Uso del hábitat por la hormiga mediterránea *Aphaenogaster senilis,* en Doñana: competencia y explotación de los recursos

TESIS DOCTORAL

Ángel Barroso Rodríguez

2012

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
ESTACIÓN BIOLÓGICA DE DOÑANA
Departamento de Etología y Conservación de la Biodiversidad

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Uso del hábitat por la hormiga mediterránea *Aphaenogaster senilis* en Doñana: competencia y explotación de los recursos

Memoria presentada por Ángel Barroso Rodríguez para
optar al grado de Doctor en Ciencias Biológicas

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DEDICATORIA

A mis padres
José Barroso y Antonia Rodríguez

Para Lupe

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ÍNDICES

Índice de contenidos

Índice de imágenes

CAPÍTULO 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

Índice de tablas

CAPÍTULO 1

CHAPTER 3

CHAPTER 4

CHAPTER 5

CAPÍTULO 1

Introducción general y objetivos

1.1 INTRODUCCIÓN

La enorme abundancia de las hormigas, combinada con su ubicuidad, convierte a estos organismos en elementos importantes en la mayoría de los ecosistemas terrestres. Tal importancia se ve reflejada en el gran número de interacciones en las que pueden jugar un papel relevante: interacciones competitivas, tróficas, mutualismos, respuestas a cambios ambientales... (Lach et al. 2010). Pero las hormigas son también importantes por su estatus de superorganismos que las sitúan entre los niveles de organización de individuo y ecosistema (Hölldobler & Wilson 2008), por ello las influencias mutuas que se producen entre su organización social y los factores ambientales, son también temas de interés en investigación tanto en ecología como en biología evolutiva.

El uso de los recursos alimentarios, las interacciones mutualistas planta-animal relacionadas con la dispersión de semillas, los patrones de distribución espacial y la dispersión colonial son temas típicos en ecología con repercusión en biología evolutiva. Esta tesis estudia aspectos de los temas anteriores para el caso de la hormiga mediterránea *Aphaenogaster senilis* en una zona del sur de España.

1.1.1 Dietas en hormigas

En los trópicos muchas especies de hormigas tienen una alimentación especializada (cortadoras de hojas, depredadores especialistas).En cambio, en las zonas templadas la mayoría de las especies son omnívoras, combinando de forma variable alimento de origen animal (presas vivas, cadáveres, excrementos y exudados de homópteros) y vegetal (partes sólidas y néctar u otros líquidos) (Stradling 1978; Tobin 1994; Blüthgen & Feldhaar 2010). No obstante, mediante el uso de isótopos estables se ha podido demostrar que, además de las hormigas granívoras, hay otras especies que son extremadamente herbívoras (por ejemplo, especies del género *Camponotus*), ya que presentan valores muy bajos de δ^{15} N (Gibb & Cunningham 2011).

La dieta de las hormigas varía en relación a factores, tanto internos (de la colonia) como externos (ambientales). Sin embargo, en pocas especies se conoce con detalle la dieta y puede reconstruirse su papel trófico (Blütghen & Feldhaar 2010). La dieta se relaciona también con otros aspectos ecológicos importantes como la productividad de la colonia, o la razón de sexos. Igualmente, para el caso de las hormigas dispersoras de semillas, la carencia de información sobre la alimentación natural limita la comprensión del alcance del fenómeno y del grado de dependencia de las hormigas respecto a las plantas dispersadas.

El estudio de la dieta se ha realizado, generalmente, analizando las presas traídas al nido por las obreras. Sin embargo, aspectos como en qué grado los alimentos son aprovechados, o quién se los come, pueden ser estudiados de forma más adecuada mediante el uso de isótopos estables, proporcionando con ambos métodos una imagen más completa de la alimentación. (Caut et al. datos no publicados).

