salzmanniana* (Asteraceae), an annual species endemic to the W Mediterranean and NW African coastal areas. Samples covered the total distributional range of the species, with 8 populations in SW Spain and 6 populations in NW Morocco. Using nine primer combinations, we obtained 546 fragments in *H. salzmanniana* and its sister species *H. arachnoidea* of which 487 (89.2%) were polymorphic. The neighbour-joining tree shows that the populations S of the Loukos river in Morocco are clearly differentiated, having more polymorphic, private, and rare fragments, and higher genetic diversity, than all the other populations. The southernmost populations in Morocco, S of the river Sebou, form a large panmictic population. They are probably the oldest populations that have been relatively unaffected by stochastic processes resulting from Pleistocene glaciations. Northward migration of populations during this period may have resulted in loss of genetic diversity in specific regions, perhaps due to bottlenecks caused by rise in sea level during interglacial periods, and, in some cases, by changes in the breeding system.

Introduction

At the end of the Miocene, around 5.33 Ma BP, the Strait of Gibraltar opened, allowing contact between the Atlantic Ocean and the Mediterranean basin, and initiating the end of the "Messinian Salinity crisis" (Krijgsman *et al.* 1999). This event caused the separation of the Iberian Peninsula and African continental areas.

Subsequently, the climatic oscillations caused by Quaternary glaciations and their effects on sea levels, which were lower during glacial periods and raised during interglacial periods, are likely to have had a major influence on the vegetation on either side of the Strait of Gibraltar (Cheddadi *et al.* 2005). During these glacial events, the sea level was approximately 120–150 m lower than at present (Pou 1989; Yokohama *et al.* 2000) thus revealing islands, which could have facilitated biological communication between Africa and Europe in the extreme W Mediterranean area, near the Strait of Gibraltar (Collina-Girard 2001). During interglacial periods, the sea level rose again, submerging the islands and Europe and Africa were once again physically isolated.

Within this dynamic situation, lower sea levels would have favoured the expansion of populations of species into the newly exposed coastland areas and also the migration of populations between Africa and Europe, and vice-versa. During interglacial, however, the rise in sea level must have caused the extinction of some populations and a reduction in size of others as a result of the flooding of land previously colonized. For coastal species, river estuaries and the flanks of mountains may have acted as refugial areas during interglacial periods. The Quaternary glacial episodes are likely to have modelled the genetic structure and distribution of present-day coastal species.

Various studies have used diverse molecular genetic markers (ITS, *mt*DNA, *cp*DNA-RFLPs, allozymes, and microsatellites) to look at phylogeographic patterns in species that occur on both sides of the Strait of Gibraltar. Most of these have been with animal groups, such as amphibians (Busack 1986; García-París & Jockusch 1999; Fromhage *et al.* 2004; Martínez-Solano *et al.* 2004; Veith *et al.*

2004), reptiles (Harris *et al.* 2002; Carranza *et al.* 2006), scorpions (Gantenbein & Largiadèr 2003; Gantenbein 2004), and beetles (Palmer & Cambefort 2000; Sanmartín 2003). All these groups date back late in Tertiary and, consequently, the Betic crisis at c. 16–14 Ma BP as well as the Messinian salinity crisis at c. 5.59–5.33 Ma BP, which provided a land bridge between Iberia and North Africa, are invoked as possibilities for faunal exchange (Veith *et al.* 2004; Palmer & Cambefort 2000; Sanmartín 2003). The fragmentation of the Betic region at c. 12–10 Ma BP, the opening of the Betic Strait at c. 10–8 Ma BP, as well as the opening of the Strait of Gibraltar at c. 5.33 Ma BP are supposed to have led to vicariance speciation in these genera (Busack 1986; García-París & Jockusch 1999; Fromhage *et al.* 2004; Martínez-Solano *et al.* 2004; Gantenbein & Largiadèr 2003; Gantenbein 2004). Moreover, recent dispersal across the Strait of Gibraltar via rafting on vegetation has also been suggested (Veith *et al.* 2004; Harris *et al.* 2002; Carranza *et al.* 2006). Few plant species occurring on both sides of the Strait of Gibraltar have been investigated, including some woody taxa such as *Frangula alnus* (Hampe *et al.* 2003), *Quercus ilex* (Lumaret *et al.* 2002; Petit *et al.* 2005), and *Abies pinsapo* (García *et al.* 1993), and also various coastal and semi coastal herbacous taxa, such as *Cakile maritima* (Clausing *et al.* 2000; Kadereit *et al.* 2005), *Saxifraga globulifera* (Vargas *et al.* 1999) and *Androcymbium gramineum* (Caujapé-Castells & Jansen 2003). In the slowly evolving trees, the current genetic structure is thought to reflect population divergence that predates the onset of the Mediterranean climate in the Pliocene (Petit *et al.* 2005). The Strait of Gibraltar is an effective barrier to gene flow in these taxa (Hampe *et al.* 2003; Lumaret *et al.* 2002; Petit *et al.* 2005). In the Betic-Rifian *Saxifraga globulifera* (Saxifragaceae), the N African populations are likewise isolated from the Iberian ones (Vargas *et al.* 1999).

In this study we have used Amplified Fragment Length Polymorphism (AFLP) analyses to study the phylogeography of *Hypochaeris salzmanniana* (Lactuceae, Asteraceae), a predominantly self-incompatible species endemic to the maritime

sands of the Atlantic coast on either side of the Strait of Gibraltar (Ortiz *et al.* 2006). Using a molecular clock, Tremetsberger *et al.* (2005) estimated the divergence between *Hypochaeris* and *Leontodon*, the most closely related genus, at 6.6 Ma BP (95% C.I. due to uncertainty of branch lengths = 4.0–9.3 Ma BP); and for sect. *Hypochaeris* (Tremetsberger, unpublished data) at 0.8 Ma BP (95% C.I. due to uncertainty of branch lengths = 0.5–1.4 Ma BP). Thus, unlike the studied species mentioned above, the evolutionary expansion of *H. salzmanniana* seems to have occurred during the latest phase of the glacial period. However, due to limitations of the dating method [e.g., transfer of age estimate obtained by molecular clock calculation on an independent tree; see Tremetsberger *et al.* (2005) for a discussion], these dates are tentative estimates.

The same geographical distribution as in *H. salzmanniana* is also found in other Lactuceae species, such as *Reichardia gaditana* Willk., *Crepis erythia* Pau, and *Hedypnois arenaria* (Schousboe) DC., as well as many other Asteraceae coastal species such as *Carduus myriacanthus* DC., *C. meonanthus* Hoffmanns. & Link, and *Onopordum dissectum* Murb. etc. No matter whether of Pliocene or Pleistocene age, all these species certainly have been affected by glacial and interglacial events during the last phase of the Pleistocene. To date there is no knowledge about the patterns of genetic structure in species having this particular distributional range.

AFLPs have some advantages over other methods for the analysis of DNA polymorphisms, since the technique requires no prior sequence knowledge, is highly reliable, gives a large number of genomic fragments, and requires only minimal development time (Vos *et al.* 1995; Mueller & Wolfenbarger 1999). The major disadvantage is that they are dominant markers, therefore precluding any inference of heterozygosity (Mueller & Wolfenbarger 1999). AFLPs have been established as useful genetic markers in studies of biogeographic patterns that have been shaped by Quaternary glaciations in European alpine species such as *Erinus alpinus* (Stehlik *et al.* 2002), *Phyteuma globulariifolium* (Schönswetter *et al.*

2002) and *Saponaria pumila* (Tribsch *et al.* 2002); and with three S American *Hypochaeris* species: *H. tenuifolia, H. palustris,* and *H. acaulis* (Stuessy *et al.* 2003; Tremetsberger *et al.* 2003a, b; Muellner *et al.* 2005). However, none of these studies has focused on the effects of these glaciations on the phylogeography of coastal species.

We focus on the following questions: (1) Did any areas serve as refugia for ancient populations of this species? Because of their long-lasting, uninterrupted existence at the same locality, we expect refugial populations to harbour high allelic richness and possibly also higher genetic diversity than more recently established populations (e.g., Widmer & Lexer 2001). (2) Are self-compatible populations of *H. salzmanniana* genetically depauperate in comparison to self-incompatible populations? (3) Have coastal features such as river estuaries, mountain spurs, and especially the Strait of Gibraltar functioned as significant barriers to gene flow between populations of this species? If the Strait of Gibraltar was an effective barrier prohibiting gene flow in *H. salzmanniana* as has been postulated for several animal and plant groups (see above), we would expect a strong genetic differentiation between populations in Spain and Morocco. If, on the other hand, river estuaries or mountain spurs have been more effective barriers than the Strait, we would expect to find the strongest genetic differentiation between regions separated by these barriers. We will test the genetic differentiation found in groupings defined according to these hypotheses through Analysis of Molecular Variance (AMOVA).

Materials and methods

Study species

Hypochaeris salzmanniana is a species of maritime sands or adjacent woodlands with a restricted distribution along the coast of SW Spain (from Conil to Algeciras) and the Atlantic coast of Morocco from Tanger to Kenitra (Fig. 4.1A). *H. salzmanniana* is grouped in sect *Hypochaeris*. Recent studies on the phylogeny

and genetic structure of the four species comprising the monophyletic *Hypochaeris* sect. *Hypochaeris* (*H. glabra*, *H. radicata*, *H. salzmanniana*, and *H. arachnoidea*; Tremetsberger *et al.* 2004, 2005) demonstrate that *H. salzmanniana* and *H. arachnoidea* are sister species, and this clade is sister to *H. radicata*. Both *H. salzmanniana* and *H. arachnoidea* are annual herbs with $2n = 8$ chromosomes with allopatric distributions and different habitats. *H. arachnoidea* grows in dry pastures from 100 to 1800 m a.s.l. in the mountains of Morocco and Algeria. Both species are interfertile and readily hybridize under experimental conditions (Ortiz, unpublished observations), but natural hybrids have never been found.

A first approach to the population structure of *H. salzmanniana* in Spain (Tremetsberger *et al.* 2004) showed that: three populations located to the north of the river Barbate (Conil, Barbate and Los Caños) form a panmictic group that is different from those to the south of this river (Zahara, Punta Paloma and Los Algarbes) and also from two populations in Algeciras Bay (Palmones and La Línea).

Subsequently, breeding system studies (Ortiz *et al.* 2006) on *H. salzmanniana* plants sampled from the same Spanish populations, and also from populations in Morocco, established that all plants are self-incompatible (SI) with the exception of the populations south of the river Barbate, which consist entirely of self-compatible (SC) plants, and those north of this river, where the populations are semi-compatible with SI and SC individuals co-occurring.

Study populations

We sampled 14 populations (Fig. 4.1A, Table 4.1) from throughout the distributional range of *Hypochaeris salzmanniana* during the spring of 2001 and 2003. Fresh leaves from 10 individuals per population were dried in silica gel to give a total of 140 samples. These samples included eight populations from Spain [three populations from the north side of Barbate river (NB), three from the south of this river (SB) and two from Algeciras Bay (AB)]; and six populations from

Fig. 4.4.A: Map of the Atlantic coast from the south of Spain, Strait of Gibraltar, and NW Morocco. The studied populations of *H. salzmanniana* are indicated by the numbered circles. The land elevation is shown in metres (75, 150, 225, 300 and 450 m) as well as the sea depth (100 and 200 m). Map Modified from the DMAA Center (compiled 1965, revised 1991) Morocco, TPC G-1D, St. Louis, Missouri, U.S.A.

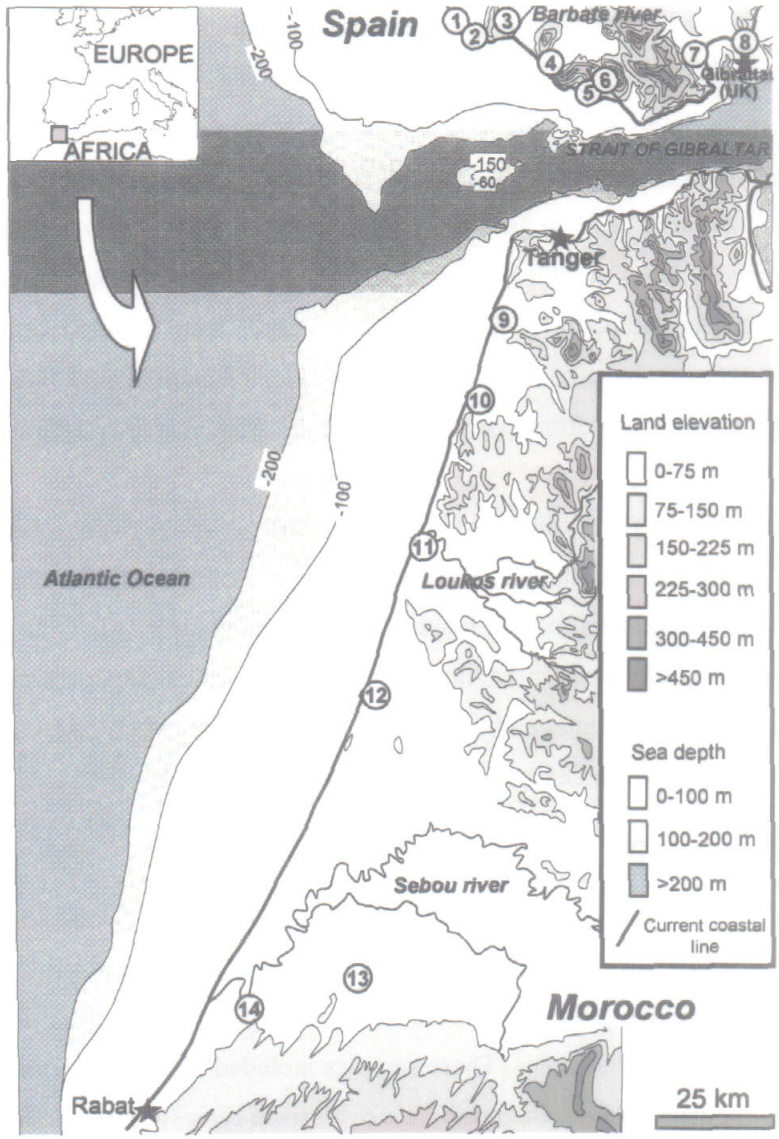
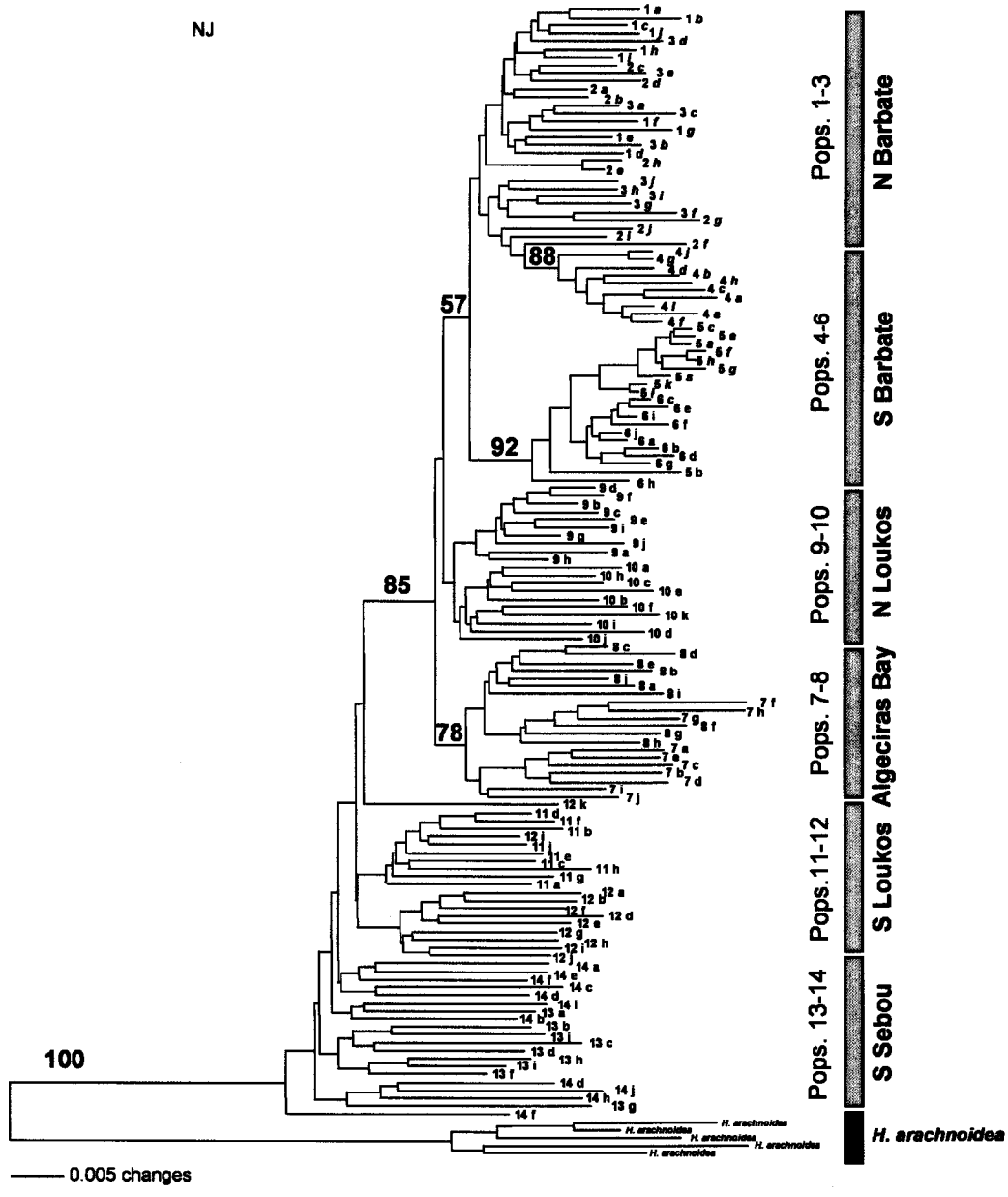


Fig. 4.1.B: NJ dendrogram of the 140 individuals analysed of *Hypochaeris salzmanniana*, rooted with *H. arachnoidea*, based on Nei & Li's genetic distance. Bootstrap values based on 1000 permutations, if higher than 50% are indicated at each node. For details of populations, see Table 4.1.



Morocco [two populations from the north of the Loukos river (NL), two populations from the south of this river (SL), and two from the south side of Sebou river (SS)]. These samples include all known populations of this species with the exception of one from the south of the Loukos river and one from the south of the Sebou river. For purposes of discussion, we initially considered these population samples in six geographical areas: three in Spain (NB, SB and AB), and three in Morocco (NL, SL and SS). Population size was estimated through direct censuses when the number of individuals was lower than 100 individuals and, if the number of individuals was higher, through the estimation of the density of individuals in transects and the extrapolation to the total surface occupied.

To root the tree we have included as an outgroup in the analysis five individuals of *H. arachnoidea* (the species closest to *H. salzmanniana*) sampled from a population at Cap de l'Eau, near Nador (Morocco).

Vouchers of all sampled populations are deposited in the Herbarium of the University of Seville (SEV, Spain) and University of Vienna (WU, Austria).

DNA isolation and AFLP analysis

Total genomic DNA was extracted from dry leaf material following a modified CTAB-protocol (Doyle & Doyle 1987) and the quality of the extracted DNA was checked on 1% TAE-agarose gels as in Tremetsberger *et al.* (2004). The average DNA concentration was estimated photometrically (UV 160 A Spectrophotometer, Shimadzu). The AFLP procedure followed established protocols (Vos *et al.* 1995; PE Applied Biosystems 1996) with modifications (Tremetsberger *et al.* 2004). We used nine primer combinations, six of which were used by Tremetsberger *et al.* (2004) for the Spanish populations, and three additional primer combinations were used in this study for better resolution. The nine final primer combinations for the selective PCR were: *MseI*-CTCG/*EcoRI*-ATC (Fam), *MseI*-CAC/*EcoRI*-ACG (Hex), *MseI*-CTA/*EcoRI*-ACC (Ned), *MseI*-CTG/*EcoRI*-ACA (Fam), *MseI*-CTC/*EcoRI*-AGG (Hex), *MseI*-CTGA/*EcoRI*-AAC (Ned), *MseI*-CAC/*EcoRI*-ACT

Table 4.1. Region, population, coordinates, collectors, population size (*N*), total number of fragments per population (Frag $_{tot}$), % polymorphic fragments ($Frag_{poly}$), private fragments ($Frag_{priv}$), rare fragments (DW), Shannon diversity (H_{SH}), average gene diversity (H_D), and distance among populations (F_{ST}) of *Hypochaeris salzmanniana* populations sampled for AFLP. (10 individuals for overall population, (see Materials and Methods).

Populations	Coordinates (N/W)	*Collectors no.*	*N*	$Frag_{tot}$	$Frag_{poly}$	$Frag_{priv}$	DW	H_{SH}	H_D	F_{ST}
SPAIN, Cádiz										
N BARBATE RIVER (NB)										*0.083***
Pop. 1. Conil-El Palmar, 10 m	*36°13′/6°04′*	ST, TS et al. 5/02	*> 10^6*	*256*	*51.17*	*6*	13.31	32.65	*0.0967*	
Pop. 2. Vejer-Barbate, 50 m	36°12′/5°56′	*ST, TS et al. 14/02*	100–500	265	51.32	4	10.71	36.88	0.1028	
Pop. 3. Caños de Meca, 10 m	36°11′/6°01′	*ST, TS et al. 24/02*	100–500	271	52.40	9	14.68	39.40	0.1097	
S BARBATE RIVER (SB)										0.530***
Pop. 4. Zahara, 10 m	36°08′/5°51′	*ST & MAO 1/03*	100–500	209	40.67	1	3.37	21.61	0.0648	
Pop. 5. Punta Paloma, 50 m	36°04′/5°41′	*ST et al. 32/02; 3/03*	< 50	206	31.55	2	5.81	16.10	0.0426	
Pop. 6. Los Algarbes, 80 m	36°04′/5°41′	*ST & MAO 2/03*	2000–20000	208	34.62	1	4.43	16.56	0.0461	
ALGECIRAS BAY (AB)										0.119***
Pop. 7. Palmones, 10 m	36°10′/5°25′	*ST, TS et al. 33/02*	600–1000	262	59.16	3	12.98	43.37	0.1231	
Pop. 8. La Línea, 10 m	36°09′/5°20′	*ST, TS et al. 35/02*	100–500	250	56.40	4	10.18	36.41	0.1053	
MOROCCO										
N LOUKOS RIVER (NL)										0.214***
Pop. 9. c. Tanger, 10 m	35°35′/5°59′	*ST, TS et al. 18/03M*	< 50	227	42.29	2	5.16	24.00	0.0702	
Pop. 10. Asilah, 10 m	35°29′/6°01′	*ST, TS et al. 20/03M*	600–1000	267	54.31	4	8.87	37.56	0.1064	
S LOUKOS RIVER (SL)										0.156***
Pop. 11. Larache, 20 m	35°07′/6°09′	*ST, TS et al. 31/03M*	100–500	286	55.24	13	24.15	41.61	0.1206	
Pop. 12. M.–Bousselham, 10 m	34°43′/6°15′	*ST, TS et al. 51/03M*	100–500	301	59.47	16	25.52	48.39	0.1365	
S SEBOU RIVER (SS)										0.057***
Pop. 13. La Mamora, 37 m	34°15′/6°19′	*ST, TS et al. 71/03M*	2000–20000	322	68.32	18	32.43	57.19	0.1590	
Pop. 14. c. Kènitra, 46 m	34°13′/6°35′	*ST, TS et al. 53/03M*	> 10^7	340	69.41	26	46.40	62.89	0.1675	

(Fam), *MseI*-CTC/*EcoRI*-ATC (Hex) and *MseI*-CTG/*EcoRI*-AAC (Ned). The fluorescence-labelled selective amplification products were run on an automated sequencer (ABI 377, Perkin Elmer). Raw data were scored and exported as a presence/absence matrix using ABI Prism GeneScan® Analysis Software 2.1 (PE Applied Biosystems) and Genographer (version 1.6.0, © Montana State University 2001; available at http://hordeum.oscs.montana.edu/genographer/).