1.1.2 Dispersión de semillas por hormigas

Las hormigas están entre los principales dispersores de semillas. Hasta ahora, los estudios sobre la dispersión de semillas por las hormigas se han centrado sobre la verdadera mirmecocoria. Las semillas mirmecócoras poseen un apéndice (elaiosoma) rico en lípidos, que induce al transporte de la diáspora hasta el nido (Brew et al. 1989; Hughes et al. 1994; Boulay et al. 2006). Después de consumir el elaiosoma, las hormigas desechan la semilla intacta junto a otros restos de comida. Al transportar las semillas vulnerables poco después de su liberación, las hormigas permiten la colonización de nuevos hábitats (Gorb & Gorb 2003), la reducción de la competencia intraespecífica y de la mortalidad por depredación y por el fuego (Hughes & Westoby 1992; Espadaler & Gómez 1997; Manzaneda et al. 2005; Boulay et al. 2007a, 2009a). Determinadas especies de hormigas, por ejemplo *Aphaenogaster rudis* de los bosques caducifolios de Norteamérica, tienen una importancia mucho mayor como dispersoras que el resto de su comunidad (Zelikova et al. 2008; Ness et al. 2009). A éstas se les ha denominado especies dispersoras clave, “keystone species” (Gove et al. 2007) y presentan una cierta combinación de características: tamaño relativamente grande, abundancia, amplia distribución, y comportamiento subordinado. Son generalmente las primeras en descubrir el alimento, pero carecen de capacidad para defenderlo ante otras especies dominantes de menor tamaño que realizan reclutamiento en masa. Por ello, transportan las semillas con elaiosoma hasta el nido, en vez de alimentarse del mismo *in situ*. En el caso de *A. rudis*, Ness et al (2009) han demostrado que se trata de la única especie dispersora de una amplia agrupación de hierbas mirmecócoras, dándose un grado de especialización en esta interacción que es comparable a la de los mutualismos obligados planta-animal del bosque tropical.

Las hormigas no sólo dispersan las semillas portadoras de elaiosoma. Por ejemplo, las hormigas granívoras, aunque se comen la mayor parte de las semillas que recolectan, pierden una cierta cantidad en el camino de regreso al nido (Retana et al. 2004; Arnan 2006, Arnan et al. 2011), las olvidan en las cámaras de almacenamiento o las sacan intactas a las pilas de desechos (Brown & Human 1997; Retana et al. 2004, Oliveras et al. 2008). Este proceso es generalmente conocido como dispersión accidental o diszoocoria. El balance entre depredación y dispersión depende de características tanto de las plantas como del medio, y del comportamiento de forrajeo de las hormigas (Arnan et al. 2010). La producción de una cantidad grande de semillas por parte de la planta (Andersen 1989) y la baja disponibilidad de hábitats favorables para la germinación y el desarrollo (Andersen 1989; Levassor et al.1990) reducen el efecto de la depredación y favorecen la dispersión. Además, las hormigas granívoras emplean la estrategia de recoger la mayor cantidad posible de semillas durante los limitados periodos en que ello es posible (estrategia de “voracidad recolectora” Azcárate & Manzano 2011), de manera, que a menudo recolectan más semillas de las que se pueden comer. Existe una creciente evidencia del papel como dispersoras de las hormigas granívoras que pone en cuestión su estatus único de depredadoras de semillas (Wolf & Debussche 1999; MacMahon et al. 2000; Retana et al. 2004; Arnan et al. 2011).

La dispersión de frutos carnosos por las hormigas es frecuente en los trópicos, particularmente en el Nuevo Mundo, donde la verdadera mirmecocoria es rara (Roberts & Heithaus 1986; Böhning-Gaese et al. 1999; Pizo & Oliveira 2000; Christiani et al. 2007; Christiani & Oliveira 2009, 2010). La mayoría de estas plantas (arbustos o árboles) son dispersadas primariamente por aves. Las hormigas son atraídas por los restos de pulpa, y transportan hasta el nido los frutos o las semillas, bien procedentes de los excrementos de aves, o tras su caída al suelo.