The AFLP datamatrix was submitted to TreeBase (study accession no. SN2812).

Statistical analyses

The presence/absence matrix originated with the nine primer combinations was imported into PAUP* (version 4.0b10; Sunderland, MA: Sinauer Associates). To represent overall genetic relationships among all individuals analysed of *H. salzmanniana*, we constructed a dendrogram applying the Neighbor-Joining method (NJ) in conjunction with Nei and Li's (1979) genetic distances with the sister species *H. arachnoidea* used to root the tree. Support for each node was tested by 1000 bootstrap replicates. The UPGMA distance and Jaccard algorithms were also applied to the data matrix and resulted in a very similar dendrogram (data not shown).

As a measure of within-population diversity, we assessed the total number of fragments per population ($Frag_{tot}$), the percentage of polymorphic fragments ($Frag_{poly}$), as well as the number of private fragments ($Frag_{priv}$; confined to one population or metapopulation) for all populations of *H. salzmanniana*. We also assessed the number of fragments that were shared exclusively between pairs or groups of populations (shared private fragments), and we looked for "rare fragments", i.e. fragments that were found in less than 10% of the individuals within a population (Stehlik *et al.* 2002). The latter were used to calculate another index of diversity DW ("frequency-down-weighted marker values"). For each population, the number of occurrences of each AFLP marker in that population

was divided by the number of occurrences of that particular marker in the total dataset. Finally these values were summed (Schönswetter & Tribsch 2005).

As another measure of genetic variability, we also calculated the average gene diversity (H_D; Arlequin version 3.01; Excoffier, Laval, Schneider 2005): $H_D=1-\sum x_i^2$, where x_i is the population frequency of each "allele" (1 or 0) at locus *i*. The average gene diversity is the average of this quantity across all loci (Lowe *et al.* 2004). H_D differences among metapopulations were assessed with a general linear model (GLM; SAS Institute Inc. 1989) considering the metapopulation as a fixed effect. The Shannon diversity index is widely used in Ecology and applied also as a measure of genetic diversity: it was calculated as $H_{Sh} = -\sum[p_i.\ln(p_i)]$, where p_i is the relative frequency of the i^{th} fragment in a population (FAMD 1.02, Schlüter & Harris 2006).

A statistical approach to genetic differentiation was the analysis of molecular variance (AMOVA; Arlequin version 3.01; Excoffier, Laval, Schneider 2005) that we undertook with three different groupings of the *H. salzmanniana* populations. The first grouping (a) has two hierarchical levels and describes differentiation among all populations of the species. Groupings (b) and (c) have three hierarchical levels and each describes differentiation among two geographical areas: between Spanish populations N of the Strait of Gibraltar (NB, SB, AB) and Moroccan populations S of the Strait (NL, SL, SS; grouping b); and between Spanish and Moroccan populations N of the Loukos river (NB, SB, AB, NL) and the extension of the Rif mountain in N Morocco and Moroccan populations S of this geographical barrier (SL, SS; grouping c). In this way, we aimed to test the two different hypotheses (groupings b and c) of which geographical barrier had the largest effect on genetic differentiation in *H. salzmanniana*. The AMOVA-derived fixation index F_{ST} (Arlequin version 3.01; Excoffier, Laval, Schneider 2005) describes the reduction in heterozygosity within populations relative to the total population (Wright 1951) and is an indirect approach to estimate gene flow. The confidence interval of the F_{ST} values was determined through bootstrapping

(20000 replicates) as implemented in Arlequin version 3.01 (Excoffier, Laval, Schneider 2005).

To evaluate the correlation between F_{ST} and geographic distance between populations in *H. salzmanniana*, we used two-tailed Mantel tests based on Spearman correlations (on 10^6 random permutations). We also tested whether there was a correlation between the average genetic diversity and the population size using a Spearman non-parametric correlation. For basic statistical analyses (GLM and Spearman correlation) we used JMP® 4.0.1 (SAS Institute Inc. 1989) except for Mantel tests where we used XLSTAT-Pro 7.5.3 (© Addinsoft).

Results

Population level

The nine primer combinations employed with samples from 14 populations of *H. salzmanniana* and its sister species *H. arachnoidea* generated a total of 546 fragments, ranging from 60 to 500 bp, with an average of 60.7 fragments per primer combination, of which 487 (89.2%) AFLP markers were polymorphic. The number of fragments for each primer combination (with percentage of polymorphisms within parenthesis), were: *MseI*-CTCG/*EcoRI*-ATC: 52 (90.4%), *MseI*-CAC/*EcoRI*-ACG: 66 (93.9%), *MseI*-CTA/*EcoRI*-ACC: 93 (91.4%), *MseI*-CTG/*EcoRI*-ACA: 59 (89.8%), *MseI*-CTC/*EcoRI*-AGG: 72 (93.1%), *MseI*-CTGA/*EcoRI*-AAC: 62 (95.2%), *MseI*-CAC/*EcoRI*-ACT: 51 (72.5%), *MseI*-CTC/*EcoRI*-ATC: 46 (87.0%) and *MseI*-CTG/*EcoRI*-AAC: 45 (82.2%). This number of fragments was sufficient to distinguish all individuals as separate phenotypes.

Our analysis of AFLPs for the eight Spanish populations effectively duplicated those made by Tremetsberger *et al.* (2004), i.e. we did a new analyses of AFLP fragments from the same samples from the same populations. Our analyses were made with three extra primer combinations in addition to those used in this previous study. The results show high congruence with, and replicate results for

part of the population system of *Hypochaeris salzmanniana* and provide confidence in the AFLP parameters employed in these studies.

H. salzmanniana shows a very well supported large cluster (Fig. 4.1B) with 85% BS, that separates four populations situated to the south of the river Loukos (SL and SS, from Larache to Kenitra), from all of the rest. The remaining populations form three groups: the first, which is well supported with 78% BS, comprises the populations from Algeciras Bay (AB, 7–8) in Spain; the second consists of a not very well supported cluster with the populations from the north of the Loukos river (NL, 9–10) in Morocco; and the third, weakly supported at 57% BS, includes the populations from the Atlantic coast of Spain. Within the latter, one can distinguish an independent branch with 92% BS that includes the populations at Punta Paloma (5) and Los Algarbes (6), whereas the population at Zahara (4), although well supported with 88% BS, is immersed in the group formed by the three populations from the north of Barbate (NB, 1–3).

The population at Kenitra (14) presents the largest genetic diversity for all parameters, whilst the population at Punta Paloma (5) is the least diverse. The most diverse population at Kenitra is also the largest population ($> 10^7$ individuals), and that at Punta Paloma is a very small population (less than 50 individuals) (Table 4.1). However, over all populations there is no correlation between size and average genetic diversity (Spearman $\rho = 0.4045$, $P = 0.1514$, n = 14).

The Mantel tests, which compared the pairwise distances between populations (in direct line in km) with their respective F_{ST} values, indicated that there is no correlation between these parameters ($r = 0.059$; P = 0.725). This correlation is significant ($r = 0.466$; P = 0.004) when removing from the analysis the populations from the south of the river Barbate (SB, 4–6).

Regional level

The results of the Analyses of Molecular Variance (AMOVA; Table 4.2) reveal that the regions separated by the Loukos river and the extension of the Rif

mountain in Morocco (grouping c) are more strongly genetically differentiated (AMOVA-derived F_{ST} = 0.176; 95% confidence interval = 0.140–0.212) than those separated by the Strait of Gibraltar (grouping b; AMOVA-derived F_{ST} = 0.119; 95% confidence interval = 0.093–0.148). As the upper 1-tailed critical value (α = 0.05) for grouping (b) was of 0.143, genetic differentiation in grouping (c) (0.176) was significantly larger than in grouping (b).

Table 4.2. Results of Molecular Variance Analyses (AMOVA) of AFLP data (Squared Euclidean Distance) from 14 populations of *Hypochaeris salzmanniana*. Groupings b and c were used to test the effectiveness of geographic barriers in *H. salzmanniana* by maximizing the percentage of variation among regions. For abbreviations of populations and regions see Fig. 4.1 and Table 4.1. d.f.: degree of freedom; SS: mean sum of squares.

Grouping	N	Source of variation	d.f.	SS	Variance components	% of variance
a [1–14]	14	Among populations	13	2065.293	13.34695	34.45
		Among individuals	126	3200.300	25.39921	65.55
b [NB, SB, AB], [NL, SL, SS] Strait of Gibraltar	2	Among groups	1	468.805	4.89657	11.93
		Among populations	12	1507.880	10.76414	26.22
		Among individuals	126	3200.300	25.39921	61.86
c [NB, SB, AB, NL], [SL, SS] Extension of the Rif mountain in Morocco	2	Among groups	1	557.413	7.55573	17.58
		Among populations	12	1507.880	10.02575	23.33
		Among individuals	126	3200.300	25.39921	59.09

The S Sebou populations (13–14) had the highest significant average gene diversity (Tukey-Kramer HSD test, q*= 2.85; α = 0.05). The average gene diversity of S Loukos populations (11–12) was not statistically different from that

of the N Barbate and from the Algeciras Bay populations, and the average gene diversity of the S Barbate populations (4–6) was not statistically different from N Barbate populations (1–3) and N Loukos populations (9–10) (Fig. 4.2).

With respect to the numbers of shared private fragments between regions, the region to the south of the Sebou river (SS) is the only one to share fragments with

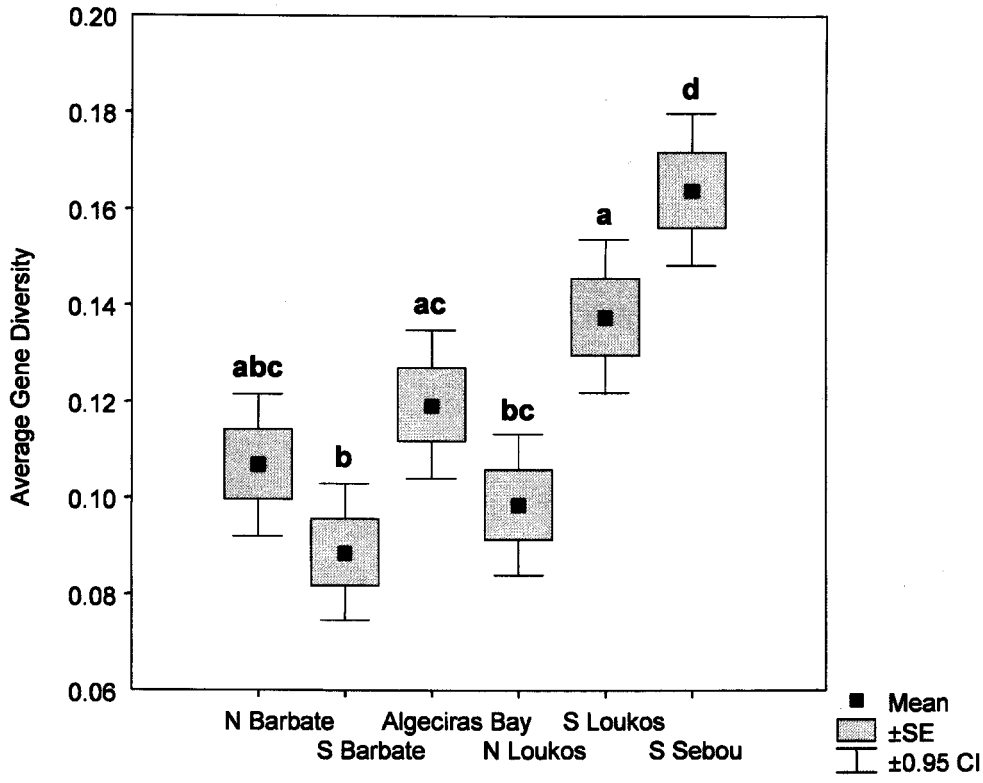


Fig. 4.2. Average gene diversity of geographical regions of *Hypochaeris salzmanniana*. Plots with the same letter are not significantly different at a 5% confidence level.

all other regions (Table 4.3), followed by N Barbate (NB), which shares private fragments with all populations except those of the N Loukos (NL).

The group of populations that possesses most private fragments are S Sebou and S Loukos (54 and 32 fragments, respectively), and those with the least number

of private fragments are S Barbate and N Loukos (6 and 7 private fragments, respectively).

The values of the pairwise fixation index (F_{ST}) between regional groups (Table 4.3) indicate that the following populations are most related: S Sebou and S Loukos (F_{ST} = 0.09), N Barbate and S Barbate (F_{ST} = 0.11), N Barbate and N Loukos (F_{ST} = 0.14), and Algeciras Bay and N Loukos (F_{ST} = 0.14). S Barbate is the metapopulation that is least related to the others.

Table 4.3. Pairwise shared private fragments ($Frag_{sh}$), and pairwise fixation index (AMOVA-derived F_{ST}) with 95% confidence interval between regions and private fragments ($Frag_{priv}$) of each region, in *Hypochaeris salzmanniana* based on analysis of 490 AFLP fragments (excluding the outgroup *H. arachnoidea*.

$Frag_{sh}$ \ F_{ST}	N Barbate	S Barbate	Algeciras Bay	N Loukos	S Loukos	S Sebou
N Barbate	-	0.11 (0.07–0.15)	0.19 (0.15–0.23)	0.14 (0.10–0.18)	0.28 (0.23–0.32)	0.27 (0.22–0.32)
S Barbate	2	-	0.18 (0.12–0.25)	0.16 (0.09–0.22)	0.28 (0.22–0.34)	0.27 (0.21–0.33)
Algeciras Bay	1	1	-	0.14 (0.10–0.19)	0.25 (0.20–0.30)	0.21 (0.16–0.26)
N Loukos	0	0	0	-	0.18 (0.13–0.24)	0.21 (0.16–0.25)
S Loukos	2	0	0	1	-	0.09 (0.06–0.12)
S Sebou	8	5	2	4	19	-
$Frag_{priv}$	20	6	13	7	32	54

Discussion

The Neighbour-Joining dendrogram (Fig. 4.1B) shows that the populations of *H. salzmanniana* are genetically structured into two geographical areas: those to the south of the Sebou river at La Mamora and Kenitra (pops. 13–14) have a high

affinity with those to the south of the river Loukos at Larache and M. Bousselham (pops. 11–12; Fig. 4.1B, Table 4.1), whilst in contrast, the Moroccan populations at Tanger and Asilah (9–10) link with all Spanish populations (Algeciras Bay, and south and north of the Barbate river). Clustering among Spanish populations corresponds to that obtained by Tremetsberger *et al.* (2004) with six primer combinations, but in this study using nine primer combinations, the groups are more strongly supported. Possibly, the extension of the Rif mountain that reaches the Atlantic coast in the region of Larache (see Fig. 4.1A) may have acted as a natural frontier separating the coastal areas to its N (pops. 1–10; territories of the Betic-Rifian Cordillera) from those to its S (pops. 11–14; valleys of the rivers Loukos and Sebou) during interglacial periods.

Effectively, the populations to the south of the Loukos river valley share the highest parameters of genetic diversity, particularly with regard to the number of private fragments and rare fragments found in them. On the assumption that such fragments accumulate through time and are therefore a measure of population antiquity (Stehlik *et al.* 2002; Schönswetter & Tribsch 2005), we can assume that the populations south of the Sebou river (SS) and south of the Loukos river (SL) are the oldest populations in this species system. That the Sebou populations are the only ones to have some pairwise "shared private fragments" with all other populations located in N Morocco and the Iberian Peninsula also supports this view. This reasoning is similar to that used to support the antiquity proposed for *Hypochaeris palustris* in the Coastal Cordillera of Chile (Muellner *et al.* 2005), and for eastern populations of *Saponaria pumila* in the European Alps (Tribsch *et al.* 2002).

These southern Moroccan populations also represent the largest extant populations of *H. salzmanniana* (>10^7 individuals), and it is probably significant that they are located in *Quercus suber* woodlands, adjacent to the sea coast (see Fig. 4.1A), a habitat similar to that of its Moroccan sister species, *H. arachnoidea.* The ancestor of *H. salzmanniana* (and also of *H. arachnoidea*) may have been an

annual herb of *Quercus* woodlands in N Africa. During the earlier glaciations of the Pleistocene, these forests, mainly *Quercus suber*, expanded to occupy the newly emerged lowlands, as a consequence of the decrease of the sea level. In these new habitats, the *Hypochaeris* populations neighbouring to the sea coast could expand and diversify. Such events could have originated the present-day *H. salzmanniana*. Subsequently, during the marine transgressions in the interglacial periods, many of these coastal populations of *H. salzmanniana* would have been extinguished, whilst others became isolated in riverine estuaries and the slopes of the Betic-Rifian Mountains. Consecutive glacial and interglacial periods would favour new expansions, reductions and local extinctions of some populations, and migrations in other cases, all helped to model the genetic structure and breeding system of this species. The fact that in south Sebou populations, we found a higher genetic diversity and higher number of private and rare fragments in populations that live in *Quercus* woodlands supports the view that such woodlands may be the primitive habitat of *H. salzmanniana*.

These factors lead us to believe that these southern populations are older than those of the northern group. Moreover, *H. arachnoidea* shares more fragments with the south Sebou populations (16 shared private fragments) than with the other regions (11 with south Loukos, 1 with north Loukos, 4 with Algeciras Bay, 1 with south Barbate, and 8 with north Barbate), and this further supports the view that this southern Moroccan area is probably ancestral for both *H. salzmanniana* and its sister taxon *H. arachnoidea*.

Based on Tremetsberger *et al.* (2005), we consider that the period for active evolutionary expansion of *Hypochaeris* sect. *Hypochaeris* most probably occurred during the more recent glaciations of the Pleistocene. We hypothesize, therefore, that the species *H. salzmanniana* originated in southern Morocco, as indicated by fragment data, and migrated northwards along the shifting coastline to the area of the Strait of Gibraltar. At glacial maximum, with a much lower sea level prevailing, islands were established in the area of the present Strait (see Fig. 4.1A),

thus providing 'stepping stones', and we can assume that *H. salzmanniana* migrated from north Africa into southern Spain via these temporary land connections and further migrated along the much more extensive SW Spanish coastline.

The extant north Moroccan populations (NL) and Spanish populations (AB, SB and NB) of *H. salzmanniana* all show much lower values than the other populations for private and rare fragments (Table 4.1), which indicates that the northwards migratory history of this species may have been affected adversely by diverse genetic bottlenecks and founder effects. Such population expansions and reductions were also accompanied in some areas by changes in breeding system (Ortiz *et al.* 2006).

We consider below in more detail the possible factors that may have affected the genetic diversity in these northerly populations.

North Loukos and Algeciras Bay populations

The populations NL (Asilah and Tanger, 9–10) and AB (Palmones and La Línea, 7–8) are small, with only 50–1000 individuals. However, as in the southern Moroccan populations (SL, 11–12; SS, 13–14), NL and AB comprise only self-incompatible plants (Ortiz *et al.* 2006), i.e. with obligate outbreeding. We might expect these outcrossing populations to show high levels of genetic diversity. In fact, whilst average gene diversity is reasonably high (mean H_D = 0.088 and 0.114 respectively), in comparison with the southern Moroccan populations (overall mean H_D = 0.146), values for private and rare fragment numbers are much lower than for SL and SS. On the other hand, the values for polymorphic fragments and Shannon diversity index are relatively high. These characteristics indicate that both the NL and AB populations are probably old populations that have suffered bottleneck events and fragmentation since the peak of expansion of *H. salzmanniana*, perhaps in part due to historic changes in the coastline, with rising sea levels allowing mountain uplands to have greater influence, and possibly in part due to more recent human perturbations.

South Barbate populations

These three populations, Zahara (4), Punta Paloma (5), and Los Algarbes (6), occupy an area bounded by the river Barbate and an extension of the Sierra de San Bartolomé near to Tarifa, the latter being the most southerly extension of the Iberian Peninsula. All three populations are genetically depauperate, suggesting that they might have originated through recent colonization. However, the NJ dendrogram (Fig. 4.1B) and the Fixation index (Table 4.1) indicate that these populations are not homogeneous: the population at Zahara is closer, genetically and geographically (see Fig. 4.1A, B), to the north Barbate ones (pops. 1–3), and we assume that this population has been derived by a relatively recent colonization event from the north Barbate area. The other two populations, Punta Paloma and Los Algarbes, are likely to be much older. In this context, it is of interest that Punta Paloma was signalled as a locality with ancient populations of *Senecio gallicus*, and therefore as a possible Quaternary refuge by Comes & Abbott (1998, 2000). However , the pairwise Fixation index (F_{ST} = 0.37) calculated for these two populations, Punta Paloma (5) and Los Algarbes (6), is surprisingly high considering that they are only 1 km distant from each other.

It is significant that the three populations south of the Barbate river are the only fully self-compatible (SC) populations found in *H. salzmanniana* (Ortiz *et al.* 2006). A selfing mating system might arise as a consequence of bottleneck or founder events, which dramatically reduce the number of individuals in a population. This reduction in population size could enhance the probability that a low-frequency self-compatibility allele of the S-Locus might become homozygous and eventually fixed in the populations by chance. Moreover, a selfing mating system would explain the depauperate genetic structure of these populations regardless of their age and origin. Low levels of genetic diversity as a consequence of selfing have been reported in the SC species *Lasthenia maritima* (Heliantheae, Asteraceae), a species derived from the self-incompatible (SI) *L. minor* (Crawford *et al.* 1985), and in SC populations of *Leavenworthia crassa* (Brassicaceae) in

comparison with SI populations of this species (Liu *et al.* 1999). Effectively, the probable consequences of the selfing breeding system in these south Barbate populations means that we do not have reliable indicator genetic parameters (private and rare fragment values, etc.) to hypothesise whether they are all relatively recent in origin, or whether some are recent and others are old.

North Barbate populations

The populations 1–3 (NB) of *H. salzmanniana* from Conil, Vejer and Caños de Meca are particularly interesting. The population at Conil (1) is large (over 10^6 individuals) with the other two being small satellite populations. The NJ dendrogram (Fig 1b) and the Fixation index (0.083) (Table 4.1) indicate that effectively these three are subpopulations of a single population that may have been recently fragmented, possibly due to urban expansion in this coastal area (Ortiz *et al.* 2003). Despite the very large population in the NB region, the values for rare and private fragments are surprisingly much lower than those found in the only other large population of *H. salzmanniana* at Kenitra (pop. 14). Even if the NB populations had passed through a bottleneck event, as it expanded to its present large size, one would expect genetic heterozygosity to regain previous levels, although perhaps with a drastic reduction in the number of alleles (Nei *et al.* 1975; Allendorf 1986) and rare and private fragments.

Again, the breeding system is important. All of these N Barbate populations (1–3) of *H. salzmanniana* are 'semi-compatible' in that they comprise a mix of SI and SC individuals in a 1:1 proportion (Ortiz *et al.* 2006). This fact was unknown to us when these populations were originally sampled for leaf material, but by random sampling one would expect approximately half of our 30 leaf samples to come from SC plants and the other half from SI individuals. A consequence of this population structure would be a mixed mating system that would lead to a marked diminution in heterozygosity and in the parameters used to estimate genetic diversity (Fontdevila & Moya 1999). Since *H. salzmanniana* is predominantly a SI

species, as its sister *H. arachnoidea* is (Ortiz *et al.* 2006), it is likely that selfing is a recent development in these N Barbate populations.

The Strait of Gibraltar: how effective as a barrier?

Analysis of Molecular Variance (AMOVA) with three hierarchical levels revealed that the genetic differentiation between the areas to the north and south of the extension of the Rif mountains in N Morocco explain 17.6% of the variance encountered, whereas the differentiation between those populations delimited just by the Strait of Gibraltar, explain only 11.9% (see Table 4.2). This indicates that when the coastal migration pathway in W Morocco, and island "stepping stones" across the Strait were eliminated by rising seas, mountain ranges became important barriers that must be considered explaining the phylogeography of *Hypochaeris salzmanniana.*

If we assume that *Hypochaeris salzmanniana* is of Pleistocene age (Tremetsberger, unpublished data; see also Tremetsberger *et al.*, 2005), it is likely that one of the glaciations of this epoch facilitated expansion of Moroccan populations across the Strait of Gibraltar into the Iberian peninsula. During each of the Pleistocene glaciations, the sea level in the western Mediterranean region was lower than today (Pou 1989; Yokohama *et al.* 2000), thereby reducing the distance between European and African coasts; moreover, emergent islands that were present periodically during successive glacial periods in the extreme west of the Mediterranean, in the Strait of Gibraltar area, must have favoured contact between the two continents (Collina-Girard 2001). The Strait of Gibraltar, therefore, would not have been a major geographic barrier as it is at present.

Once established in the southern Iberian peninsula, the populations of *H. salzmanniana* seem to have undergone further vicissitudes. With retreat of the ice sheets, and rising sea levels, the populations N and S of Barbate (1–6) on the one hand, and in Algeciras Bay (7, 8) on the other hand, are likely to have been separated by the steep barrier of the Betic Cordillera (Fig. 4.1A). This could account for the similarities in genetic structure between the Algeciras Bay and N

Loukos populations vis-a-vis those further north. Moreover, the extension of the Sierra de San Bartolomé seems to have effectively isolated the populations at Punta Paloma (5) and Los Algarbes (6), thereby leading to decrease in population size, genetic depauperation, and self-compatibility. Whilst the Rif and Betic mountain ranges seem to have formed important barriers, this does not seem to have been the case for rivers. Our results show that the populations on both sides of the river Sebou (SS and SL) are closely related, and the same is true for the populations from both sides of the river Barbate (NB and SB; see Table 4.3). Therefore, these rivers do not seem to have been insurmountable barriers to gene flow.

In conclusion, our results suggest that (1) *Hypochaeris salzmanniana* originated in Morocco from its common ancestor with *H. arachnoidea*; (2) the species spread northwards i.e. from Morocco to Spain; (3) during this expansion, a loss of within-populational genetic variability occurred, in part probably due to bottleneck events; (4) self-compatibility in SB populations (4–6) is also associated with a loss of genetic variability; and (5) historically, the Strait of Gibraltar was not a major barrier for *H. salzmanniana.*

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5 Phylogeography of the invasive weed *Hypochaeris radicata* (Asteraceae): from Moroccan origin to world-wide introduced populations

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Abstract

In an attempt to delineate the area of origin and migratory expansion of the highly successful invasive weedy species *Hypochaeris radicata,* we analysed amplified fragment length polymorphisms from samples taken from 44 populations. Population sampling focused on the C and W Mediterranean area, but also included sites from northern Spain, W and C Europe, SE Asia and South America. The six primer combinations applied to 213 individuals generated a total of 517 fragments of which 513 (99.2%) were polymorphic. The neighbour-joining tree presented two major clusters, and five subclusters, and these divisions were supported by the results of Bayesian analyses. One major cluster included the heterocarpic populations from Morocco, southern Spain and the Central Mediterranean area, and the other, with one important exception, included all of the homocarpic populations from C and N Spain, W and C Europe, and also those from Asia and South America. Analyses of fragment parameters indicate that the oldest populations of *H. radicata* are located in Morocco, and that the species expanded from this area in the late Quaternary via at least three migratory routes, the earliest of which seems to be the SW Sierra Morena area, with subsequent colonizations to the Central Mediterranean region and the Betic Sierras area in southern Spain. Homocarpic populations that probably had their origin in the Sierra Morena region, subsequently spread across C and N Spain. The latter have provided the source for introduced populations of this highly successful species in W and C Europe and world-wide.

Introduction

A basic evolutionary phenomenon, particularly in post-glacial Europe, is the gradual expansion of the area of distribution of species (Hewitt 1999). The spread of many species outward from their original area has increased notably due to human activity, and such facilitated expansions of area can sometimes result in invasive populations. In their natural habitat, the expansion of species may be constrained by factors such as competition with other taxa, or physical environmental parameters. If the limits of natural dispersal can be overcome, however, a species may be able to very successfully colonize new habitats in which the plants tend to be more vigorous and individuals often more abundant than in their native area.

Various authors have noted that there are very few valid generalisations to explain the extraordinary success of some invasive species (Daehler & Strong 1993, Blossey & Nötzold 1995; but see review of consequences of reproductive diversity for plant invasion by Barrett et al. 2008), although various factors have been proposed, such as the new habitat providing more favourable conditions, the absence of predators (Crawley 1987), or as a consequence of the re-allocation of resources due to trade-offs in biomass allocation (Blossey & Nötzold 1995). Other authors, e.g., Neuffer and Hurka (1999), have suggested that invasive species are genetically pre-adapted to their new habitats. In recent times, the spread of domesticated grazing animals from Europe to extensive pastures in the Americas and Australia (Baker 1974) has favoured the exchange and establishment of weeds in areas that had not been previously exposed to such grazing pressure.

Although the majority of successful invasive plant species involve self-compatibility or marked vegetative reproduction (Baker 1974, Rambuda et al. 2004), there are some examples of self-incompatible (SI) species that have achieved notable colonizing success (Lafuma & Maurice 2007). Since the major genetic bottlenecks that usually accompany founding events can cause the total breakdown of SI systems, to yield self-compatible individuals (Reinartz & Les

1994), the survival of SI probably requires some flexibility in the system (Hiscock 2000). In many SI species, some plants produce a low proportion of seeds from self-pollen, a phenomenon called pseudo-self-compatibility (PSC; Nettancourt 1977), and such PSC may be particularly advantageous in small, newly established populations that have little *S*-allele diversity (Levin 1996). During founding events, mating may be restricted due to the reduced number of *S*-alleles in the initial population (Byers & Meagher 1992; but see Brennan et al. 2006), and selection may favour the spread of PSC individuals that will allow the population to increase. Although the offspring of PSC parents may suffer the effects of inbreeding depression, this is likely to be outweighed by the reproductive assurance achieved (Hiscock 2000). Subsequently, if the population size and *S*-allelic diversity increase, as when aided by the arrival of new genotypes via recolonization events, or perhaps by mutation, the level of PSC may decline (Levin 1996). This can have important evolutionary consequences, especially when stochastic processes are involved, since in general, PSC populations are likely to have higher fixation probabilities (Levin 1996).

The amount of genetic variation in a species and its distribution among and within populations is determined by a large number of factors, such as the breeding system, historical events (e.g. habitat availability, population size, migration between populations), and many ecological factors. Nei *et al.* (1975) argued that the loss of heterozygosity depends not only on a small population size, but also on a low population growth rate. If the population growth is fast, the reduction in heterozygosity is likely to be low, even if the number of founders is few.

The family Asteraceae comprises around 8.4 % of the World flora and around 14.4 % of the European flora (Pyšek 1998). In a study of the alien species in 26 local floras worldwide, Pyšek (1997) concluded that members of the Asteraceae were present in all alien floras, with an average contribution of 13.5 %, and were the second most represented family, after the Poaceae. Several authors have

argued that the Asteraceae possess ideal features for colonizers, i.e. high reproductive rate, specialised dispersal structures, and a diversity of metabolic products providing protection from grazing, etc. (Pyšek 1997, 1998; Caño *et al.* 2007).

Hypochaeris radicata is a very successful colonizing species that now has a presence on virtually all continents. Beyond its probable native area in the Mediterranean region, where it occurs in relatively sparse populations associated with humid evergreen woodland, *H. radicata* is a successful invasive weed in northern and central Europe, where it is apparently very flexible with regard to soil requirements and growth conditions (Turkington & Aarssen 1983), and recent studies in SE Australia showed that *H. radicata* is one the most common dicotyledonous plants of temperate perennial pastures (Dellow *et al.* 2002). Likewise, Doi *et al.* (2006) found *H. radicata* in all parts of temperate Japan, commonly in grasslands, with a distribution that is still expanding, and this species is also very common in South America (Cabrera 1971, 1978, 1987) with populations comprising large numbers of individuals. The timing of this remarkable expansion is largely unknown, but it is likely to have occurred in recent times. In the *Flora Brasilensis* (Eichler 1884) there is no record of *H. radicata* for Brazil at that time, although it is now abundant in the south of this country, and similar recent colonization events have been noted for this species in New Zealand (Esler & Astridge 1987) and Lord Crowe Island in the Pacific Ocean (Pickard 1984).

AFLPs (amplified fragment length polymorphism) have been established as useful genetic markers in studies of genetic diversity and biogeographical patterns that have been shaped by Quaternary climatic changes in Mediterranean plant species such as *Anthyllis montana* (Kropf et al. 2002), *Armeria pungens* (Piñeiro *et al.* 2007), *Limonium dufourii* (Palacios *et al.* 1999), and other coastal plant species (Kadereit *et al.* 2005) and with three South American species of *Hypochaeris*: *H. tenuifolia, H. palustris,* and *H. acaulis* (Muellner *et al.* 2005; Tremetsberger *et*

al. 2003a, 2003b), and also with *Hypochaeris salzmanniana*, a species of the NW Moroccan – SW Iberian Atlantic coast (Ortiz *et al.* 2007; Tremetsberger *et al.* 2004)

In the present study we extend AFLP analyses of Tremetsberger *et al.* (2004) to study the phylogeography of the cosmopolitan weed *Hypochaeris radicata* that is one of the World's most common plant species. We focus on the following questions: (i) What is the ancestral area of *H. radicata*? (ii) What is the natural area of distribution of this species? (iii) What are the sources of the introduced populations of this species? (iv) Are the colonizing populations genetically depauperate with respect to the populations in the natural area?

Materials and Methods

Study species

Hypochaeris radicata L. (common catsear or false dandelion) is a short-lived perennial herb, with high seed production despite the fact that throughout its range it largely maintains a homomorphic sporophytic self-incompatibility system (Parker 1975, Pico *et al.* 2004, Ortiz *et al.* 2006). *H. radicata* is found on all continents (except Antarctica): in the Mediterranean area its principal habitat is *Quercus* woodlands and also upland wet pastures, where it occurs in small populations. Out of this area, *H. radicata* is found in more disturbed habitats, such as cultivated grassland and meadows, where it can occur as an aggressive weed in populations that comprise large numbers of individuals.

Plant material

A total of 213 individuals were sampled from 44 populations (Table 5.1, Fig. 5.1). The most detailed sampling was from the W and C Mediterranean area: 11 populations from Morocco, 6 from central Mediterranean area, 17 from the Iberian Peninsula, 3 from West and Central Europe, 3 from Asia, and 4 from South America were sampled. In general, 3–5 individuals were analysed in each

Table 5.1. Localities; geographical coordinates; collection no.; total n° fragments ($Frag_{tot}$); % polymorphic fragments ($Frag_{poly}$); private fragments ($Frag_{priv}$), values given in brackets if they are fixed; rare fragments index (DW); average gene diversity (H_D); for individual populations and the average per group; number of samples for the general analysis, and in brackets a higher number of samples used for the diversity purposes (N_{AFLP}), and AMOVA derived F_{ST} of *Hypochaeris radicata* AFLP samples

Localities	Coordinates (Long/Lat)	*Col. no.*	$Frag_{tot}$	$Frag_{poly}$	$Frag_{priv}$	DW	H_D	N_{AFLP}	F_{ST}
Morocco group									
1. Tanger, Cap Spartel	35°45'/5°54'	*ST et al. 1/03M*	175	52.57	3	10.3	0.0847	5	
2. Tetouan, Larache	35°07'/6°09'	*ST et al. 38/03M*	177	54.80	6	12.8	0.0882	5	
3. Tetouan, c. Bab-Taza	35°03'/5°12'	*ST et al. 454/03M*	177	55.37	6	12.1	0.0932	5	
4. Tetouan, c. Bab-Berred	34°59'/4°52'	*ST et al. 538/03M*	174	50.57	5	12.4	0.0941	4	
5. Meknés, 24 Km S Timahdite	33°25'/5°11'	*ST et al. 660/03M*	195	58.97	10	18.0	0.1037	5	
6. Meknés, 15 Km S Azrou	33°24'/5°13'	*ST et al. TK 63*	182	57.69	6	11.4	0.0952	5	
7. Meknés, Col du Zad	32°57'/5°08'	*ST et al. 709/03M*	191	62.30	5	10.4	0.1079	5	
8. Ksar Es Souk, 5 Km S of Boumia	32°41'/5°05'	*ST et al. 275/03M*	161	54.04	0	2.9	0.0843	5	
9. Beni-Mellal, Jbel Tassemit 1.	32°19'/6°16'	*ST et al. 244/03M*	166	50.60	2	7.5	0.0785	5	
10. Beni-Mellal, Jbel Tassemit 2.	32°18'/6°16'	*ST et al. 238/03M*	165	47.88	0	4.2	0.0892	5	
11. Beni-Mellal, Khemis-des-Oulad-Ayad.	32°13'/6°46'	*ST et al. 224/03M*	160	51.87	2	7.4	0.0793	5	
Mean ± SE			**174.8 ± 3.5**	**54.24 ± 1.27**	**4.1 ± 0.9**	**9.9 ± 1.3**	**0.091 ± 0.003**		**0.28**

Localities	Coordinates (Long/Lat)	*Col. no.*	$Frag_{tot}$	$Frag_{poly}$	$Frag_{priv}$	DW	H_D	N_{AFLP}	F_{ST}
Central Mediterranean group									
12. Italy, Foggia, Gargano	41°50'/15°43'	*KT s/n*	140	38.57	1	4.8	0.0499	5	
13. Italy, Calàbria, Palmi	38°21'/15°50'	*KT s/n*	139	33.81	0	4.3	0.0472	5	
14. Italy, Calàbria, Aspromonte	38°09'/15°52'	*KT s/n*	151	45.69	0	5.2	0.0607	5	
15. Sicily, Palermo, La Pizzuta	37°59'/13°15'	*Castroviejo 5692*	133	27.07	2	4.7	0.0356	5	
16. Sicily, Nebrodi	37°53'/14°31'	*Ehrendorfer 5FE*	135	25.19	1	3.6	0.0367	4	
17. Tunisia, Jendouba, Ain Draham	36°30'/8°46'	*Ehrendorfer s/n*	160	48.75	3	7.0	0.0716	5(19)	
Mean ± SE			**143 ± 4.3**	**36.51 ± 3.93**	**1.2 ± 0.5**	**4.9 ± 0.5**	**0.050 ± 0.006**		**0.44**
Betic Sierras (S Spain)									
18. Córdoba, Ctra. Luque-Carcabuey	37°30'/4°16'	*ST & AO s/n*	174	46.55	4	7.7	0.0762	5	
19. Córdoba, Sierra de Rute	37°30'/4°15'	*ST, AO et al. s/n*	183	50.27	0	9.2	0.0913	5	
20. Málaga, Ronda, La Nava	36°40'/5°03'	*ST & AO*	178	49.44	1	6.6	0.0839	5	
21. Málaga, Gaucín	36°31'/5°18'	*ST, TS et al. 40*	170	46.47	1	6.6	0.1019	3	
22. Cádiz, Alcalá de los Gazules	36°27'/5°43'	*AO s/n*	175	49.14	1	7.1	0.0797	5	
23. Cádiz, Chiclana	36°25'/6°06'	*AO 89/02*	166	42.77	1	4.4	0.0751	4	
24. Cádiz, Vejer, Montenmedio	36°16'/5°58'	*ST et al. 87/01*	181	50.28	2(1)	11.7	0.0836	5(20)	
Mean ± SE			**175.3 ±2.3**	**47.85 ± 1.04**	**1.6 ± 0.5**	**7.6 ± 0.9**	**0.084 ± 0.004**		**0.25**
Doñana N.P. (Spain, Huelva)									
25. P.N. Doñana, El Corchuelo	37°12'/6°42'	*ST, MAO et al.*	181	49.17	2	10.0	0.0847	5	
26. P.N. Doñana, El Acebrón.	37°08'/6°32'	*ST, TS et al.*	158	32.91	1(1)	11.7	0.0495	5(20)	
Mean ± SE			**169 ± 11.5**	**41.04 ± 8.13**	**1.5 ± 0.5**	**10.8 ± 0.8**	**0.067 ± 0.018**		**0.51**

Localities	Coordinates (Long/Lat)	*Col. no.*	$Frag_{tot}$	$Frag_{poly}$	$Frag_{priv}$	DW	H_D	N_{AFLP}	F_{ST}
North and Central Spain									
27. La Coruña, Outeiro	42°48'/8°55'	*SO s/n*	159	40.25	3	8.8	0.0592	5	
28. Navarra, Yesa, Bigüenzal	42°35'/1°10'	*Villar s/n*	177	51.98	1	4.5	0.0866	5	
29. Madrid, campus UAM	40°26'/3°42'	*ST s/n*	170	50.00	1	3.8	0.1096	3	
30. Valencia, El Saler	39°22'/0°19'	*MAO & KT 5/04*	191	58.12	2	9.5	0.1060	5	
31. Jaen, Santa Elena, Despeñaperros	38°23'/3°31'	*MAO & KT 4/04*	199	61.31	2	10.0	0.1153	5	
Mean ± SE			**179 ± 7.2**	**52.3 ± 3,6**	**1.8 ± 0.4**	**7.3 ± 1.3**	**0.095 ± 0.01**		**0.24**
SW Sierra Morena (Spain, Huelva)									
32. Huelva, Aracena	37°53'/6°32'	*MAO et al. s/n*	184	57.07	4	9.6	0.0955	5	
33. Huelva, Santa Ana La Real	37°52'/6°42'	*ST, TS et al.*	170	51.18	1	4.4	0.0840	5(20)	
34. Huelva, Valverde	37°34'/6°45'	*ST, TS et al.*	164	49.39	1	5.0	0.0750	5	
Mean ± SE			**172.7 ± 6**	**52.55 ± 2.32**	**2 ± 1**	**6.3 ± 1.6**	**0.085 ± 0.006**		**0.11**
West and Central Europe									
35. France, Toulouse	43°36'/1°26'	*MAO s/n*	174	43.10	1	4.7	0.0837	5	
36. Netherlands, Konijnendijk	52°02'/6°26'	*Mix s/n*	105	51.43	0	3.4	0.0910	5(20)	
37. Austria, Tirol, Reith im Alpbachtal	47°24'/11°52'	*TS s/n*	111	57.66	1	4.5	0.1070	5(20)	
Asia									
38. Taiwan, Houhuan Mountain	24°14'/121°09'	*TS s/n*	157	46.50	1	3.9	0.0670	5(20)	
39. S Korea, Cheju Island, Chonwang	33°30'/126°31'	*TS 17511*	160	55.00	1	3.2	0.0836	5(20)	
40. S Korea, Cheju Island, Seogwipo	33°15'/126°33'	*TS 17520*	178	60.11	1	3.6	0.0959	5(20)	
South America									
41. Argentina, Jujuy, Yala	24°07'/65°23'	*TS et al. 18056*	154	51.30	0	2.1	0.0770	5(20)	
42. Argentina, Río Negro, Cerro Tronador	41°10'/71°49'	*TS et al. 18053P*	172	52.91	0	2.4	0.0862	5(20)	
43. Chile, Santa Barbara	37°39'/72°01'	*TS et al. s/n*	137	43.80	0	1.5	0.0557	5(20)	
44. Chile, Region X, Volcán Choshuenco	39°49'/72°04'	*TS 15826*	158	54.43	0	1.2	0.0824	5(17)	
Mean ± SE			**150.6 ± 8**	**51.62 ± 1.8**	**0.5 ± 1.7**	**3.0 ± 0.4**	**0.083 ± 0.004**		**0.25**

population, depending on availability, but for some 13 populations we were able to study larger sample sizes of 17–20 individuals per population (see Table 5.1) Overall the samples focus on N and C Morocco - SW Iberian Peninsula because this is an area where *Hypochaeris* sect. *Hypochaeris* has a centre of diversity (Tremetsberger *et al.* 2005), but they also provide a reasonable coverage of the remarkably widespread areas where this species is found. For all samples, fresh leaves of the plants were dried in silica gel. To root the tree we used three individuals of *H. glabra* as an outgroup (the basal species of the section) in the AFLP analysis. Vouchers of all sampled populations are deposited in the Herbarium of the University of Seville (SEV, Spain) and/or University of Vienna (WU, Austria).