A diferencias de lo que ocurre en los bosques caducifolios de Europa y América, en la región mediterránea se conocen pocas especies de plantas dispersadas por hormigas (ya que son escasas las especies cuyas semillas tienen elaiosoma), y en su lugar, las aves y los mamíferos parecen ser los principales dispersores animales. El papel de las hormigas en la dispersión de frutos carnosos es un tema controvertido (Herrera 2001) y poco estudiado. La hormiga granívora *Messor minor* dispersa a varias plantas arbustivas ornitócoras en el sur de Italia (Aronne & Wilcock 1994). El transporte de frutos carnosos también se ha observado en España en los géneros *Cataglyphis* y *Aphaenogaster*

(Traveset 1994; Hulme 1997; Bas et al. 2009), pero la importancia de este fenómeno es prácticamente desconocida.

1.1.3 Dispersión de las colonias

Las hormigas son modelos interesantes en ecología de poblaciones debido a que las diferentes especies han desarrollado diversos modos de dispersión en relación con la fundación de la colonia (Bourke & Franks 1995; Hölldobler & Wilson 1990).

Fundación independiente

Por un lado, las reinas vírgenes de muchas especies tienen largas alas funcionales, activadas por una musculatura torácica hipertrofiada, que les permiten volar a distancias relativamente largas (Peeters & Ito 2001). Después del apareamiento, que se suele realizar durante un vuelo nupcial, se despojan de sus alas y, casi de inmediato, empiezan a buscar una ubicación del nido para iniciar una nueva colonia por sí mismas. Si bien la fundación independiente expone a las reinas a una tasa de mortalidad importante debido a la depredación (Adams & Tschinkel 2001; Boulay et al. 2007d; Wiernasz & Cole 2003) y a la competencia con las colonias establecidas, tiene la ventaja de garantizar el flujo genético suficiente para evitar la endogamia (Helmkampf et al. 2008) y favorecer la dispersión.

Fundación dependiente o por fisión colonial

Por otro lado, algunas especies de hormigas fundan nuevas colonias por fisión colonial. Aquí, las reinas, o bien tienen pequeñas alas no funcionales, o carecen completamente de alas (Amor et al. 2011; Molet & Peeters 2006; Molet et al. 2008). Abandonan su nido madre acompañadas por un grupo de obreras para fundar una nueva colonia a una distancia cercana del nido materno. Esta estrategia aumenta la supervivencia de la reina durante la fundación colonial pero, al mismo tiempo, limita la distancia a la que la nueva colonia puede establecerse (Pamilo 1991). Es de esperar que la fisión provoque una estructura genética alta y un flujo genético limitado, incluso en distancias cortas (Giraud et al. 2000; Clémencet et al. 2005; Berghoff et al. 2008) dando como resultado una relación genética alta entre las colonias vecinas (viscosidad de la población), que a su vez, ocasione una mayor competencia por los recursos locales (Wilson et al. 1992; West et al. 2001).

1.1.4 Patrones de distribución espacial y migraciones coloniales

Las colonias de hormigas se parecen en cierto sentido a las plantas porque sus nidos están enraizados en el suelo. La distribución espacial de los nidos (y colonias) puede seguir varios patrones: regular, aleatorio y agregado.

Las poblaciones de hormigas están controladas generalmente por procesos competitivos dependientes de la densidad (Hölldobler & Wilson 1990). Esto se pone de relieve porque, a escala local, las colonias se distribuyen frecuentemente siguiendo un patrón regular, más que patrones aleatorios o agregados, indicando que cada colonia necesita un área exclusiva alrededor de su nido en la que no pueden establecerse otras colonias (Boulay et al. 2007d). El patrón regular sugiere competencia por algún recurso: alimento, lugares adecuados de nidificación... Puede deberse también a la mayor mortalidad de las colonias jóvenes que se sitúan cerca de colonias adultas de la misma especie (Ryti & Case 1992), o al robo de larvas entre colonias cercanas (Adams & Tschinkel 1995). La migración de las colonias puede ser una respuesta a la competencia y producir de forma rápida patrones regulares (Adams & Tschinkel 2001).