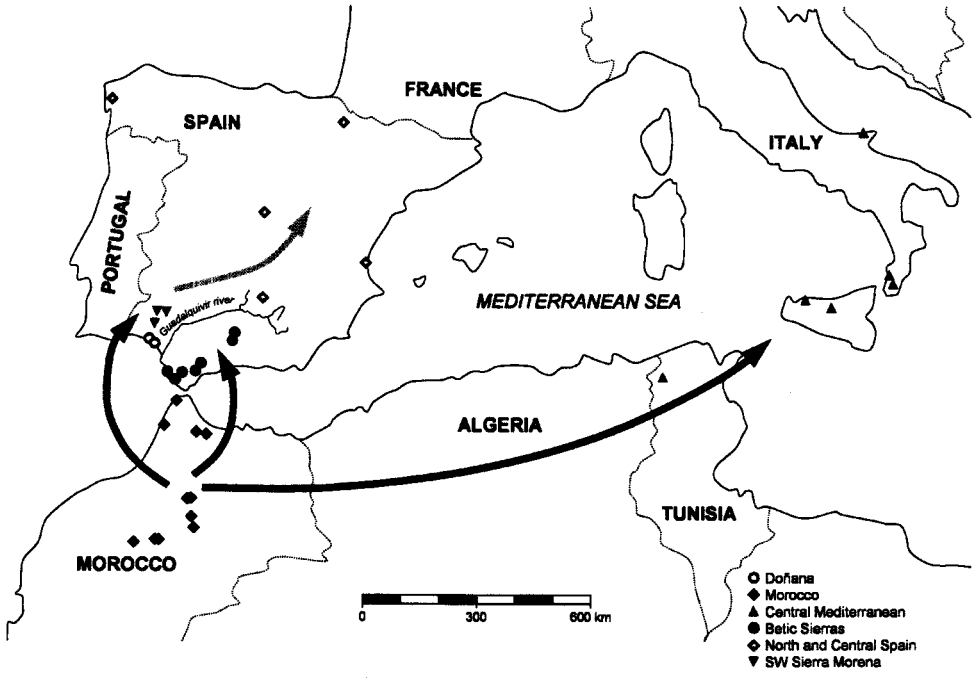

Figure 5. 1. Map showing sampling localities for *Hypochaeris radicata* in the W Mediterranean region. Black symbols show heterocarpic populations, white symbols homocarpic populations. Lines and arrowheads in black show postulated initial expansions of heterocarpic populations from Morocco; that in grey indicates subsequent European expansion of homocarpic populations.

DNA isolation and AFLP analysis

Total genomic DNA was extracted from dry leaf material following the CTAB protocol (Doyle & Doyle 1987) with modifications (Tremetsberger *et al.* 2003b, 2004, Ortiz *et al.* 2007). The AFLP procedure followed established protocols (Vos *et al.* 1995) with modifications (Tremetsberger *et al.* 2003b, 2004, Ortiz *et al.* 2007). The six primer combinations for the selective PCR were those selected by Tremetsberger *et al.* (2004): *MseI*-CTCG/*EcoRI*-ATC (Fam), *MseI*-CAC/*EcoRI*-ACG (Hex), *MseI*-CTA/*EcoRI*-ACC (Ned), *MseI*-CTG/*EcoRI*-ACA (Fam), *MseI*-CTC/*EcoRI*-AGG (Hex), *MseI*-CTGA/*EcoRI*-AAC (Ned). Four of the primer combinations [*MseI*-CTGA/*EcoRI*-AAC (Ned), *MseI*-CTCG/*EcoRI*-ATC (Fam), *MseI*-CTG/*EcoRI*-ACA (Fam), and *MseI*-CAC/*EcoRI*-ACG (Hex)] were applied to a larger sample (17–20 individuals) per population. The fluorescence labelled selective amplification products were separated on a 5% polyacrylamide gel with an internal size standard (GeneScan®-500 ROX, PE Applied Biosystems) on an automated sequencer (ABI 377, Perkin–Elmer). Amplified fragments from 50 to 500 base pairs were scored, and exported as a presence/absence matrix using ABI Prism GeneScan® Analysis Software 2.1 (PE Applied Biosystems) and Genographer (version 1.6.0 © Montana State University 2001; available at http://hordeum.oscs.montana.edu/genographer/).

Statistical data analyses

The presence/absence matrix developed with the six primer combinations was imported into PAUP* (version 4.0b10 Sunderland, MA: Sinauer Associates) with which we constructed a dendrogram applying the Neighbor-Joining method (NJ) in conjunction with Nei & Li (1979) genetic distances. Support for each node was tested by 10000 bootstrap replicates. As a measure of within-population diversity, we assessed the total number of fragments per population, the percentage of polymorphic fragments, as well as the number of private fragments for all populations of *H. radicata* (Table 5.1). We used FAMD v. 1.1 (Schlüter & Harris 2006) to

exchange between different file formats and to calculate the fixed and private fragments in the populations. We also assessed the number of fragments that were shared exclusively between pairs or groups of populations (shared private fragments, Table 5.2), and we looked for "rare fragments", i.e. those that were found in less than 10% of the individuals within a population (Stehlik *et al.* 2002) and calculate DW ("frequency-down-weighted marker values") as proposed by Schönswetter & Tribsch 2005. As another measure of genetic variability, we also calculated the average gene diversity (Arlequin v. 3.01; Excoffier *et al.* 2005).

We used the results for the populations involving larger sample sizes (pops. 17, 24, 26, 33, 36–44) to check those using samples of 3-5 individuals per population. General Linear Model (GLM) analyses were applied to detect differences in the average gene diversity, the number of polymorphic fragments and the number of private fragments when genotyping five or twenty individuals in the same populations.

A linear correlation between genetic and geographic distances, the standardized Mantel test, was used in Mediterranean populations of *H. radicata* (pops 1–34). A Nei & Li's distance matrix among all pairwise combinations of populations was compared to geographic distances (in km) among populations (with distances within populations set to 0; XLSTAT-Pro v. 7.5.2).

In order to examine the population structure of *Hypochaeris radicata*, we conducted two approaches based on statistical inference with Bayesian clustering methods using STRUCTURE v. 2.2 (Pritchard *et al.* 2000, available at http://pritch.bsd.uchicago.edu/structure.html), and BAPS v. 4.14 (Corander & Marttinen 2006 available at http://www.abo.fi/fak/mnf/mate/jc/software/baps.html). In STRUCTURE we chose the admixture ancestry model, and the correlated allele frequencies option (Falush *et al.* 2007). We ran the simulation with a burn-in period of 10^5 and a run length of 10^5 MCMC, with K from 1 to 5 and 10 iterations, and from 6 to 12 with 6 iterations, which showed a stabilised result. To choose the most accurate value of K we followed Evanno *et al.* (2005).

The results were compared with those provided by the other Bayesian clustering method, BAPS. This simulation was run from $K = 2$ to $K= 50$ as the maximum number of diverged groups, with four replicates for each K. We used the option "clustering of individuals" with the following settings: minimal size of clusters at four individuals; 100 iterations to estimate the admixture coefficients for the individuals; 200 simulated reference individuals from each population; and 20 iterations. We did not use the Austrian and Dutch populations (pops 36–37) in Bayesian inference because they presented some missing data that could disturb the results.

Results

AFLP profiles

The six primer combinations applied to 213 individuals of *H. radicata* generated a total of 517 fragments, ranging from 55 to 500 bp, with an average of 86.2 fragments per primer combination, of which 513 (99.2%) were polymorphic. The number of fragments for each primer combination was: *MseI*-CTCG/*EcoRI*-ATC: 77, *MseI*-CAC/*EcoRI*-ACG: 100, *MseI*-CTA/*EcoRI*-ACC: 118, *MseI*-CTG/*EcoRI*-ACA: 80, *MseI*-CTC/*EcoRI*-AGG: 59, *MseI*-CTGA/*EcoRI*-AAC: 83. This number of fragments was sufficient to distinguish all 213 individuals as separate phenotypes.

Identification of population structure

The Neighbour-joining method revealed two distinct clusters (Fig. 5.2). The first major group includes three separate subclusters: the Moroccan (89% BS), the central Mediterranean (100% BS), and the southern Spanish populations of the Betic Sierras (98% BS). The second major group, with 59% BS, includes two subclusters: the first (56% BS) grouped all NC Spain and SW Sierra Morena, together with the remaining European and all Asian and the South American populations (pops. 35–44), whilst the second subcluster (with 100% BS)

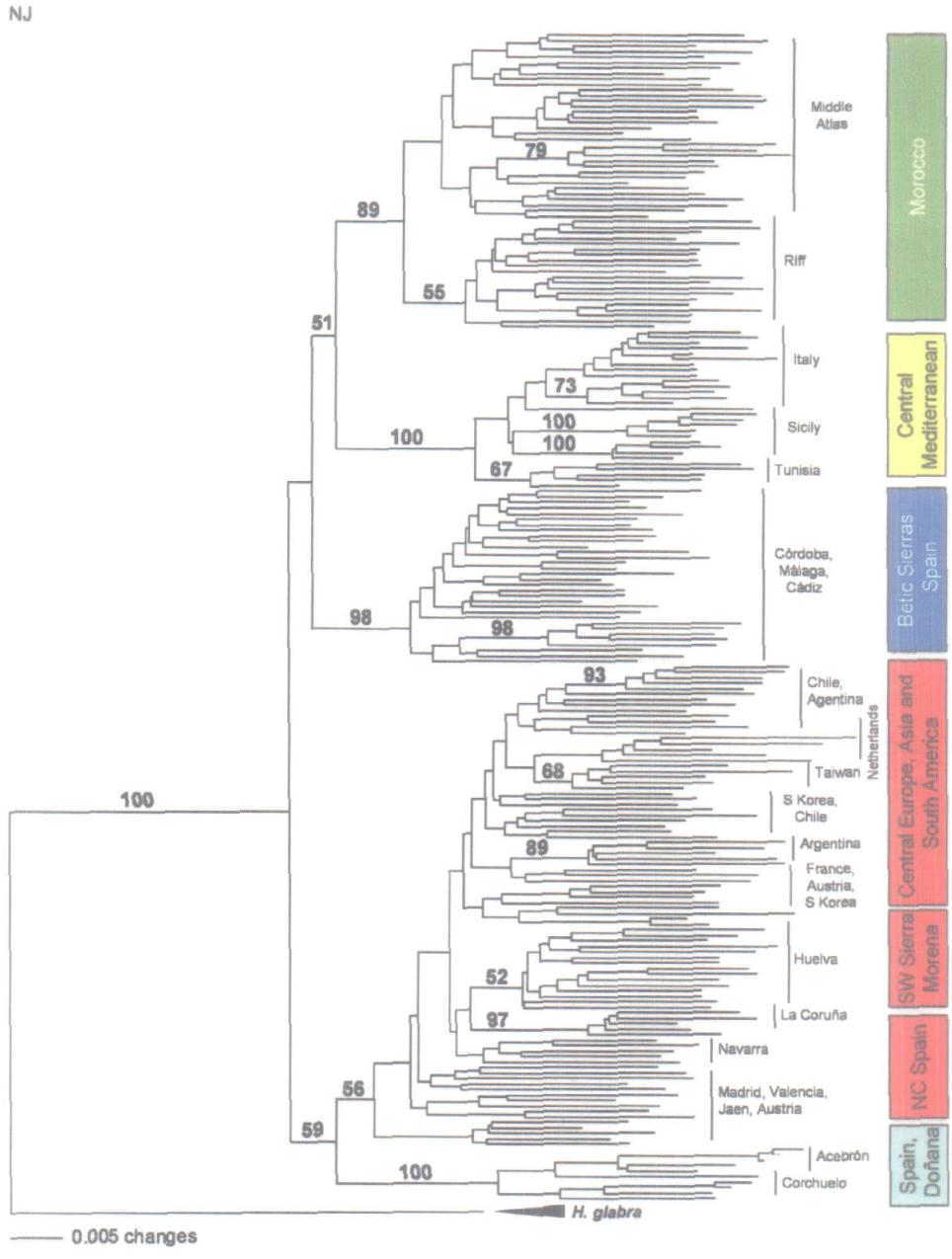

Figure 5. 2. NJ dendrogram of the 140 individuals analysed of *Hypochaeris radicata*, rooted with *H. glabra*, based on Nei & Li's genetic distance. Bootstrap values based on 10000 permutations, if higher than 50%, are indicated at each node. For details of populations, see Table 5.1.

comprised the two populations from the Doñana National Park. When the Bayesian clustering conducted with STRUCTURE was challenged with $K = 2$ to $K = 11$, the $K = 2$ groups gave a high probability, followed by $K = 5$. All the individuals were assigned to their original populations. The first Bayesian grouping, with $K = 2$ (Fig. 5.3a), separates the Moroccan, C Mediterranean and Betic Sierras populations from those from Doñana, NC Spain, SW Sierra Morena, W Europe, Asia and South America, confirming results of the NJ tree. It is notable that Spanish populations appear in both clusters divided by the Guadalquivir River, whilst the two populations from Doñana at the estuary of the Guadalquivir River into the Atlantic Ocean occupy an intermediate position. At $K = 5$ (Fig. 5.3b) divide the populations from Morocco, Betic Sierras, C Mediterranean, and Doñana into separate groups. The BAPS program results (Fig. 5.3c) were largely coincident with the STRUCTURE analysis, showing an optimal grouping for $K = 5$.

Genetic diversity

The results of the estimated genetic diversity patterns are shown in Table 5.1. The average gene diversity varied from 0.0356 in Palermo (pop. 15) to 0.1153 in Santa Elena (pop. 31); polymorphic fragments varied from 25.19% in Nebrodi (pop. 16), to 62.30% in Bab Berred (pop. 4). The Moroccan populations (pops. 1–11) showed the highest average gene diversity, followed closely by the NC Spanish populations (pops. 27–31), whereas the C Mediterranean populations (pop 12–17) showed the lowest values. The population with the highest number of private fragments was Timahdite (pop. 5) with 10 unfixed private fragments. Only two populations showed fixed private fragments, both from southern Spain: El Acebrón (pop. 26) and Montenmedio (pop. 24).

We compared the results for samples with 5 individuals per population, with those using the larger population samples of 17–20 individuals from the same 13 populations (see Table 5.1). The average gene diversity was not significantly different among populations ($F_{1,12} = 3.06$, $P = 0.1055$), whilst, unsurprisingly, the

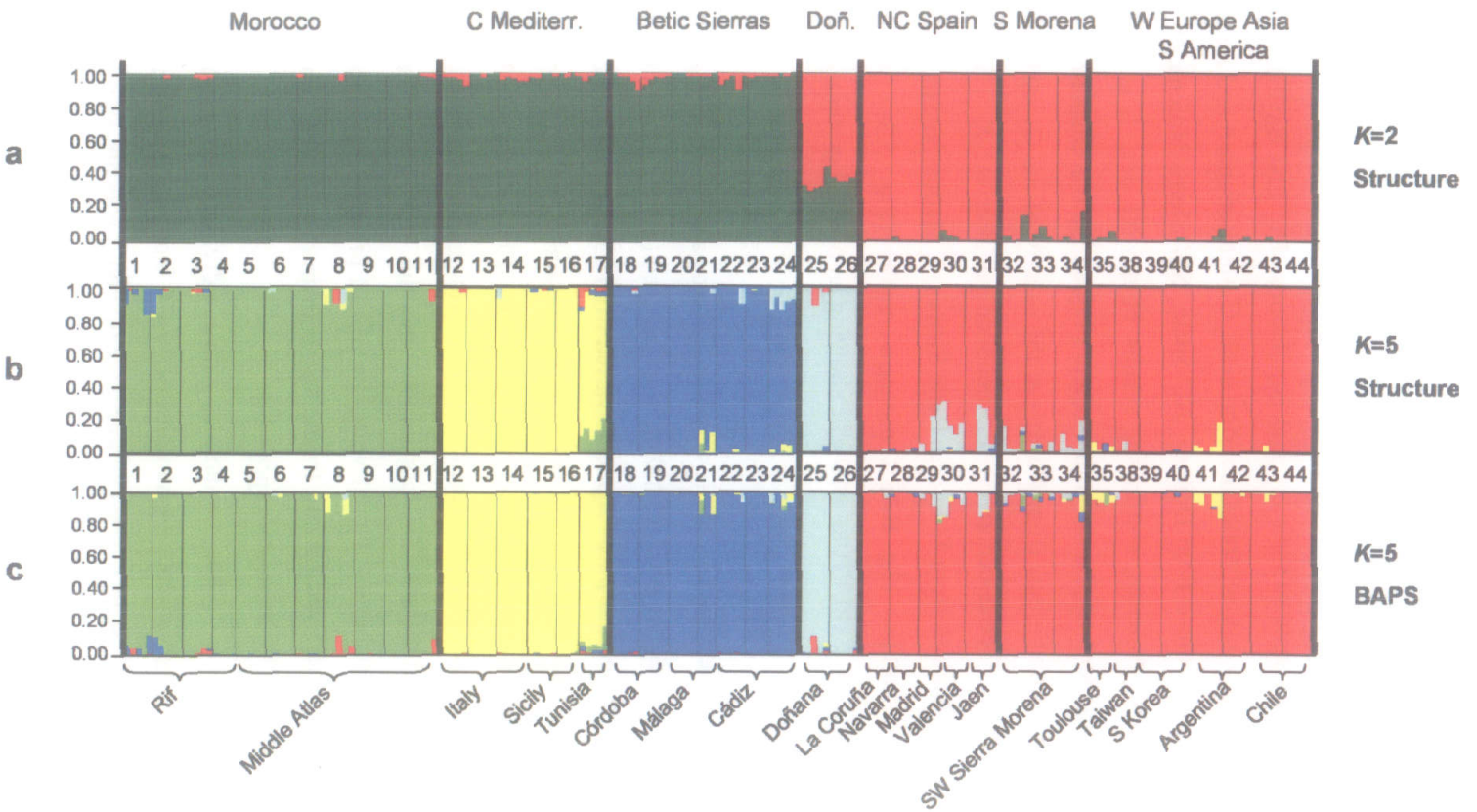

Figure 3. Population structure of *Hypochaeris radicata* inferred by Bayesian clustering of the AFLP data the by programs STRUCTURE and BAPS.

number of polymorphic and private fragments was higher in the 17–20 individual samples compared to those with 5 ($F_{1,12}$ = 12.81, P = 0.0038 and $F_{1,12}$ = 8.99, P = 0.0111, respectively). The sample of 5 individuals per population therefore appears sufficient to accurately estimate genetic diversity within populations.

Relationships between geographical groups

The groups of populations with most private fragments were those from Morocco and Betic Sierras (73 and 23, respectively; Table 5.2), and those with the least number of private fragments were those from the SW Sierra Morena, Doñana area, and W & C Europe, Asia and S America (9, 8, and 7, respectively). The group formed by W & C European, Asian and South American populations do not share more than 2–4 private fragments with most other groups, but strikingly, share 9 with the NC Spanish group. The Moroccan group has more shared private fragments with all other regions, and the Doñana populations share the least private fragments with all the other groups (Table 5.2). With the AMOVA-derived F_{ST} (Table 5.1), the Doñana (F_{ST} = 0.51) and C Mediterranean populations (F_{ST} = 0.44) showed the highest values.

The results of the Mantel test showed that there is a significant correlation between these parameters (r = 0.664; P < 0.001) for Mediterranean populations of *H. radicata* (i.e., accessions from W & C Europe, Asia and South America were excluded). Considering partial groupings, the correlation was significant only for the Moroccan area (r = 0.767, P < 0.001) and the NC Spain and SW Sierra Morena areas together (r = 0.638, P < 0.001). The values for the pairwise fixation index (F_{ST}) between groups (Table 5.2) are very high (> 0.2), except for the NC Spain region with respect to WC European, Asian and S American populations (F_{ST} = 0.15).

F_{ST} / $Frag_{sh}$	Morocco	Center Mediterranean	Betic Sierras	Doñana	NC Spain	SW Sierra Morena	W & C Europe, Asia and S America
Morocco	-	0.58	0.53	0.65	0.58	0.62	0.53
C Mediterranean	9	-	0.74	0.77	0.73	0.76	0.55
Betic Sierras	23	5	-	0.68	0.62	0.66	0.50
Doñana	3	2	0	-	0.57	0.66	0.44
NC Spain	6	2	2	2	-	0.26	0.15
SW S Morena	4	0	1	1	6	-	0.22
W & C Europe, Asia and S America	3	4	4	3	9	2	-
$Frag_{priv}$	**73**	**14**	**23**	**8**	**12**	**9**	**7**
N_{AFLP}	**54**	**24**	**27**	**10**	**23**	**15**	**50**

Table 5.2. Pairwise shared private fragments ($Frag_{sh}$), and pairwise fixation index (AMOVA-derived F_{ST}, Nei & Li distances) with 95% confidence interval between regions and private fragments ($Frag_{priv}$) of each region, in *Hypochaeris radicata* based on analysis of 517 AFLP fragments

Discussion

Moroccan origin of Hypochaeris radicata

In order to structure ideas on what may be considered as the natural distributional area of *H. radicata* with respect to those areas in which this plant is present as an introduction, we need to attempt to establish the probable area of origin of this species. Locating the origin of species can be problematical since key genetic parameters such as diversity estimates or number of rare fragments may also be used to determine refugial areas or suture zones (Bonin *et al.* 2007). On the assumption that private and rare fragments accumulate through time and are, therefore, a measure of population antiquity (Stehlik *et al.* 2002; Schönswetter & Tribsch 2005), we can assume that the populations of the Moroccan area of *H. radicata* are the oldest in this species system since they have the highest values of these parameters (Tables 5.1 & 5.2).

H. radicata belongs to *Hypochaeris* sect. *Hypochaeris*. Recent studies (Tremetsberger *et al.* 2005) on the phylogeny of these four species showed the section to be monophyletic, with *H. glabra* branching off at the basal node, and with *H. salzmanniana* and *H. arachnoidea* as sister species, and this cluster as sister to *H. radicata*. All of these species co-occur in Morocco. Tremetsberger *et al.* (unpubl. data) estimate the diversification of *Hypochaeris* sect. *Hypochaeris* at 1.7-2.0 Ma [95% HPD interval (program BEAST; available from http://evolve.zoo.ox.ac.uk/beast/) = 0.6–3.5 Ma], so that the evolutionary expansion of *H. radicata* seems to have occurred during the Quaternary glacial periods. Moreover, *H. arachnoidea*, *H. glabra*, and *H. salzmanniana* show heterocarpy (i.e., the flowering heads have dimorphic achenes, with the outer ones unbeaked and the inner ones with a long slender beak), as do the Moroccan populations of *H. radicata*, and also those in the Betic Sierras and Central Mediterranean areas, but not those in C and N Europe or the worldwide introduced populations - see dis-

cussion below). Thus, a Moroccan area of origin for *H. radicata* is also supported by the occurrence of populations with heterocarpic fruits in this area.

This hypothesis parallels conclusions of Ortiz *et al.* (2007), who proposed that the oldest populations of *Hypochaeris salzmanniana* are situated in Morocco, in the Sebou valley, in pastures of *Quercus suber* evergreen woodlands. Such woodlands are also the habitat of *H. radicata* in North Africa, and we suppose it to be the ancestral area of this species, from which it subsequently spread to similar habitats in southern Spain and other parts of the Mediterranean, and subsequently to cool humid pasture areas further north in Europe, and more recently, it has successfully dispersed to similar habitats on the other continents.

Natural distributional area of H. radicata

In order to determine the natural distributional area of *H. radicata*, we will attempt to reconstruct its geographical expansion out of its Moroccan home. The NJ dendrogram (Fig. 5.2), and the Bayesian analyses (Fig. 5.3a) indicate that the populations of *H. radicata* are genetically structured in two major clusters: (i) the first includes the Moroccan, Central Mediterranean and Betic Sierras populations. The plants in these areas also have a striking morphological feature in common since they all have heterocarpic fruits. (ii) The second major cluster includes all the Iberian Peninsula populations located on the northern side of the Guadalquivir River (pops. 25–34), together with all of the W and C European, Asian and South American populations (pops. 35–44). Populations in this cluster are mostly homocarpic (the floral heads produce only fruits of the long slender beaked type) but there are some important exceptions – see further discussion below. Similar groups were distinguished for *H. radicata* by Tremetsberger *et al.* (2004), in a preliminary survey of *H. salzmanniana* and related species based on a limited sampling of European and extra-European localities, but lacking populations from Morocco.

The entirely heterocarpic major cluster includes three subclusters (Figs. 2, 3b). The first (with 89% BS) comprises the Moroccan populations, which show some, albeit weakly supported (55% BS), geographical structure, since populations from the Rif mountain range can be distinguished from those from the Atlas Mountains. Little is known about the phylogeography of other species occurring in this area of Morocco, but the Rif and Atlas mountain ranges are separated by the Sebou valley, which, together with other important Moroccan river valleys, has influenced the phylogeographical structure of the congeneric species *Hypochaeris salzmanniana* (Ortiz *et al.* 2007). As discussed above, we assume these Moroccan populations of *H. radicata* represent the first expansion area of this species.

The second subcluster, which is also strongly supported (98% BS), comprises the Betic Sierras populations (S Spain); these plants are characterized by flowering heads with broad and cordate external involucral bracts with a wide scarious margin. Plants with these features were described by Boissier (1839) as *Hypochaeris platylepis*, and subsequently considered by other authors (Jahandiez & Maire 1934; Galán de Mera 1995) as a subspecies of *H. radicata*. It is probably significant that many plants of *H. radicata* from the Rif mountain range (i.e., the northernmost geographical subunit of the Moroccan subcluster) also present heads with cordate external involucral bracts, similar to those of plants from the Betic Sierras, and this led Galán de Mera (1995) to include these populations in *H. radicata* subsp. *platylepis*.

Expansion of *H. radicata* from its Moroccan homeland into southern Spain probably occurred during the glaciations of the Quaternary, when several islands, today submerged, facilitated communication between NW Morocco and the SW Iberian Peninsula (Collina-Girard 2001). It is likely that a fluctuating island "stepping stone" situation during this period permitted several expansionary pathways from Morocco into southern Spain rather than a single corridor. Again, this biogeographical scenario is very similar to that proposed for *Hypochaeris salzmanniana* (Ortiz *et al.* 2007), an annual species that is phylogenetically very close to

H. radicata (Tremetsberger *et al.* 2004; 2005). However, of all the populations in the Iberian Peninsula, those from the Betic Sierras area are closest to the Moroccan populations in the NJ dendrogram. The Moroccan populations also share a large number of private fragments (23) with the Betic Sierras populations, a value much higher than those shared with the other heterocarpic population areas in SW Sierra Morena (4) and the Central Mediterranean (9). This parameter indicates a comparatively recent dispersal from Morocco to the Betic Sierras vis-a-vis those to the latter areas.

The third subcluster (with 100% BS) includes the Central Mediterranean populations (Italy, Sicily and also Tunisia) indicating a migratory expansion from Morocco to the central Mediterranean region. Plants with heterocarpic fruits are also reported in floristic accounts from Greece (Halácsy 1902) and Turkey (Davis 1975), so that it is likely that this central Mediterranean group achieved a further expansion to the east of the Mediterranean basin. A similar phylogeographic link between populations in North Africa and those in southern Italy, Sicily has been reported for *Abies* spp. (Parducci *et al.* 2001). These authors found a common haplotype between *Abies alba* and *A. numidica* and they suggested that these species might have been in contact in the past.

The second major cluster (Figs. 2 and 3) includes all the homocarpic populations plus three heterocarpic populations in the SW Sierra Morena (pops. 32–34). The latter are weakly separated in the NJ dendrogram from the remaining NC Spanish populations (52% BS; Fig. 5.2). Since, as noted above, we regard heterocarpy as the primitive state in sect. *Hypochaeris*, we hypothesize that these heterocarpic populations from the SW of the Sierra Morena represent survivors of an early colonization from Morocco, and this view receives further circumstantial support from the fact that the SW Sierra Morena populations share four private fragments with those from Morocco but only one with the Betic Sierras. Heterocarpic populations of *H. radicata* have also been reported from Portugal (Mariz

1894) indicating that the SW part of the Iberian Peninsula was the first point of contact for plants arriving from Morocco.

A strongly supported subgroup (100% BS) consists of the two populations from the Doñana National Park area in Huelva province. This was an area of very extensive freshwater wetlands until the middle of the XIX century (Sousa & García-Murillo 2003), with *Quercus suber* woodlands surrounding the lagoons, and so effectively a typical habitat of *H. radicata*. Doñana plants are morphologically different from all the other populations of *H. radicata* in that they have linear, sub-entire and fleshy leaves, and they show marked vegetative multiplication by underground stolons such that large stands apparently of the same genet are formed. Within such a mosaic of clonal plants, a self-incompatible species such as *H. radicata* (Ortiz *et al.* 2006) is likely to encounter constraints on successful sexual reproduction, and such constraints are likely to be more severe with the homomorphic sporophytic SI found in the Asteraceae (Byers & Meagher 1992) than with other incompatibility systems. Consequently, a reduction in the genetic diversity of these populations is to be expected. Effectively, these populations show a low fruiting success in the field (Ortiz *et al.* 2006), and they do indeed show a low genetic diversity (Table 5.1).

It is not possible to decide whether the Doñana populations are derived from the SW Sierra Morena populations or whether they represent a separate introduction from Morocco. Whichever of these scenarios prevailed, the Doñana populations show a large number of rare fragments, and El Acebrón (pop. 26) is one of the few populations that present a fixed private fragment. We therefore assume that they are old populations. Moreover, the low genetic diversity present in both Doñana populations (Table 5.1), probably due to their vegetative spread and infrequent sexual reproduction (Ortiz *et al.* 2006), indicates that they might have been submitted to bottleneck events. We must assume that their homocarpy evolved *in situ* following isolation and possible bottleneck events.

The remaining N & C Spanish populations (pop. 27–31) are all homocarpic and show in general a high genetic diversity (mean H_D = 0.095) and intermediate number of rare (mean DW = 7.3) and private fragments (mean $Frag_{priv}$ = 1.8; Table 5.1). These populations show close relations to those in W and C Europe, and to weedy populations throughout the world.

As Turkington and Aarssen (1983) commented with respect to the occurrence of *H. radicata* in Great Britain, it is very difficult to establish what are native populations for such a successful invasive species. We hypothesize, on the basis of our results, that the heterocarpic ancestral populations of *H. radicata* expanded out of northern Africa via a series of stepping stones across the Strait of Gibraltar area into the southern Iberian Peninsula in the Quaternary. The fragment data indicate that this was not a simple advance into the Iberian Peninsula. A parsimonious explanation requires at least two independent colonizations in this area, the first to the SW, to establish the "Sierra Morena" and possibly Portugal foothold, and the second to the south to give rise to the Betic Sierras populations; furthermore, as noted above, an additional separate expansion probably gave rise to the Central Mediterranean populations.

The geological history of the southwest of the Iberian Peninsula provides some support for such a first phase of expansion for *H. radicata*. Until the end of the Miocene the "Guadalquivir Valley" was a marine corridor that linked the Atlantic Ocean with the Tethys Sea, at which time the south of the Iberian Peninsula consisted of fragmenting land connections with North Africa. Subsequently, when sedimentary deposits closed this marine corridor, there existed even up to historical times a large estuarine area at the mouth the Guadalquivir, called Lake Ligustinus by the Romans (Rodríguez-Ramirez *et al.* 1996). The first postulated colonization by *H. radicata* from Morocco would have been to the west of this estuarine area, and expansion from such an introduction may then have occurred into the adjacent wetlands of Doñana, where homocarpy became established. At this time, the Guadalquivir Valley was still a sufficient barrier to prevent the

heterocarpic populations subsequently established in the Betic Sierras, from migrating north. The Guadalquivir valley was likewise indicated as an effective barrier for plant (Tremetsberger *et al.* 2004; Pimentel *et al.* 2007) and animal species (Busack 1986; García-París *et al.* 1998; García-París & Jockusch 1999; Sanmartín 2003).

We propose as a tentative working hypothesis, that Morocco, and the area of the southern Iberian Peninsula with heterocarpic populations comprising the SW Sierra Morena (and also including the enigmatic homocarpic Doñana enclave) and the Betic Sierras, together with the heterocarpic populations found in the Central (and Eastern) Mediterranean, should be considered as the native area of distribution of *H. radicata.*

Introduced populations of H. radicata

AFLP data suggest that homocarpic plants of *H. radicata* had an origin in the SW Sierra Morena area and began to spread into C and N Spain at a time when the Guadalquivir River still effectively prevented northward expansion of populations in the Betic Sierras area. These homocarpic populations on the northern side of the Guadalquivir River provided the source of the subsequent expansion of *H. radicata* into central and northern Europe and more recently throughout temperate areas of the rest of the world. On this view, somewhat surprisingly, neither the populations of the Betic Sierras area, nor those of the Central Mediterranean area, seem to have contributed to the amazingly successful migratory expansion of this species in Europe and the rest of the world.

Populations from W & C Europe, Asia, and South America, are characterized by a low incidence of rare and private fragments (Table 5.1). These features indicate that they are recent in origin and have probably been submitted to bottleneck effects during colonization. However, values for total number of fragments, percentage of polymorphic fragments, and average gene diversity are intermediate in comparison to values presented by the other groups of *H. radicata*

(Table 5.1) indicating that the species is able to quickly recover genetic diversity, possibly through rapid population growth.

Exactly why homocarpic populations of *H. radicata*, should have first successfully expanded across Spain and then into northern and central Europe, and subsequently become an aggressive colonizer worldwide, is a very difficult question to answer. Homocarpy *per se* does not seem to bestow any particular advantage. The spread of homocarpic populations into central Spain was probably aided by anthropomorphic factors. The humid pastures of the Iberian plateau have been subjected to annual sheep transhumance for at least 2000 years, with vast flocks travelling north - south accompanying the forage seasons. This kind of sheep-aided dispersal would not only favour the colonization of new areas but also the repeated colonization of the same areas with different genotypes from different populations, as reported in *Crupina vulgaris* (Garnatje *et al.* 2002). These animals could have aided the dispersal of *H. radicata* achenes, in the same way as the ectozoocoric dispersal proposed for *Hypochaeris glabra* by Baker and O'Dowd (1982). However, the establishment of homocarpy might have coincided with an important change in ecological tolerance, which permitted the hitherto humid Mediterranean woodland *H. radicata* to invade temperate grasslands.

The presence of homomorphic, sporophytic self-incompatibility, likewise, does not seem to have constrained the expansion of *H. radicata* from its Mediterranean homeland. The introduced populations show, in general, a similar genetic diversity to those in the "native range" (see above). A study by Mix *et al.* (2006), in *H. radicata,* in a fragmented agricultural landscape in the Netherlands, also indicates that this species can maintain its genetic diversity between high and low density populations.

An important factor that has very likely contributed to the migratory success of this species lies in flexibility in its SI mechanism, with some pseudo-self-compatible (PSC) individuals capable of setting a small proportion of seed from selfing. There is some evidence that the proportion of PSC individuals is higher in

introduced populations of *H. radicata*: Ortiz *et al.* (2006) found only 13.3 % PSC plants (19 in a total of 138 individuals) in the area we consider native, but 27.5% (11 in 40) in samples from C & S America. Likewise, Pico *et al.* (2004) in studies on two populations in the Netherlands, found PSC in around 63% of the 30 individuals analysed. This latter value is similar to the 69% of PSC individuals (150 plants sampled) found by Becker *et al.* (2006) in 15 populations from Czech Republic, Germany, and the Netherlands). The facility to produce some self seeds and establish new founder populations could be the key to success for a self-incompatible species such as *Hypochaeris radicata.* A similar situation was found in *Senecio squalidus,* another successful invasive alien species with SI that has colonized most parts of the UK within the last 150 years (Hiscock 2000).

To summarize, our scenario for the phenomenally successful world-wide invasive weed *Hypochaeris radicata* is that this self-incompatible, perennial herb originated in a habitat of humid woodlands in Morocco. It initially expanded as a still heterocarpic species in the Quaternary into the Iberian Peninsula and the Central Mediterranean to give a stable natural area of distribution. Only recently, with the evolution of homocarpy and a tolerance for the grassland habitat, it expanded via diverse human assisted transportations across C and N Europe, Asia, Australasia, and the Americas to establish as an aggressive colonizer.

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6 Biogeographic patterns in *Hypochaeris* sect. *Hypochaeris* (Asteraceae, Lactuceae) of the western Mediterranean

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Abstract

Aim We analysed biogeographic patterns in four species of *Hypochaeris* sect. *Hypochaeris*, including possible areas of origin and microevolutionary processes that have shaped their morphology, genetics and distribution.

Location Western Mediterranean area.

Methods Amplified Fragment Length Polymorphism (AFLP) markers have been used in a total of 494 individuals belonging to 82 populations of *H. arachnoidea*, *H. glabra*, *H. radicata*, and *H. salzmanniana* to detect population structure.

Results Morocco is the ancestral area of *H. glabra*, *H. radicata* and *H. salzmanniana*, where most private and rare fragments are found. The Strait of Gibraltar is only a weak barrier separating populations in *H. radicata* and *H. glabra*, but it appears as a strong barrier among populations of *H. salzmanniana*. The Guadalquivir River (SW Spain) is an efficient barrier in *H. glabra* and *H. radicata*. *Hypochaeris arachnoidea* appears to have originated in the Atlas Mountains, subsequently descending to lower elevations.

Main conclusions La Mamora forest and the central Middle Atlas in Morocco, based on higher levels of genetic variation, appear to have been a center for the origin of sect. *Hypochaeris*. We hypothesize that the common ancestor of section *Hypochaeris* was a perennial and self-incompatible species. Increased aridity as a consequence of the appearance of the Mediterranean climate may have resulted in new habitats that encouraged speciation within the section.

Introduction

Among continental areas of the world, the Mediterranean region has undergone dramatic geomorphological and environmental changes during the past eight million years (Thompson 2003). The principal impacts have been the closing of the Mediterranean sea (at 7–6 mya), the opening of the Strait of Gibraltar (at 5.33 mya), and the definitive establishment of the Mediterranean climate (~3.5 mya), followed by Pleistocene glaciations ending 12,000 years ago (Cosson *et al.* 2005). The first impact led to the formation of numerous hypersaline lakes of different sizes in the previous Mediterranean basin, plus providing many land corridors that united southern Europe and northern Africa (Bocquet *et al.* 1978). The opening of the Strait resulted in fragmented land masses or new islands, especially in the western Mediterranean, that through time became reintegrated into northwestern Africa (Patarnello *et al.* 2007). The development of the Mediterranean climate resulted in aridity of the Mediterranean Basin, and the origin of the large N African desert (Barrón & Peyrot 2006). The impact from Pleistocene glaciations lowered sea levels, hence joining previously separated land areas (such as Corsica and Sardinia), followed by rebounding higher sea level after the ice sheet retreated northward. All of these influences have led to complex evolutionary and biogeographic patterns now revealed in the biota of the Mediterranean region, especially in the western part (Jong 1998; Véla & Benhouhou 2007).

More specific impacts in the western Mediterranean include the effects of the Messinian salinity crisis (Bocquet *et al.* 1978), the formation of the Guadalquivir River and its floodplain in southern Spain, and the development of refugia as a consequence of Quaternary glaciations. Between 5.96 and 5.33 million years ago (Duggen *et al.* 2003) the Mediterranean Sea lost contact with the Atlantic Ocean, thus offering a bridge for migration of biota north and south between Spain and Morocco. At 5.33 mya the Strait of Gibraltar opened (Krijgsman *et al.* 1999), separating Europe and Africa. A number of studies have shown that this interrup-

tion has had an important role in the formation of distinct genetic populational systems north and south, in some cases followed by migration in either direction (e.g. Vargas *et al.* 1999; Álvarez *et al.* 2000; Castella *et al.* 2000; Harris *et al.* 2002; Lumaret *et al.* 2002; Gantenbein & Largiadèr 2003; Hampe *et al.* 2003; Terrab *et al.* 2007). The present location of the Guadalquivir River was a shallow tongue of the sea five million years ago (Riding *et al.* 1998; Braga & Aguirre 2001; Cosson *et al.* 2005). As the sea retreated, the Guadalquivir region was formed by deposition (Soria *et al.* 1999) and yielded the present river with its broad floodplain. This has remained a barrier to a number of organisms despite that it became passable by land (García-París *et al.* 1998; García-París & Jockusch 1999). The Atlas Mountains of Morocco, comprising four ranges that run south-north, are known to harbour high levels of endemic taxa (Quezel 1978; Médail & Quézel 1997, 1999). It has been hypothesized that due to changing sea levels affecting lowland populations plus changing climate, these regions served as refugia during Pleistocene glaciations (Terrab *et al.*unpubl.).

To further assess the impact of these environmental changes in the western Mediterranean requires examination of additional organismic groups. The flowering plant genus *Hypochaeris* (Asteraceae, Cichorieae) is well represented in this region and serves as a useful model for further studies. The genus consists of approximately 58 species worldwide with 15 confined to the Mediterranean region, three in Eurasia, and more than 40 in South America. Of those in the Mediterranean area, section *Hypochaeris,* with four species, is centered in the Western Mediterranean. The section consists of *H. salzmanniana*, confined to the Atlantic coasts of southern Spain and Morocco, *H. arachnoidea*, restricted to the mountains of Morocco and Algeria, and *H. glabra* and *H. radicata*, both presently worldwide weeds and possibly native to the Western Mediterranean area. In this paper the species of section *Hypochaeris* are been examined closely for patterns of morphological, reproductive biological, and genetic variation that might be correlated with known geomorphological alterations in the western Mediterranean

region. In general, *Hypochaeris* is a suitable generic system in which to evaluate biogeographic patterns because of the many existing background studies already completed on the cytology and cytogenetics (Cerbah *et al.* 1995; Ruas *et al.* 1995; Cerbah *et al.* 1997; Weiss-Schneeweiss *et al.* 2003; Weiss *et al.* 2003; Weiss-Schneeweiss *et al.* 2007; Ruas *et al.* in press; Weiss-Schneeweiss *et al.* in press), DNA sequencing (Cerbah *et al.* 1998; Samuel *et al.* 2003; Tremetsberger *et al.* 2005), AFLP populational analyses (Stuessy *et al.* 2003; Tremetsberger *et al.* 2003a, 2003b, 2004, 2006; Muellner *et al.* 2005; Ortiz *et al.* 2007) and reproductive biology (Ortiz *et al.* 2006). Much attention has previously been given to the South American species, because this is where the highest level of specific diversity occurs.

We review available data for *Hypochaeris* sect. *Hypochaeris* and focus on three biogeographic patterns: (1) The impact of the Strait of Gibraltar on populations of *H. salzmanniana*, *H. radicata* and *H. glabra*; (2) the impact of the Guadalquivir River in southern Spain on population divergence in *H. radicata* and *H. glabra*; and (3) patterns of genetic divergence in *H. arachnoidea* in Morocco as relating to possible ancestral area for origin of the section.

Material and Methods

Hypochaeris *sect.* Hypochaeris

Section *Hypochaeris* is a monophyletic group (Tremetsberger et al. 2005) composed of four species: *H. glabra*, *H. radicata*, *H. arachnoidea* and *H. salzmanniana*. This section shows an interesting variability in different aspects: in life-forms, *H. radicata* is perennial whereas *H. arachnoidea*, *H. glabra*, and *H. salzmanniana* are annuals; in somatic chromosome number, *H. glabra* is $2n = 10$, whereas *H. arachnoidea*, *H. radicata*, and *H. salzmanniana* are $2n = 8$ (Tremetsberger et al. 2005); in distribution, *H. glabra* and *H. radicata* are widespread in the Mediterranean region and worldwide weeds, whereas *H. arachnoidea* is

endemic to NW Africa (Morocco and Algeria) and *H. salzmanniana* occupies an even smaller area, restricted to the Atlantic coast of Morocco and SW Spain (Cádiz). The natural habitat of these species is the understory of open *Quercus* woodland, but *H. salzmanniana* lives principally in coastal dunes.

Another important characteristic of the genus *Hypochaeris* is sporophytic self-incompatibility, requiring pollination vectors, principally bees, for pollen transport between plants. Within sect. *Hypochaeris* we encounter variability of this system: *H. glabra* is self-compatible, *H. radicata* and *H. arachnoidea* are self-incompatible, and *H. salzmanniana* presents differences at the populational level with some populations completely self-incompatible or completely self-compatible, and others mixed (Ortiz *et al.* 2006).

Sampled populations

We used a total of 494 individuals belonging to 82 populations of the four species of sect. *Hypochaeris* (Fig. 1, 2 and Appendix). The sampling of populations was concentrated on the western Mediterranean region but also included other populations from throughout the distributional ranges: 15 populations of *H. glabra* (5 from Morocco, G1–G5; 8 from the Iberian Peninsula, G6–G13; one from Canary Islands, G14; and one from Chile, G15); 44 populations of *H. radicata* [11 from Morocco, R1–R11; 5 from the Central Mediterranean area (Italy, Sicily and Tunisia), R12–R17; 17 from the Iberian Peninsula, R18–R34; 3 from West and Central Europe (France, Netherlands and Austria), R35–R37; 3 from Asia (Taiwan and South Korea), R38–R40; and 4 from South America (Argentina and Chile), R41–R44]; 9 Moroccan populations of *H. arachnoidea* (A1–A9); and 14 populations of *H. salzmanniana* (6 from Morocco, S1–S6; and 8 from Cádiz, Spain, S7–S14). Of these, all populations of *H. arachnoidea* and Moroccan and C and SE Spanish populations of *H. glabra* have been newly analysed for AFLP. Populations of *H. radicata* are those of Tremetsberger et al. (2004) and Ortiz *et al.* (in rev.)