No obstante, muchos estudios en ecología de hormigas se basan en el supuesto de que las colonias no cambian frecuentemente de hormiguero. Sin embargo, eso no es cierto para muchas especies. Hölldobler y Wilson (1990) llaman la atención sobre el elevado número de especies que realizan migraciones coloniales y la falta de información sobre ello. Aún en la actualidad, sigue existiendo una gran laguna en el conocimiento sobre ecología básica debido a la falta de investigación sobre las migraciones (McGlynn et al. 2004). Las causas de las migraciones pueden ser diversas: perturbaciones del nido, por ejemplo inundación, cambios en el microclima del nido, predación, competencia, carga de parásitos o acercamiento a fuentes de alimento. No obstante, en la mayoría de las especies la causa no es bien conocida. Preguntas cómo ¿por qué ocurren las migraciones? y ¿qué consecuencias tienen sobre las poblaciones? no tienen respuesta clara en la mayoría de los casos.

Aphaenogaster senilis es una hormiga abundante y ampliamente distribuida en gran parte de la Península Ibérica. Se reproduce por fisión colonial y cambia con frecuencia de nido (migraciones). Estas características la hacen ser un buen modelo para estudiar una serie de procesos ecológicos y evolutivos, como la dinámica espacial, dispersión, competencia y conflictos entre castas o sexos. Por otro lado, parece tener un papel destacado como dispersora de algunas semillas. Desde el punto de vista metodológico, su estudio se ve facilitado por su tamaño grande, abundancia y facilidad para la cría en laboratorio.

1.2. DESCRIPCIÓN DE LA ESPECIE

1.2.1 El género *Aphaenogaster*

Aphaenogaster es un género extenso (con 5 subgéneros), del que se conocen 176 especies vivientes y 19 especies fósiles (www.antweb.org). Están distribuidas por todo el mundo, salvo África subsahariana y América del Sur (Brown 1973), aunque la mayoría habita en la región Paleártica, en climas templados o cálidos. El género se remonta al menos al Eoceno tardío: 44 m.a. (Cagniant 1996; Bolton et al. 2006; Moreau et al. 2006). La región mediterránea es el mayor centro de diversidad de especies (con más de 100). Casi todas tienen un área de distribución reducida; lo que sugiere una dispersión lenta y una tendencia al aislamiento geográfico (Bernard 1968). Solo dos especies (*A. subterranea* y *A. splendida*) están presentes tanto en la zona mediterránea oriental como en la occidental (www.formicidae.org), hecho excepcional en los otros géneros de Myrmicinae que la humanidad ha transportado desde épocas antiguas por todo el Mediterráneo (Bernard 1968). La reproducción por fisión colonial podría explicar este patrón de distribución.

En las *Aphaenogaster* americanas se conocen especies que se reproducen por fundación independiente y tienen amplia distribución (*A. fulva* o *A. rudis*, Carroll 1975), mientras otras probablemente lo hagan por fisión (*A. floridana*, Carroll 1975). De las 15 especies japonesas, solo 3 tienen una distribución extensa, siendo el resto endemismos insulares (http://ant.edb.miyakyo-u.ac.jp). Entre las especies mediterráneas, *A. senilis* se reproduce por fisión (Ledoux 1971) y *A. iberica* probablemente también dado que las hembras son igualmente braquípteras (Tinaut & Ruano 1992).

En varias especies americanas y japonesas se ha detectado polimorfismo cromosómico y ausencia de flujo génico entre poblaciones (Crozier 1977; Watanabe & Yamane 1999) lo que supone la existencia de grupos de especies hermanas difíciles de diferenciar morfológicamente. También en especies ibéricas se han citado ejemplos de polimorfismo cromosómico (Palomeque 1993) por lo que es posible igualmente la existencia de grupos similares.