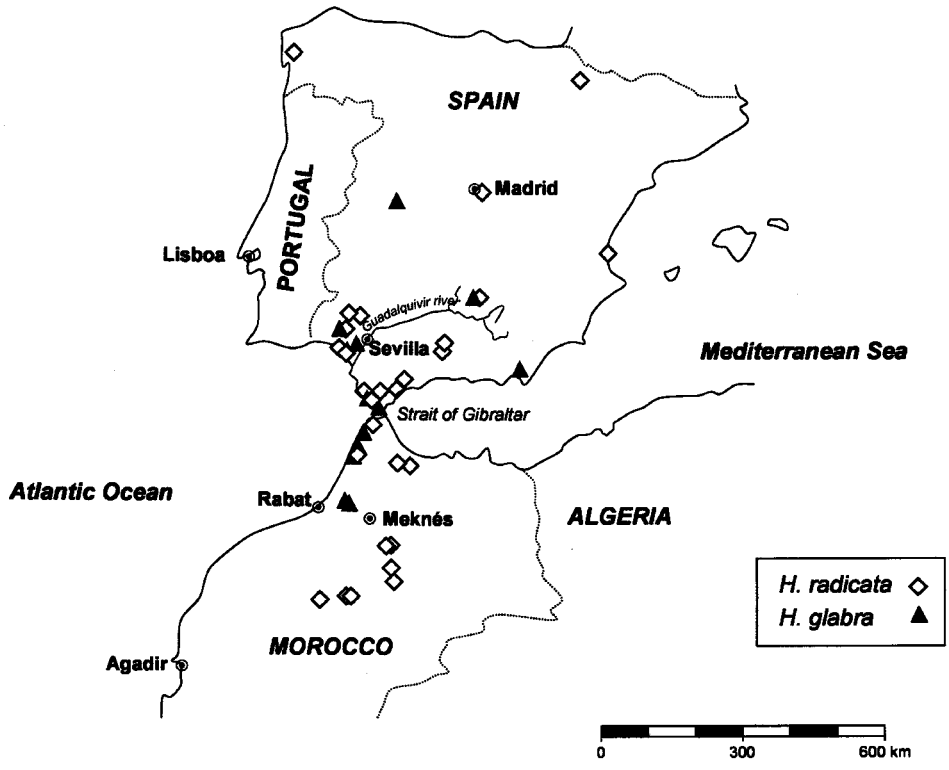

Fig. 1. Sampling localities for species of *H. glabra and H. radicata* in the W Mediterranean region.

and populations of *H. salzmanniana* are those of Tremetsberger *et al.* (2004) and Ortiz *et al.* (2007). The number of individuals sampled in each population is shown in the Appendix. Fresh leaves of the plants were collected at least one meter apart and dried in silica gel. Vouchers of all sampled populations are deposited in the Herbarium of the University of Seville (SEV, Spain) and/or University of Vienna (WU, Austria).

DNA isolation and AFLP analysis

Total genomic DNA was extracted from dry leaf material following the CTAB protocol (Doyle & Doyle 1987) with modifications. The AFLP procedure followed established protocols (Vos *et al.* 1995) with modifications (Tremetsberger *et al.*

2003a, 2004; Ortiz *et al.* 2007). The six primer combinations for the selective PCR selected by Tremetsberger et al. (2004) were applied to all four species: *MseI*-CTCG/*EcoRI*-ATC (Fam), *MseI*-CAC/*EcoRI*-ACG (Hex), *MseI*-CTA/*EcoRI*-ACC (Ned), *MseI*-CTG/*EcoRI*-ACA (Fam), *MseI*-CTC/*EcoRI*-AGG (Hex), *MseI*-CTGA/*EcoRI*-AAC (Ned) with three primers more selected by Ortiz et al. (2007): *Mse*I-CAC/*Eco*RI-ACT (Fam), *Mse*I-CTC/*Eco*RI-ATC (Hex), and *Mse*I-CTG/*Eco*RI-AAC (Ned) to get better resolution in *H. salzmanniana*. The fluorescence labelled selective amplification products were separated on a 5% polyacrylamide gel with an internal size standard (GeneScan®-500 ROX, PE Applied Biosystems) on an automated sequencer (ABI 377, Perkin–Elmer).

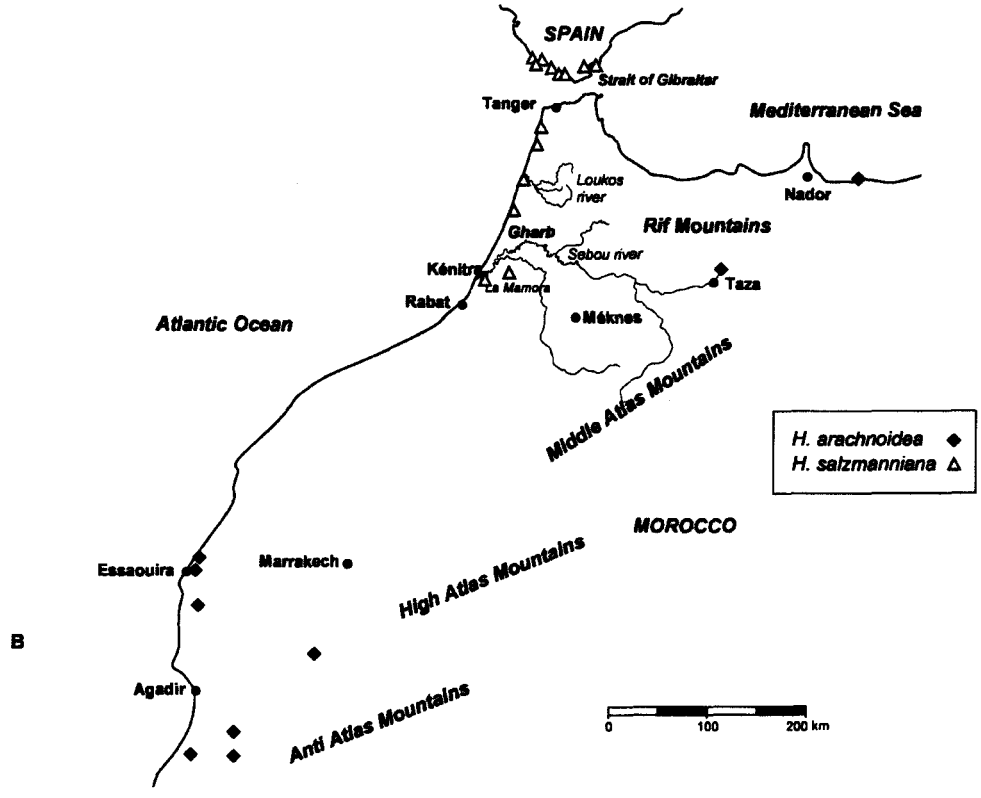

Fig. 2. Sampling localities for species of *H. arachnoidea and H. salzmanniana* in the W Mediterranean region.

Amplified fragments from 60 to 500 base pairs were scored and exported as a presence/absence matrix using ABI Prism GeneScan® Analysis Software 2.1 (PE Applied Biosystems) and Genographer (version 1.6.0 © Montana State University 2001; available at http://hordeum.oscs.montana.edu/genographer/).

Data analyses

To determine relationships among the four species of sect. *Hypochaeris*, we scored together one individual of five populations of each species, covering as much as possible the natural distributional range (*H. glabra*: G4, G5, G8, G10, and G12; *H. radicata*: R7, R21, R26, R27, and R28; *H. arachnoidea*: A1, A4, A6, A7, and A9; *H. salzmanniana*: S3, S6, S9, S11, and S13). With this presence/absence matrix, we constructed a dendrogram applying the Neighbor-Joining method (NJ) in conjunction with Nei & Li's (1979) genetic distances using PAUP* (v. 4.0b10; Sunderland, MA: Sinauer Associates). Support for each node was tested by 10,000 bootstrap replicates. We used FAMD v. 1.1 (Schlüter & Harris 2006) to change between different file formats and calculate private fragments in the species, i.e., those confined to only one species. Pairwise shared private fragments, fixed pairwise shared fragments, and pairwise fixation indices (AMOVA-derived F_{ST}; ARLEQUIN v. 3.01 (Excoffier *et al.* 2005) were assessed for each species.

In each species independently, the squared Euclidean distances matrix, based on AMOVA derived pairwise F_{ST}, was calculated with ARLEQUIN v. 3.01 (Excoffier *et al.* 2005) and imported into SPLITSTREE 4.6. (Huson & Bryant 2006) to construct a populational NJ dendrogram.

As a measure of within-population diversity, we assessed the percentage of polymorphic fragments ($Frag_{poly}$), as well as the number of private fragments ($Frag_{priv}$) for all populations of the four species of *Hypochaeris* sect. *Hypochaeris*. As another measure of genetic variability, we also calculated the average gene diversity (H_D; ARLEQUIN v. 3.01; Excoffier *et al.* 2005). We also looked for "rare fragments", i.e., those that were found in less than 10% of the individuals within a

population (Stehlik *et al.* 2002). The latter were used to calculate another index of divergence, DW ("frequency-down-weighted marker values") as proposed by Schönswetter & Tribsch (2005).

In order to examine the population structure of the four species, *H. glabra*, *H. radicata*, *H. arachnoidea*, and *H. salzmanniana*, we conducted an approach based on statistical inference with Bayesian clustering methods using BAPS v. 5.1 (Corander *et al.* 2003, 2004; Corander & Marttinen 2006); available at http://www.abo.fi/fak/mnf/mate/jc/software/baps.html), which uses stochastic optimisation instead of MCMC to find the optimal partition. The simulation was run from $K = 2$ to $K = N + 1$, where N is the number of populations analysed in each species, except for *H. radicata,* for which the simulation was run to 20, with five replicates for each K. We used the option "clustering of individuals" to estimate the admixture coefficients for the reference individuals, and this was performed with the following settings: minimal size of clusters at four individuals, 100 iterations to estimate the admixture coefficients for the individuals, 200 simulated reference individuals from each population, and 20 iterations.

To test for isolation by distance, we compared populations' pairwise F_{ST} values with their geographical distance using Mantel tests based on Spearman correlations (on 10,000 random permutations); this analysis was performed both on the entire sample and subgroups using XLSTAT-PRO 2007 (Addinsoft).

We used analyses of molecular variance (AMOVA; ARLEQUIN v. 3.01; (Excoffier *et al.* 2005) to distribute genetic variation into portions assignable to differences between predefined hierarchical groups (F_{CT}), among populations within these groups (F_{SC}), and among populations across the entire study area (F_{ST}) (Turner *et al.* 2000). We tested with AMOVA analyses the major geographic barriers in the three species *H. glabra*, *H. radicata* and *H. salzmanniana:* the Strait of Gibraltar, Guadalquivir River, and extension of the Rif Mountains.

Results

Phylogenetic relationships among the four species of sect. Hypochaeris

The AFLP primer combinations applied to five populations (one individual per population) of each of the four species of sect. *Hypochaeris* generated a variable number of fragments ranging from 32–118, of which a high percentage (72.5–100%) was polymorphic. The total number of fragments was 428, of which 406 (94.8%) were polymorphic. The NJ dendrogram with the four species of sect. *Hypochaeris* (Fig. 3) confirms the topology obtained from *rps16* intron (Tremetsberger *et al.* 2005). Each of the four species is well supported with bootstrap

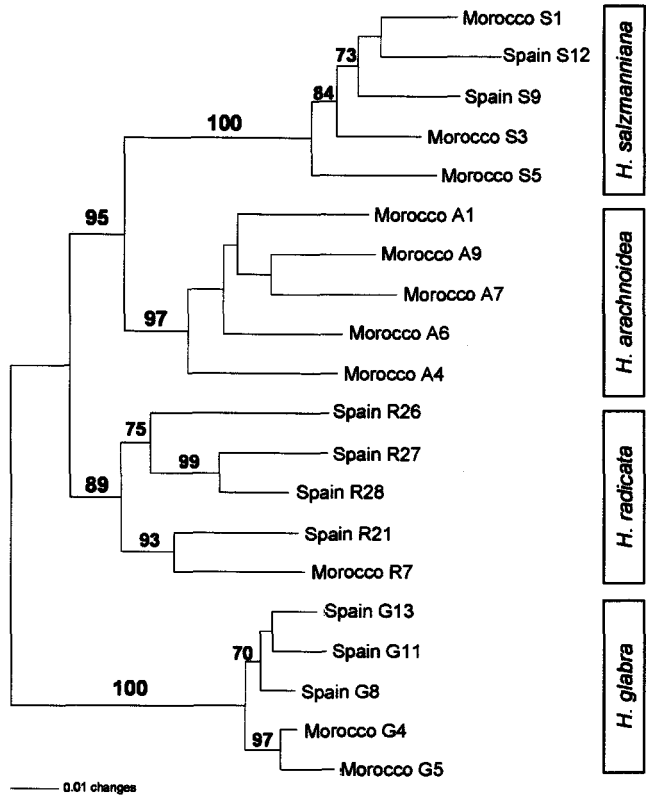

Fig. 3. NJ dendrogram of 20 individuals analysed for AFLP of *Hypochaeris glabra*, *H. radicata*, *H. salzmanniana*, and *H. arachnoidea*, based on Nei & Li's genetic distance. Bootstrap values based on 1000 permutations are indicated at each node (if greater than 50%).

values ranging from 89% (for *H. radicata*) to 100% (for *H. glabra* and *H. salzmanniana*). Based on sequence analysis (Tremetsberger *et al.* 2005), *H. glabra* was used to root the tree. *Hypochaeris arachnoidea* is sister to *H. salzmanniana* (95% BS) and *H. radicata* is sister to this latter group.

The species which showed the most private fragments (76, of which 10 were fixed) was *H. salzmanniana* (see Table 1). *Hypochaeris glabra* contains the lowest number of private fragments (52), but the highest number of fixed private fragments (20). *Hypochaeris radicata* shares most fragments with the other three species (a total of 45) and *H. glabra* is the species that shares the least private fragments with other species (a total of 26). Most private fragments are shared between *H. salzmanniana* and *H. arachnoidea* (20), indicating a close relationship. The species with a weaker relationship, based on AMOVA_derived F_{ST}, are *H. glabra* and *H. salzmanniana* ($F_{ST} = 0.67$).

Table 1 Pairwise shared private fragments (values below diagonal), pairwise fixation index (AMOVA-derived *F*ST; above diagonal), and private fragments (F_{priv}) applied to five populations (one individual per population) of each species of sect. *Hypochaeris*. Based on analysis of a total of 428 AFLP fragments. Numbers in parentheses refer to fixed fragments.

	H. glabra	*H. radicata*	*H. salzmanniana*	*H. arachnoidea*
H. glabra	–	0.56	0.67	0.60
H. radicata	13 (1)	–	0.44	0.34
H. salzmanniana	5	18	–	0.45
H. arachnoidea	8	14	20 (2)	–
F_{priv}	52 (20)	64 (3)	76 (10)	62 (4)

Phylogeographical patterns within each species

The six AFLP primer combinations applied to *H. glabra* yielded a total of 242 fragments, of which 81.8% were polymorphic. The NJ unrooted dendrogram and the Bayesian analysis, conducted with BAPS, applied to *H. glabra* (Fig. 4A),

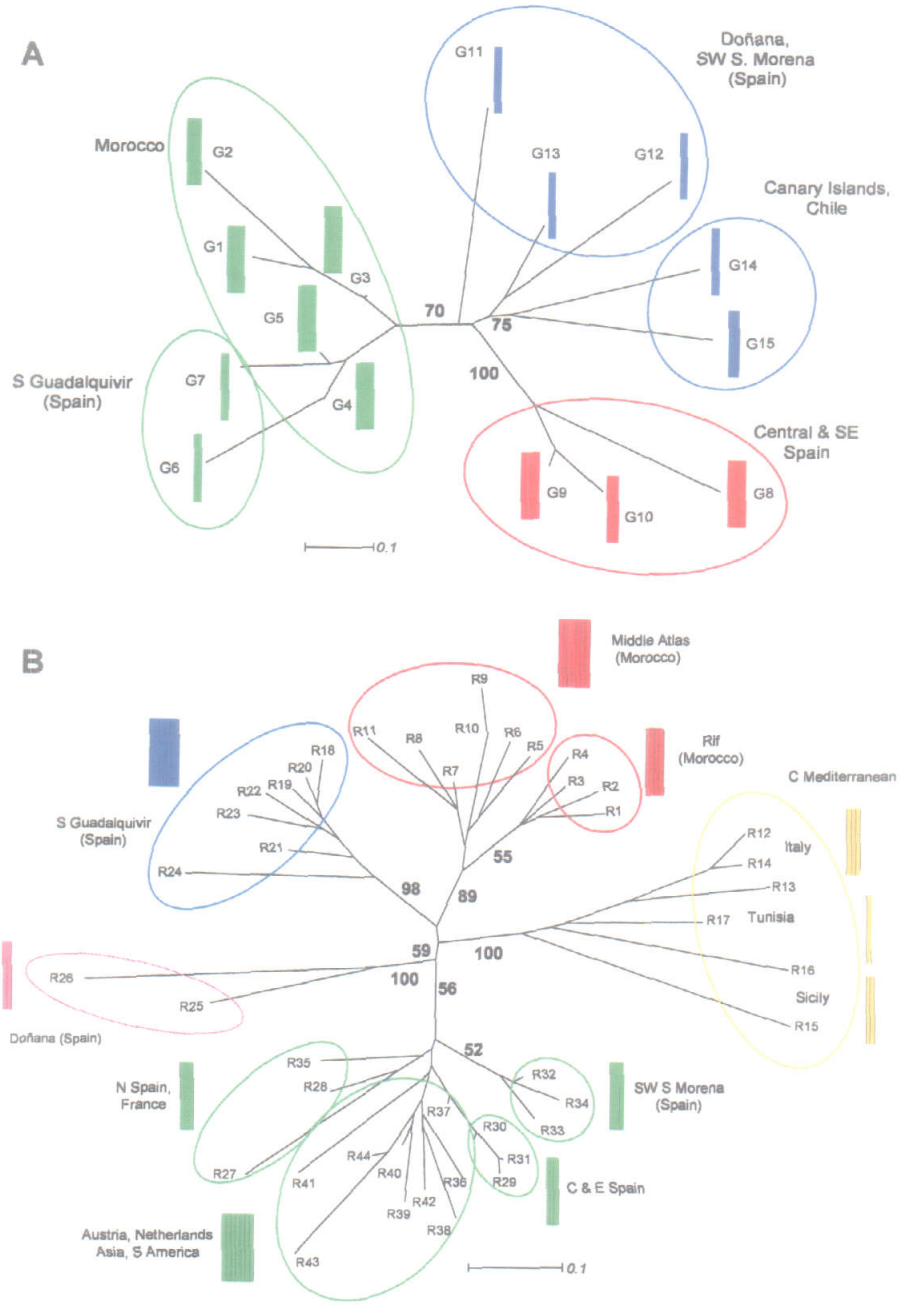

Fig. 4. NJ unrooted dendrograms, based on AMOVA-derived F_{ST} values of *Hypochaeris glabra* (A) and *H. radicata* (B) based on Nei & Li's genetic distance. Bootstrap values based on 10000 permutations are indicated at each node (if greater than 50%).

showed three main clusters: the first (70% BS) includes the populations of Morocco (G1–G5) and Spanish populations S Guadalquivir River (Cádiz; pops. G6–G7); the second cluster includes C & SE Spanish populations (G8–G10; 100% BS); and the remaining populations form a distinct Bayesian cluster, but without bootstrap support with the NJ method, grouping Doñana and SW Sierra Morena (SW Spain, pops. G11–G13) together with Canary Islands (pop. G14) and Chile (pop. G15; the latter two supported by 75% BS). The main divide within the species is between Morocco and S Guadalquivir River (Spain), on the one hand, and Doñana, SW Sierra Morena, introduced populations, and C & SE Spain, on the other (70% BS). Genetic diversity measures for each population of *H. glabra* are shown in Table 2. The highest numbers of polymorphism and average gene diversity are found in Moroccan populations ($\%F_{poly}$ = 28.61 and H_D = 0.091) and C & SE Spanish populations ($\%F_{poly}$ = 28.55 and H_D = 0.091; Fig. 5). The highest number of rare fragments and private fragments is found in the Moroccan populations (DW = 5.68 and F_{priv} = 4.8) and the lowest number in Spanish populations, S Guadalquivir River (DW = 0.15 and F_{priv} = 0).

Hypochaeris radicata population structure was analysed in detail by Ortiz et al. (in rev.). These data were used to construct a NJ unrooted tree and a Bayesian clustering (Fig. 4B). The main clusters were: Morocco; C Mediterranean; S Guadalquivir (Spain), Doñana N.P. (Spain); and the last one which includes SW Sierra Morena, N, C & E Spain, and the introduced accessions of the species. The averaged genetic diversity parameters are shown in Fig. 5. Morocco appears as the group with the highest diversity.

Hypochaeris salzmanniana population structure was analysed in detail by Ortiz et al. (2007). The results of the NJ unrooted dendrogram (Fig. 6A) showed five main clusters: Algeciras Bay (Spain), Sierra San Bartolomé (Spain), south Loukos river (Morocco), north Loukos river (Morocco) and Barbate (Spain), these latter two grouped in the same Bayesian cluster. The averaged genetic diversity

parameters are shown in Fig. 7. Morocco, south Loukos, appears as the group with the highest diversity.

Table 6. 2. Total nº fragments (F_{tot}), % polymorphic fragments (F_{poly}), fixed fragments (F_{fixed}), private fragments (F_{priv}), rare fragments (DW), average gene diversity (H_D) and number of samples (N_{AFLP}) of *Hypochaeris glabra* and *H. arachnoidea* populations sampled for AFLP.

	Species, localities	F_{tot}	F_{poly} (%)	F_{priv}	DW	H_D	N_{AFLP}
	H. glabra						
G1	Morocco. Tanger.	141	22.69	2	3.1	0.0677	5
G2	Asilah.	133	10.53	1	1.3	0.0306	4
G3	Larache	162	40.74	5	6.5	0.1339	5
G4	Kenitra, La Mamora 1.	167	40.12	12(1)	10.5	0.1298	5
G5	Kenitra, La Mamora 2.	145	28.96	4	7	0.0939	5
G6	S Guadalquivir, Spain, Barbate	112	-	0	0	-	2
G7	Punta Paloma	119	10.08	0	0.3	0.0489	2
G8	C & SE Spain, Cáceres	145	12.41	0	0	0.0326	5
G9	Jaen, Santa Elena.	142	47.18	5	5.5	0.1404	5
G10	Almería, Tabernas.	142	26.06	0	1.2	0.1007	3
G11	SW Sierra Morena, Spain, Valverde.	126	3.17	0	3.5	0.0163	2
G12	Doñana, Spain, Hinojos.	119	5.04	0	1.8	0.0245	2
G13	P.N. Doñana.	129	15.50	2	3.8	0.0816	2
G14	Introduced, Spain, Canary Islands.	125	6.40	1(1)	2.7	0.0326	2
G15	Chile, Ñuble.	124	10.48	0	2.5	0.0354	3
	H. arachnoidea						
A1	Taza.	160	60.62	24(2)	27.9	0.0836	9
A2	Essaouira, Moulay-Bouzerktour.	198	67.17	8	18.0	0.1106	10
A3	Essaouira.	197	64.97	4	13.8	0.1049	10
A4	J. Amsittene.	217	66.36	12	26.7	0.1140	10
A5	Anti Atlas, Biougra.	230	69.56	5	20.3	0.1549	7
A6	Tafraoute.	240	72.92	23	43.4	0.1441	10
A7	Nador, H Atlas, A Atlas, Nador.	190	60.00	9	18.3	0.0998	9
A8	Tifnit.	224	70.09	17	30.6	0.1331	10
A9	Tizi-N-Test.	235	73.62	20	31.2	0.1516	9

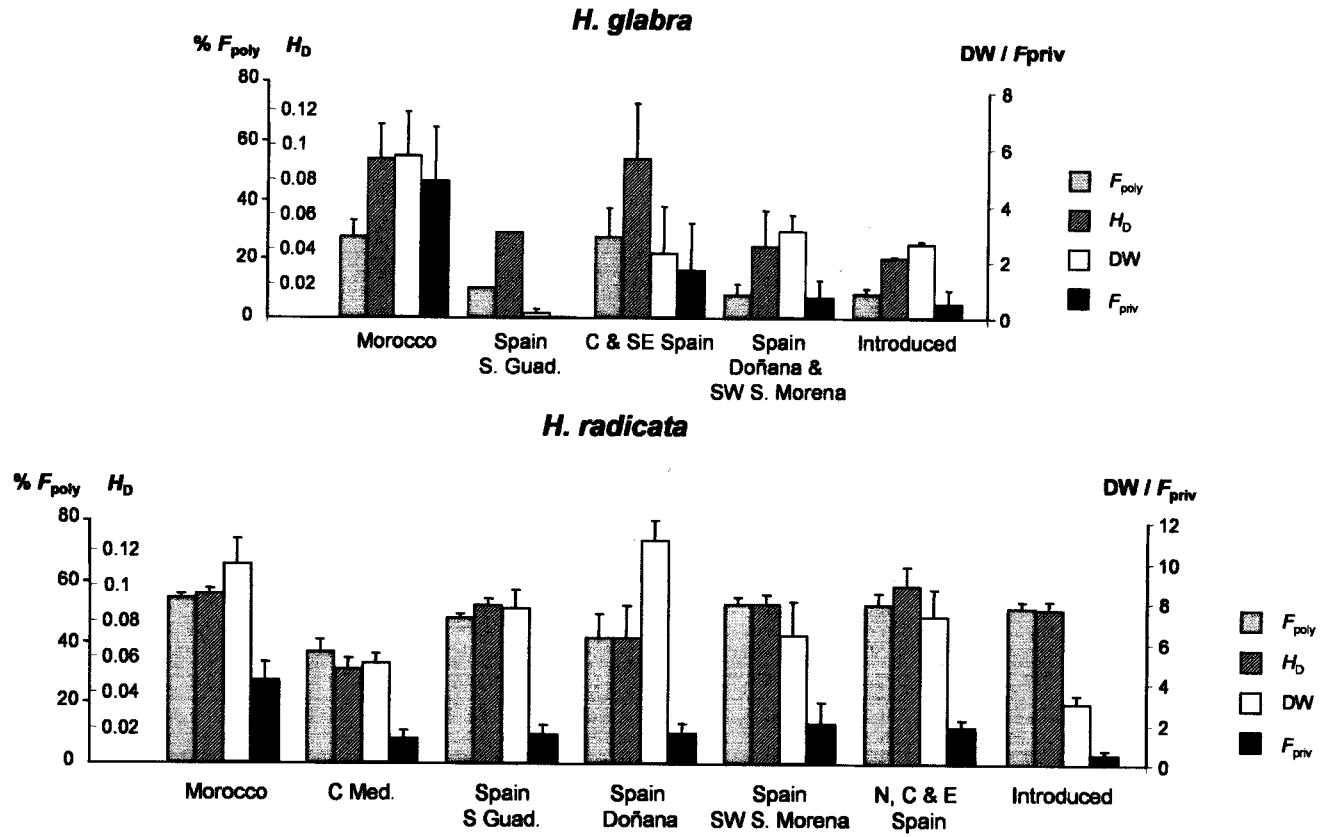

Fig. 5. Percentage of polymorphic fragments (F_{poly}), average gene diversity (H_D), index of rare fragments (DW), and private fragments (F_{priv}) averaged for each population within groups defined by Bayesian clustering and geographical occurrence in *Hypochaeris glabra* and *H. radicata*. For details of number of populations and individuals studied see Appendix.

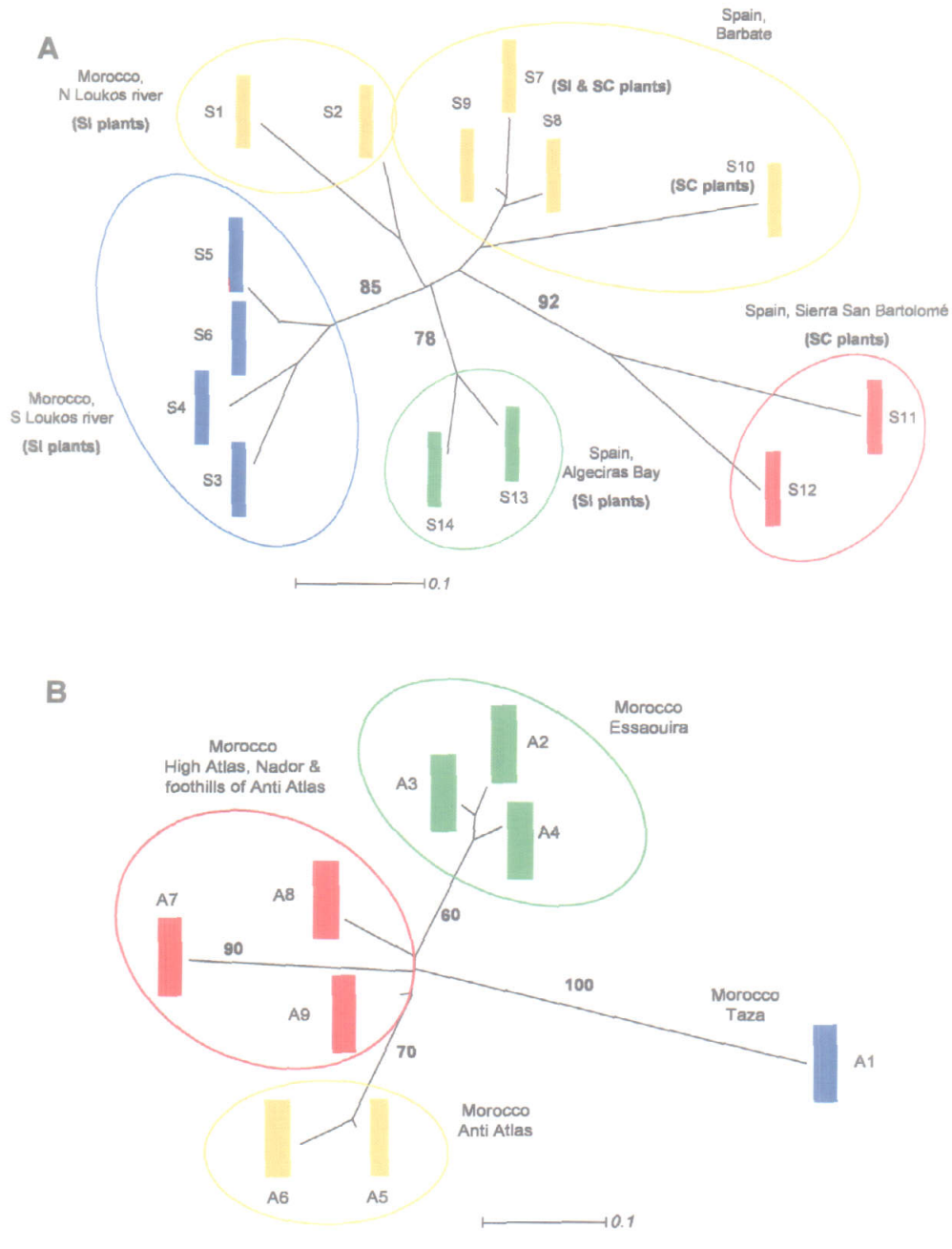

Fig. 6. NJ unrooted dendrograms, based on AMOVA-derived F_{ST} values of *Hypochaeris H. salzmanniana* (C), and *H. arachnoidea* (D), based on Nei & Li's genetic distance. Bootstrap values based on 10000 permutations are indicated at each node (if greater than 50%).

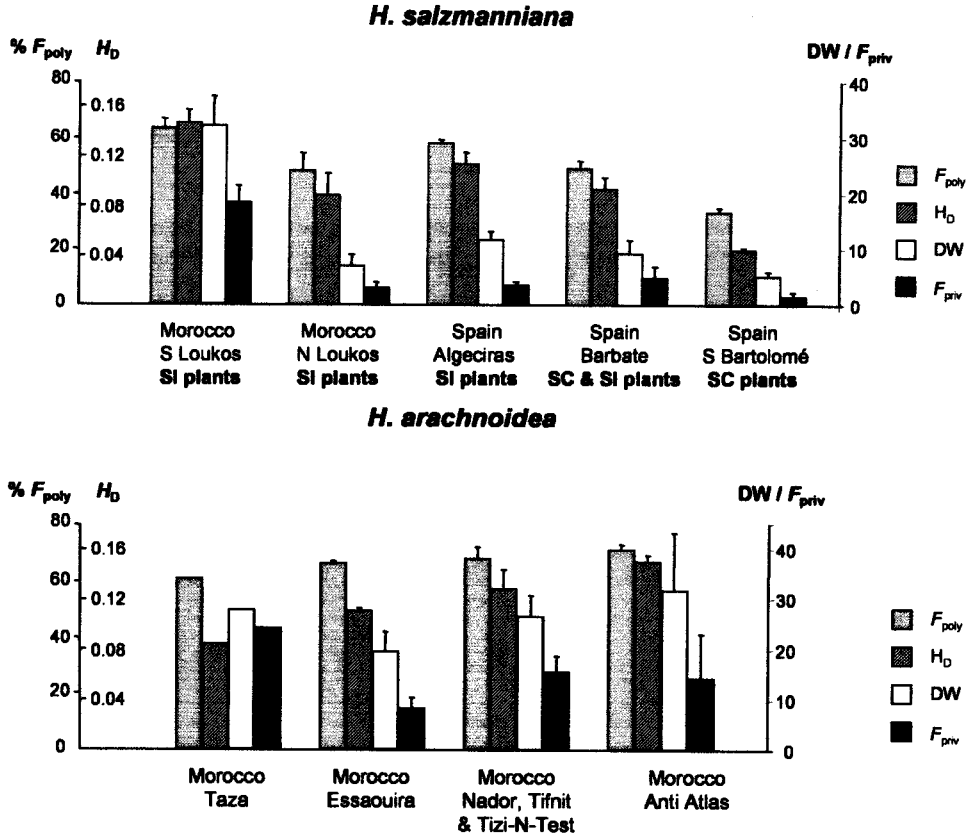

Fig. 6.7. Percentage of polymorphic fragments (F_{poly}), average gene diversity (H_D), index of rare fragments (DW), and private fragments (F_{priv}) averaged for each population within groups defined by Bayesian clustering and geographical occurrence in *Hypochaeris salzmanniana* and *H. arachnoidea*. For details of number of populations and individuals studied see Appendix.

The six AFLP primer combinations applied to *H. arachnoidea* yielded a total of 499 fragments, of which 93.2% were polymorphic. *H. arachnoidea* presents four main clusters (Fig. 6B), the first from Taza, Rif Mountains (A1; 100% BS), the second from Essaouira, S Moroccan Atlantic coast (pops. A2–A4; 60% BS), and the third from the Anti Atlas Mountains (pops. A5–A6; 70% BS). The last Bayesian cluster (not a group in the NJ tree) comprises geographically dispersed populations from Nador, in the Mediterranean coast of Morocco (A7), Tiznit, in the foothills of the Anti Atlas Mountains, close to the Atlantic coast (pop. A8), and Tizi-N-Test from High Atlas Mountains (pop. A9). Genetic diversity measures for each population of *H. arachnoidea* are shown in Table 2. The highest number of polymorphism, average gene diversity, and rare fragments is found in the Anti Atlas populations (pops. A5 to A6; % F_{poly} = 71.24; H_D = 0.1495; DW = 31.85; Fig. 7). The highest number of private fragments is found in Taza (pop. A1; F_{priv} = 24).

Testing the Strait of Gibraltar and Guadalquivir River as barriers

The analyses of molecular variance between the potential biogeographic barriers Strait of Gibraltar, Guadalquivir River, and extension of the Rif Mountains (Table 3) show that the Strait of Gibraltar is not the major barrier to gene flow within any of the three species distributed across it. In *H. glabra* and *H. radicata*, the main barrier coincides with the Guadalquivir River in Andalusia, Spain. The Guadalquivir River accounts for 35 % variance in *H. glabra* (compared to 27 % variance across the Strait of Gibraltar). In *H. radicata*, the Guadalquivir River and Strait of Gibraltar account for similar levels of genetic variance (20 % and 19 %, respectively). In *H. salzmanniana*, the Strait of Gibraltar accounts for very little variance (12 %). Rather, the extension of the Rif Mountains to the Atlantic coast in Morocco is a much more important barrier in *H. salzmanniana*, accounting for 18 % of variance.

Table 3. Comparison of molecular variance (AMOVA) across the major geographic barriers Strait of Gibraltar, Guadalquivir River and Loukos river in the three species *H. glabra*, *H. radicata* and *H. salzmanniana*.

	Source of variation	d.f.	SS	Variance comp.	% of variance	*F*-values*
H. glabra						
a [1–15]	Among pops.	14	894.90	15.79	61.74	F_{ST} = 0.62
	Among indiv.	37	361.98	9.78	38.26	
b **Strait of Gibraltar** [Morocco], [S Guadalquivir + Doñana + SW S. Morena + C & SE Spain + Introduced]	Among groups	1	261.46	7.90	27.03	F_{CT} = 0.27
	Among pops.	13	633.44	11.54	39.48	F_{SC} = 0.54
	Among indiv.	37	361.98	9.78	33.48	F_{ST} = 0.66
c **Guadalquivir River** [Morocco + S Guadalquivir], [Doñana + SW S. Morena + C & SE Spain + Introduced]	Among groups	1	327.50	10.77	35.27	F_{CT} = 0.35
	Among pops.	13	567.40	9.99	32.70	F_{SC} = 0.50
	Among indiv.	37	361.98	9.78	32.03	F_{ST} = 0.68
H. radicata						
d [1–44]	Among pops.	43	3797.81	15.08	49.56	F_{ST} = 0.50
	Among indiv.	169	2593.23	15.34	50.44	
e **Strait of Gibraltar** [Morocco + C Mediterranean] [S Guadalquivir + Doñana + SW S. Morena + N, C & E Spain + Introduced]	Among groups	1	724.17	6.42	19.04	F_{CT} = 0.19
	Among pops.	42	3073.64	11.95	35.45	F_{SC} = 0.44
	Among indiv.	169	2593.23	15.34	45.51	F_{ST} = 0.55
f **Guadalquivir River** [S Guadalquivir + Morocco + C Mediterranean] [Doñana + SW S. Morena + N, C & E Spain + Introduced]	Among groups	1	797.79	6.86	20.29	F_{CT} = 0.20
	Among pops.	42	3000.02	11.59	34.30	F_{SC} = 0.43
	Among indiv.	169	2593.23	15.34	45.41	F_{ST} = 0.55
H. salzmanniana						
g [1–14]	Among pops.	13	2065.29	13.35	34.45	F_{ST} = 0.34
	Among indiv.	126	3200.30	25.40	65.55	
h **Strait of Gibraltar** [Barbate + S. Bartolomé + Algeciras Bay], [N Loukos + S Loukos]	Among groups	1	468.80	4.90	11.93	F_{CT} = 0.12
	Among pops.	12	1507.88	10.76	26.22	F_{SC} = 0.30
	Among indiv.	126	3200.30	25.40	61.86	F_{ST} = 0.38
i **Loukos river** [Barbate + S. Bartolomé + Algeciras Bay + N Loukos], [S Loukos]	Among groups	1	557.41	7.56	17.58	F_{CT} = 0.18
	Among pops.	12	1507.88	10.03	23.33	F_{SC} = 0.28
	Among indiv.	126	3200.30	25.40	59.09	F_{ST} = 0.41

Discussion

Phylogeographic patterns in H. glabra, H. radicata, *and* H. salzmanniana

Hypochaeris glabra and *H. radicata* show very similar biogeographical patterns. Based on high numbers of private and rare fragments, we infer a Moroccan origin for *H. glabra* as well as for *H. radicata*. From here, *H. glabra* appears to have first dispersed to the region N of the Guadalquivir River (SW S. Morena, Doñana, C & SE Spain), from where the species dispersed further worldwide. The same basic pattern can be inferred for *H. radicata* (Ortiz *et al.* in rev.). The second dispersal of *H. glabra* from Morocco across the Strait of Gibraltar was to the region S of the Guadalquivir River as evidenced by strong genetic similarity between populations in these two regions. A second dispersal from Morocco to the region S of the Guadalquivir River (Betic Sierras) has also been inferred for *H. radicata* (Ortiz *et al.* in rev.). Whereas the two Doñana populations of *H. radicata* are strongly divergent genetically as well as morphologically different (Ortiz *et al.* 2006, in rev.), this is not the case for the Doñana populations of *H. glabra*. The latter species is widespread in the Doñana area, whereas populations of *H. radicata* are isolated and restricted to humid zones close to lagoons. The closing of the Strait of Gibraltar during the Messinian salinity crisis, forming a land bridge between southern Spain and northwestern Africa and remaining closed until 5.33 mya (Cosson *et al.* 2005), is excluded as a possible explanation for the similarity of populations of *H. glabra* and *H. radicata* on both sides of the Strait of Gibraltar, because the entire section *Hypochaeris* is evolutionarily younger (Tremetsberger *et al.* 2005, unpubl.), estimated to be of only Pliocene or Pleistocene age [1.7 to 2.0 mya (95% HPD = 0.6 to 3.5 mya); Tremetsberger *et al.* (unpubl.)].

As with *H. glabra* and *H. radicata*, *H. salzmanniana* is hypothesized to have originated in Morocco (Ortiz *et al.* 2007). The species appears to have migrated north from its southern Moroccan origin in *Quercus* woodlands. The main genetic division within the species is between populations on either side of the extension

of the Rif Mountains to the Atlantic coast in northern Morocco. From northern Morocco, the taxon is hypothesized (Ortiz *et al.* 2007) to have crossed the Strait of Gibraltar during Pleistocene cooler periods, when the sea level was considerably lower due to large amounts of water being retained in the ice sheets (Collina-Girard 2001). *Hypochaeris salzmanniana* developed differences in its compatibility system during its northward migration into Spain. It consists of completely self-incompatible individuals in all Moroccan and Algeciras Bay populations (S1–S6; S13–S14), mixed populations with self-compatible and self-incompatible individuals in the three Barbate populations (S7–S9), and all self-compatible individuals in the Sierra San Bartolomé and Zahara populations (S10–S12).

***Pleistocene glacial impact on* H. glabra, H. radicata, *and* H. salzmanniana**

Lowering of the sea level in the Strait of Gibraltar during Pleistocene glacial phases has been estimated at c. 130 m (Pou 1989; Yokoyama *et al.* 2000; Patarnello *et al.* 2007). Even though the European and African landmasses were not directly connected by a continuous land bridge, their coasts were considerably closer than today, especially on the Atlantic side of the Strait of Gibraltar, where the sea floor is not as deep as on the Mediterranean side. Emergent islands that were present periodically during glacial periods in the Strait of Gibraltar area have also favored contact between the two continents (Collina-Girard 2001).

The Strait of Gibraltar appears as a modest barrier to gene flow in *H. glabra* and *H. radicata*, but only a very weak barrier in *H. salzmanniana*. However, at least two dispersals from Morocco to the Iberian Peninsula are also inferred for *H. glabra* and *H. radicata* (this paper and Ortiz *et al.*, in rev.). *Hypochaeris salzmanniana* is a coastal species growing on sand dunes along the beaches and might have been better adapted to conditions presented by the exposed sea floor during glacial periods than *H. glabra* and *H. radicata*. The habitat of *H. glabra* is normally on sandy soils in woodlands, and not in coastal dunes as

H. salzmanniana. *Hypochaeris radicata* is confined to more humid habitats in the understory of *Quercus* woodland in the Mediterranean region.

***Impact of the Guadalquivir River on* H. glabra *and* H. radicata**

The Guadalquivir River begins in the Cazorla mountain range (Jaen) and flows into the Gulf of Cádiz, in the Atlantic Ocean, adjacent to the Doñana marshy lowlands. The Guadalquivir Basin separates the Sierra Morena (NW) and the Betic Cordillera (SE). Before the Messinian salinity crisis at the end of the Miocene, today's Guadalquivir Basin was a marine corridor (Betic Strait) that linked the Atlantic Ocean with the Tethys Sea. The Betic Strait filled up with Miocene and Pliocene sediments, originating periodically flooded marshlands, which persisted up to historical times as a large estuarine area, called Lacus Ligustinus by the Romans (Rodríguez-Ramirez *et al.* 1996). Thus, 2000 years ago the coastline was further inland than today. The freshwater wetlands were more extensive until the middle of the 19th century (Sousa & García-Murillo 2001, 2003). Today, the Doñana National Park adjacent to the estuarine of the Guadalquivir River is a remnant of this extensive marshland (Asensi & Díez-Carretas 1987).

Hypochaeris glabra and *H. radicata* show the same principal genetic division within the species across the Guadalquivir River (Spain) in the western Mediterranean region. In *H. radicata*, the morphologically and genetically divergent Doñana populations have an intermediate position between the two groups N and S of the Guadalquivir River. We hypothesize, on the basis of our results, that ancestral populations of *H. glabra* and *H. radicata* expanded out of northern Africa across the Strait of Gibraltar area into the southern Iberian Peninsula during the Quaternary (Ortiz *et al.* 2007). Dispersal into the Iberian Peninsula did not follow a continuous pattern. First, both species arrived to the northwestern side of the Guadalquivir River, probably because the coastline was more extensive and consequently closer to Morocco at that time. These populations expanded corresponding to the actual groups Doñana, Sierra Morena, N, C & E Spain, and

possibly Portugal. In a second more recent dispersal event, *H. glabra* and *H. radicata* reached the Iberian Peninsula more eastward to give rise to the southern Guadalquivir populations.

Interestingly, the Guadalquivir River is still a modern barrier preventing the admixture of populations. Today, the Guadalquivir Basin is an important agricultural area, in which *H. glabra* and especially *H. radicata* are seldom found. Possibly, this discontinuity in the distributional area is sufficient to prevent mixing of the two populational systems. The Sierra Morena to the NW of the Guadalquivir River and the Betic Cordillera to its SE offer rather different soil conditions. The Sierra Morena offers acidic Precambrian and Palaeozoic terrains, whereas the Betic Cordillera offers predominantly calcareous Mesozoic and Neogene terrains. *Hypochaeris radicata* from the Sierra Morena grows frequently and abundantly in the understory of *Quercus* forests, as well as in anthropogenically modified sites on different substrates in the rest of the Iberian Peninsula N of the Guadalquivir River. Many populations in this region are homocarpic (except for populations from Sierra Morena) in contrast to populations from the Betic Cordillera (S of the Guadalquivir River), which are heterocarpic (Ortiz et al., unpubl.). In the Betic Cordillera, *H. radicata* grows in the understory of humid *Quercus* forests close to water sources and is less common and less abundant than N of the Guadalquivir River. Moreover, *H. radicata* from the Betic Cordillera has broad and cordate external involucral bracts with a wide scarious margin in contrast to populations N of the Guadalquivir River, which have linear external involucral bracts without a scarious margin. Therefore, though the two populational systems N and S of the Guadalquivir River are fully crossable (Ortiz et al., pers. observ.), they might have evolved independently and adapted to different environmental conditions. Migrants across the Guadalquivir Basin might therefore be less competitive and do not establish on the other side. In *H. glabra*, an ecological distinction between the two populational systems N and S of the Guadalquivir River is less evident than in *H. radicata* and neither are we able to discern any morphological differences.

Differentiation across the Guadalquivir Basin has been documented in several animals (Busack 1986; García-París *et al.* 1998; García-París & Jockusch 1999; García-Paris *et al.* 2003; Sanmartín 2003) and in only two other plant genera apart from *Hypochaeris* (*Anthoxanthum*, Pimentel *et al.* 2007; *Senecio*, Comes & Abbott 1998). For instance, in *Discoglossus* and *Salamandra* (Amphibia), lineages are hypothesized to have been isolated by the opening of the Betic Strait or later due to the formation of the fluvial system during the Pliocene, and that this isolation has been maintained until recently by the Guadalquivir River Basin (García-París *et al.* 1998; García-París & Jockusch 1999).

Ancestral Morocco

Morocco has been inferred as the ancestral area of *H. radicata* and *H. salzmanniana* based on the presence of many private and rare AFLP fragments in this region (Ortiz *et al.* 2007, in rev.). *Hypochaeris radicata* has most private and rare fragments in the Rif, where it is found in wet pastures associated with *Quercus suber* forests, and in the central Middle Atlas where grows in wet pastures associated with forests of *Cedrus atlantica*. Ortiz *et al.* (2007) inferred *H. salzmanniana* to have originated in the southern part of its distributional range in Morocco, in the *Quercus suber* forests of La Mamora, close to Kenitra in the northwestern foothills of the Middle Atlas. *Hypochaeris glabra* also has the highest number of private and rare fragments in the La Mamora population (G4), where the species lives in the understory of the *Quercus suber* forest in mixed populations with *H. salzmanniana*. The second La Mamora population (G5) and the close Larache population (G3) also have comparatively high values for private and rare fragments indicating that the La Mamora area may be ancestral for *H. glabra*. The region of the La Mamora forest seems to have played an important role in the diversification within the section, which might relate to arid-wet cycles starting at 2.3 mya (Suc 1984).

The present distribution of *H. arachnoidea* is in NW Africa throughout the Atlas Mountains in Morocco and Algeria in dry, open woodland (Oberprieler 2002; Oberprieler & Vogt 2002; Förther & Podlech 2003). The probable origin of *H. arachnoidea* therefore is in the Atlas Mountains, though the exact location is difficult to determine, mainly because of a limited sampling (populations from Algeria and parts of the Moroccan Middle Atlas have not been collected). Moreover, unlike in the other three species of section *Hypochaeris*, the genetic grouping in *H. arachnoidea* does not follow a clear geographical pattern. The Bayesian clustering groups three populations of the region of Essaouira (A2–A4) together, as well as two populations from the Anti Atlas (A5–A6). The third cluster comprises only one population, Taza (A1), and the fourth cluster comprises very distant populations, namely Tiznit (A8) from the foothills of the Anti Atlas, Tizi-N-Test (A9) from the High Atlas, and Nador (A7) from the Mediterranean coast. We hypothesize dispersal over long distances to explain genetic similarities in the fourth cluster, possibly associated with human migrations.

Mountain populations of *H. arachnoidea* have higher levels of private and rare fragments than populations closer to the sea. The mean number of private fragments per population in mountain populations (A4 to A6, A8 to A9) is 17. In populations close to the sea [Moulay-Bouzerktour (A2) and Essaouira (A3) at the Atlantic coast and Nador (A7) at the Mediterranean coast] it is seven. Similarly, the mean number of rare fragments is 30.0 in mountain populations and 16.7 in populations close to the sea. Within the group of populations growing in the geographic vicinity of Essaouira, the mountain population J. Amsittene (A4) at 360 m also has higher values of private and rare fragments than populations close to the coast (A2 and A3) at 90 and 100 m. We hypothesize, therefore, that *H. arachnoidea* originated in a mountain habitat, and that the Atlas Mountains, at lower elevation, served as a Pleistocene refugium, from where *H. arachnoidea* descended. The Atlas Mountains are known for their high level of endemicity (Quezel 1978; Fennane & Ibn Tattou 1998). This is also evident in other species of

Hypochaeris, e.g., *H. angustifolia* and *H. leontodontoides* also being endemic to the Atlas Mountains (Galán de Mera & Vicente Orellana 1998a, 1998b). The Atlas Mountains have also been hypothesized as a refugium for species in other plant genera (Lumaret *et al.* 2002; Hampe *et al.* 2003; Terrab *et al.* 2006).

As a summary, we provide the following overview of evolutionary and biogeographic relationships in section *Hypochaeris*. According to the pattern of phylogenetic relationships (Fig. 2), *H. glabra* was the first species to diverge within the section. *Hypochaeris radicata* diverged from the common ancestor of *H. arachnoidea* and *H. salzmanniana*, and finally, *H. arachnoidea* and *H. salzmanniana* differentiated. We hypothesize that the common ancestor of section *Hypochaeris* was perennial and self-incompatible, because the majority of outgroup taxa in Hypochaeridinae have these features. We hypothesize that *H. glabra* originated as an adaptation to the new dry, sandy habitats of the lowlands generated by the newly established Mediterranean climate, thereby becoming annual and self-compatible. The ancestor of *H. radicata*, *H. arachnoidea*, and *H. salzmanniana*, however, remained perennial and self-incompatible in more humid habitats, which might have been present close to water sources, e.g., in the Sebou estuarine (the previous Rifian Corridor and the region called Gharb today), or in adjacent mountainous areas close to the central Middle Atlas. We hypothesize that there was a second adaptation to dry habitats, possibly in one of the following arid cycles, namely of the ancestor of *H. arachnoidea* and *H. salzmanniana*, again accompanied by a change to the annual condition, whereas *H. radicata* remained in the humid habitats of the Middle Atlas adjacent to La Mamora forest (as shown by high numbers of private and rare fragments in this region), thereby maintaining the perennial habit and self-incompatible breeding system. The next speciation event separated *H. arachnoidea* as a species adapted to dry mountain habitats in the Atlas Mountains and *H. salzmanniana* as a species adapted to sandy and dry habitats at lower elevation (La Mamora forest, where most private and rare fragments are found) and subsequently moving northward

along the Atlantic coast. Whereas *H. arachnoidea* and the oldest populations of *H. salzmanniana* remained self-incompatible, self-compatibility appeared in Spanish populations of the latter (Ortiz *et al.* 2006, 2007). Change of perennial to annual habit as an adaptation to dry environments with concordant change to a self-compatible breeding system in response to the rise of the Mediterranean climate has also been documented in *Bellis microcephala* (Fiz *et al.* 2002). Similarly, in Centaureinae (Asteraceae), for which the major modern or derived clades are inferred to have differentiated during transition from Pliocene to Pleistocene, aridisation is also thought to have favored development of biennial and annual species independently in different groups (Hellwig 2004).

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Appendix. Species, geographical – Bayesian groups, populations, localities, geographical coordinates, collector numbers, and sample sizes for analyzed populations of *Hypochaeris arachnoidea*, *H glabra*, *H. radicata*, and *H. salzmanniana*. (*AO* = A. Ortiz; *CB* = C.M. Baeza; *CM* = C. Mix; *FE* = F. Ehrendorfer; *KT* = K. Tremetsberger; *SC* = S. Castroviejo; *SO* = S. Ortiz; *ST* = S. Talavera; *TS* = T. Stuessy).

***H. arachnoidea* Poir.**

MOROCCO: TAZA—A1: c. Taza, 500 m, 34°19'/3°57', *ST 585/03M* (9). **ESSAOUIRA—A2:** c. Moulay-Bouzerktour, 90 m, 31°38'/9°39', *ST 98/03M* (10); **A3:** Essaouira, 100 m, 31°30'/9°41', *ST 92/03M* (10); **A4:** Essaouira-Agadir, J. Amsittene, 360 m, 31°11'/9°39', *ST 117/03M* (10). **ANTI ATLAS—A5:** c. Biougra, 980 m, 30°06'/9°13', *ST 138/03M* (7); **A6:** 33 km to Tafraoute, 1400 m, 29°48'/9°13', *ST 153/03M* (10). **NADOR, HIGH ATLAS & FOOTHILLS OF ANTI ATLAS—A7:** Nador, Cap de l'Eau, 50 m, 35°08'/2°25', *NL 1267* (9); **A8:** 15 km N to Tifnit, 160 m, 29°49'/9°37', *ST 180/03M* (10); **A9:** 25 km to Tizi-N-Test, 932 m, 30°47'/8°23', *ST 181/03M* (9).

***H. glabra* L.**

MOROCCO—G1: Tanger, Cap Spartel, 35°35'/5°59', *ST 13/03M* (5); **G2:** Asilah - Souk-Khémis-Es-Sahel, 35°17'/6°03', *ST 21/03M* (4); **G3:** Larache - Ksar el Kbir, 35°07'/6°09', *ST 34/03M* (5); **G4**: Kenitra, La Mamora, 34°12'/6°16', *ST 75/03M* (5); **G5**: Kenitra, La Mamora, 34°08'/6°09', *ST 88/03M* (5). **SPAIN, S GUADALQUIVIR—G6**: Cádiz, Vejer-Barbate, 36°12'/5°56', *TS 15* (2); **G7:** Cádiz, Punta Paloma, 36°04´/5°41´, *TS 31* (2); **C & SE SPAIN—G8**: Cáceres, Aldeanueva de la Vera, 40°08´/5°42´, *AO 88/01* (5); **G9**: Jaen, Santa Elena, Despeñaperros, 38°23'/3°31', *AO & KT 3/04* (5); **G10**: Almería, Tabernas, 37°02´/2°22´, *ST s/n & AO & KT 6/04* (3); **SPAIN, SW S. MORENA—G11**: Huelva, Valverde, 37°34'/6°45', *TS 45* (2); **SPAIN, DOÑANA—G12**: Huelva, Hinojos, Pino Gordo, 37°17´/6°22´ *ST s/n* (2); **G13**: Huelva, P.N. Doñana, 37°08'/6°32', *ST s/n* (2); **INTRODUCED—G14**: Canary Islands, Gran Canaria, 28°06'/15°25', *TS s/n* (2); **G15**: Chile, Región VIII, Ñuble, 36°36'/72°07', *CB 3924* (3).

***H. radicata* L.**

MOROCCO—R1: Tanger, Cap Spartel, 35°45'/5°54', *ST 1/03M* (5); **R2**: Tetouan, Larache, 35°07'/6°09', *ST 38/03M* (5); **R3**: Tetouan, c. Bab-Taza, 35°03'/5°12', *ST 454/03M* (5); **R4:** Tetouan, c. Bab-Berred, 34°59'/4°52', *ST 538/03M* (4); **R5:** Meknés, 24 km S Timahdite, 33°25'/5°11', *ST 660/03M* (5); **R6**: Meknés, 15 km S Azrou, 33°24'/5°13', *TK 63* (5); **R7**: Meknés, Col du Zad, 32°57'/5°08', *ST 709/03M* (5); **R8**: 5 km S of Boumia, 32°41'/5°05', *ST 275/03M* (5); **R9**: Beni-Mellal, Jbel Tassemit, 32°19'/6°16', *ST 244/03M* (5); **R10**: Beni-

Mellal, Jbel Tassemit, 32°18'/6°16', *ST 238/03M* (5); **R11:** Marrakech-Khemis-des-Oulad, 32°13'/6°46', *ST 224/03M* (5). **C MEDITERRANEAN—R12**: Italy, Foggia, Gargano, 41°50'/15°43', *KT s/n* (5); **R13:** Italy, Calàbria, Palmi, 38°21'/15°50', *KT s/n* (5); **R14**: Italy, Calàbria, Aspromonte, 38°09'/15°52', *KT s/n* (5); **R15**: Sicily, Palermo, La Pizzuta, 37°59'/13°15', *SC 5692* (5); **R16**: Sicily, Nebrodi, 37°53'/14°31', *FE 5* (4); **R17**: Tunisia, Jendouba, Ain Draham, 36°30'/8°46', *FE s/n* (5). **SPAIN, S GUADALQUIVIR —R18:** Córdoba, Luque-Carcabuey, 37°30'/4°16', *ST & AO s/n* (5); **R19**: Córdoba, Sierra de Rute, 37°30'/4°15', *ST s/n* (5); **R20:** Málaga, Ronda, La Nava, 36°40'/5°03', *ST s/n* (5); **R21:** Málaga, Gaucín, 36°31'/5°18', *ST 40* (3); **R22**: Cádiz, Alcalá de los Gazules, 36°27'/5°43', *MAO s/n* (5); **R23:** Cádiz, Chiclana, 36°25'/6°06', *MAO 89/02* (4); **R24**: Cádiz, Vejer, Montenmedio, 36°16'/5°58', *ST 87/01* (5). **DOÑANA, SPAIN—R25**: Huelva, P.N. Doñana, El Corchuelo, 37°12'/6°42', *ST s/n* (5); **R26**: Huelva, P.N. Doñana, El Acebrón, 37°08'/6°32', *ST s/n* (5). **N, C & E SPAIN—R27**: La Coruña, Outeiro, 42°48'/8°55', *SO s/n* (5); **R28:** Navarra, Yesa, Bigüenzal, 42°35'/1°10', *LV s/n* (5); **R29**: Madrid, campus UAM, 40°26'/3°42', *ST s/n* (3); **R30**: Valencia, El Saler, 39°22'/0°19', *OT 5/04* (5); **R31**: Jaen, Santa Elena, Despeñaperros, 38°23'/3°31', *OT 4/04* (5). **SW SIERRA MORENA, SPAIN—R32**: Huelva, Aracena, 37°53'/6°32', *AO s/n* (5); **R33:** Huelva, Santa Ana La Real, 37°52'/6°42', *ST s/n* (5); **R34**: Huelva, Valverde, 37°34'/6°45', *ST s/n* (5). **INTRODUCED—R35:** France, Toulouse, 43°36'/1°26', *MAO s/n* (5); **R36:** Netherlands, Konijnendijk, 52°02'/6°26', *CM s/n* (5); **R37**: Austria, Tirol, Reith im Alpbachtal, 47°24'/11°52', *TS s/n* (5); **R38:** Taiwan, Houhuan Mountain, 24°14'/121°09', *TS s/n* (5); **R39**: South Korea, Cheju Island, Chonwang, 33°30'/126°31', *TS 17511* (5); **R40:** South Korea, Cheju Island, Seogwipo, 33°15'/126°33', *TS 17520* (5); **R41**: Argentina, Jujuy, Yala, 24°07'/65°23', *TS 18056* (5); **R42:** Argentina, Río Negro, Cerro Tronador, 41°10'/71°49', *TS 18053P* (5); **R43**, Chile, Santa Barbara, 37°39'/72°01', *TS s/n* (5); **R44:** Chile, Region X, Volcán Choshuenco, 39°49'/72°04', *TS 15826* (5).

***H. salzmanniana* DC.**

MOROCCO, N LOUKOS RIVER—S1: Tanger, 35°35'/5°59', *ST 18/03M* (10); **S2:** Asilah, 35°29'/6°01', *ST 20/03M* (10); **MOROCCO, S LOUKOS RIVER—S3:** Larache, 35°07'/6°09', *ST 31/03M* (10); **S4:** Moulay Bousselham, 34°43'/6°15', *ST 51/03M* (10); **S5:** La Mamora Forest, 34°15'/6°19', *ST 71/03M* (10); **S6:** c. Kènitra, 34°13'/6°35', *ST 53/03M* (10). **SPAIN, CÁDIZ, BARBATE—S7:** Conil-El Palmar, 36°13'/6°04', *ST 5/02* (10); **S8**: Vejer-Barbate, 36°12'/5°56', *ST 14/02* (10); **S9**: Caños de Meca, 36°11'/6°01', *ST 24/02* (10); **S10:** Zahara. *ST 1/03*, 36°08'/5°51' (10); **SPAIN, CÁDIZ, SIERRA SAN BARTOLOMÉ**: **S11:** Punta Paloma, 36°04'/5°41', *ST 32/02, 3/03* (10); **S12:** Los Algarbes, 36°04'/5°41', *ST 2/03* (10); **SPAIN, CÁDIZ, ALGECIRAS BAY**: **S13:** Palmones. 36°10'/5°25', *ST 33/02* (10); **S14:** La Línea, 36°09'/5°20', *ST 35/02* (10).

Conclusiones

1. El género *Hypochaeris* se divide en cinco secciones: *Hypochaeris* sect. *Phanoderis* (todas las especies sudamericanas del género Hypochaeris e *H. angustifolia*), sect. *Amblachaenium* (*H. maculata*, *H. uniflora*, *H. grandiflora*), sect. *Metabasis* (*H. cretensis*, *H. oligocephala*), sect. *Seriola* (*H. leontodontoides*, *H. achyrophorus*, *H. rutea*, *H. laevigata*, *H. saldensis*) y la sect. *Hypochaeris* (*H. glabra*, *H. radicata*, *H. salzmanniana*, *H. arachnoidea*).

2. *Hypochaeris* sect. *Hypochaeris* es un grupo monofilético, hermano de la sección *Seriola*, compuesto por cuatro especies: *H. glabra*, *H. radicata*, *H. salzmanniana* e *H. arachnoidea*, siendo *H. salzmanniana* e *H. arachnoidea* hermanas de *H. radicata*, e *H. glabra* la especie basal de la sección.

3. *Hypochaeris* sect. *Hypochaeris* presenta un sistema de autoincompatibilidad esporofítico, siendo *H. glabra* la única especie completamente autocompatible de la sección, *H. radicata* e *H. arachnoidea* autoincompatibles e y presentando *H. salzmanniana* poblaciones con todos los individuos autocompatibles, poblaciones con todos los individuos autoincompatibles y poblaciones mixtas con unos individuos autocompatibles y otros autoincompatibles.

4. Existe correlación entre parámetros florales de las especies de la sección *Hypochaeris* y su grado de incompatibilidad. La especie autocompatible de la sección (*H. glabra*) presenta un diámetro del capítulo más pequeño, un número de flores por capítulo menor y está menos tiempo en antesis que las especies autoincompatibles (*H. radicata* e *H. arachnoidea*). Este mismo patrón se ha encontrado entre las poblaciones de *H. salzmanniana*. Las poblaciones autocompatibles tienen también menor diámetro, menor

número de flores y están menos tiempo en antesis que las poblaciones autoincompatibles, poniéndose también de manifiesto este fenómeno en el seno de las poblaciones mixtas, entre los individuos autocompatibles y los autoincompatibles.

5. Las poblaciones autoincompatibles de *H. salzmanniana* son las que presentan mayor diversidad genética (Marruecos y Bahía de Algeciras) y las autocompatibles son las más empobrecidas genéticamente (Punta Paloma, Los Algarbes y Zahara) mientras que las poblaciones mixtas presentan una diversidad genética intermedia. La aparición de la autocompatibilidad en esta especie puede haber sido una consecuencia de la pérdida de diversidad genética en el locus *S* debido a cuellos de botella poblacionales y deriva génica.

6. *Hypochaeris salzmanniana, H. radicata* e *H. glabra* presentan mayor diversidad genética y mayor número de alelos raros y privados en los alrededores del alcornocal de La Mamora, (*H. salzmanniana* e *H. glabra*) y en la zona central del Medio Atlas (*H. radicata*), ambos situados en Marruecos, lo que nos lleva a proponerlas como las áreas más primitivas de cada una de las especies.

7. El Estrecho de Gibraltar no ha sido una barrera infranqueable en ninguna de las tres especies de la sección que se distribuyen a los dos lados de sus aguas (*H. glabra*, *H. radicata*, *H. salzmanniana*) siendo el valle del Guadalquivir en *H. glabra* e *H. radicata*, y el valle del Loukos en *H. salzmanniana*, las principales barreras biogeográficas en estas especies.

8. El ancestro de la sección *Hypochaeris* pudo ser una hierba perenne y autoincompatible que vivió en el entorno del Atlas Medio y el alcornocal de La Mamora, en Marruecos. El incremento de la aridez, como consecuencia

de la implantación del clima mediterráneo, fomentó la aparición de nuevos hábitats favoreciendo un fenómeno de especiación. *H. glabra* pudo originarse como una adaptación a zonas más abiertas del bosque, arenosas, *H. radicata* permaneció en las zonas más húmedas, ocupando las dos especies más recientes los ambientes más xéricos, *H. arachnoidea* el interior e *H. salzmanniana* las arenas litorales.

UNIVERSIDAD DE SEVILLA

Reunido el tribunal en el día de la fecha, integrado por los abajo firmantes, para evaluar la tesis doctoral de D. Mª Angeles Ortiz Herrera titulada Biosistemática del género Hyprocharis sect. Hyprocharis: implicaciones filogeográfica y evolutiva

acordó otorgarle la calificación de SOBRESALIENTE CUM LAUDE (POR UNANIMIDAD)

Sevilla, a 29 de abril de 2008

Vocal,

Vocal,

Vocal,

Presidente,

Secretario,

Pablo Vargas

Doctorando,