Las *Aphaenogaster* son hormigas de tamaño mediano o grande, esbeltas y monomórficas. Se encuentran en una gran diversidad de hábitats, pero más a menudo en zonas boscosas, en sus márgenes y en zonas áridas. Anidan en el suelo, a veces bajo piedras, troncos o raíces. El tamaño de la colonia oscila entre unos pocos cientos, y dos mil o tres mil individuos (Wilson & Höldobler 1990; Boulay et al. 2010). Las colonias de algunas especies cambian con frecuencia de nido (por ejemplo *A. araneoides,* McGlynn 2003, 2004), en cambio, en otras como *A. cockerelli* el nido es permanente (Höldobler & Wilson 1990).

Tienen una dieta omnívora (Tobin 1994; Cerdá et al. 1988). Han perdido la capacidad para realizar trofalaxia (Delage & Jaisson 1969; Agboga 1982), pero compensan en parte esta carencia con la capacidad para transportar líquidos en la superficie de pequeños restos: hojas, palitos y piedrecitas, aprovechando la tensión superficial (Fellers & Fellers 1976; Tanaka & Ono 1978; Agboga 1982, 1985). Aunque otros autores han interpretado este comportamiento como protección ante la inundación (Höldobler & Wilson 1990), más recientemente se ha confirmado su función genuinamente alimentaria (Banschbach 2006) en la que se especializa un grupo de obreras en cada colonia. Como en otros géneros, las obreras ponen frecuentemente huevos tróficos que sirven de alimento para las larvas y la reina (Bruniquel 1972; Höldobler & Wilson 1990).

Poseen un sistema de reclutamiento en grupo que les permite transportar presas colectivamente. Se han descrito dos formas de reclutamiento que difieren significativamente en el comportamiento y en la naturaleza química de las feromonas de pista. En las *Aphaenogaster* norteamericanas del grupo *Novomesso*r, la obrera exploradora, tras encontrar una presa grande, libera feromonas al aire que pueden atraer a otras compañeras en una distancia de hasta 2 m, al mismo tiempo que produce un sonido por estridulación. En cambio, en *A. rudis* la obrera exploradora, después de encontrar una presa grande, regresa al nido dejando un rastro de feromonas. En el nido recluta a un grupo de compañeras que siguen su rastro hasta encontrar la presa. Estas diferencias sugieren que ambos grupos están poco emparentados filogenéticamente (Höldobler & Höldobler 1978; Attygalle 1998).

Algunas especies son importantes dispersoras de plantas mirmecócoras en el Mediterráneo, Norteamérica y Madagascar (Smallwood & Culver 1979; Böhnning-Gaese et al. 1999; Boulay et al. 2007a, 2009a; Zelikova et al. 2008; Bas et al. 2009).

De la mayoría de las especies solo existe una escueta información taxonómica. Las especies más estudiadas son *A. rudis* (195 citas en la base de datos Formis) y *A. fulva, A. subterranea, A. senilis* y *A. cockerelli* (con más de 100 citas cada una en la misma base de datos).

1.2.2 *Aphaenogaster senilis*

Distribución geográfica

Aphaenogaster senilis, Mayr 1853, está incluida en el subgénero *Aphaenogaster* (s. str.) Mayr. Se distribuye por la región mediterránea occidental (Cagniant et al. 1991; revisión en formicidae.org): Península Ibérica, litoral mediterráneo francés hasta Villefranche, Baleares (Mallorca y Menorca), Cerdeña, Canarias (Fuerteventura y Gran Canaria) y Azores (Cerdá et al. 1988; Cagniant et al. 1991; Cargiant 1992, 1996; www.formicidae.org) (**Fig. 1.1**). En la Península Ibérica, parece estar ausente en la mayor parte del tercio norte y en amplias zonas de la mitad este (**Fig. 1.1**). Las poblaciones ibéricas e insulares son biométricamente homogéneas, mientras en Marruecos existe mucha más diversidad y se distinguen 8 poblaciones diferentes (Cagniant et al. 1991). Probablemente se trata de un complejo de especies cuyo origen está en Marruecos (Cagniant 1996).

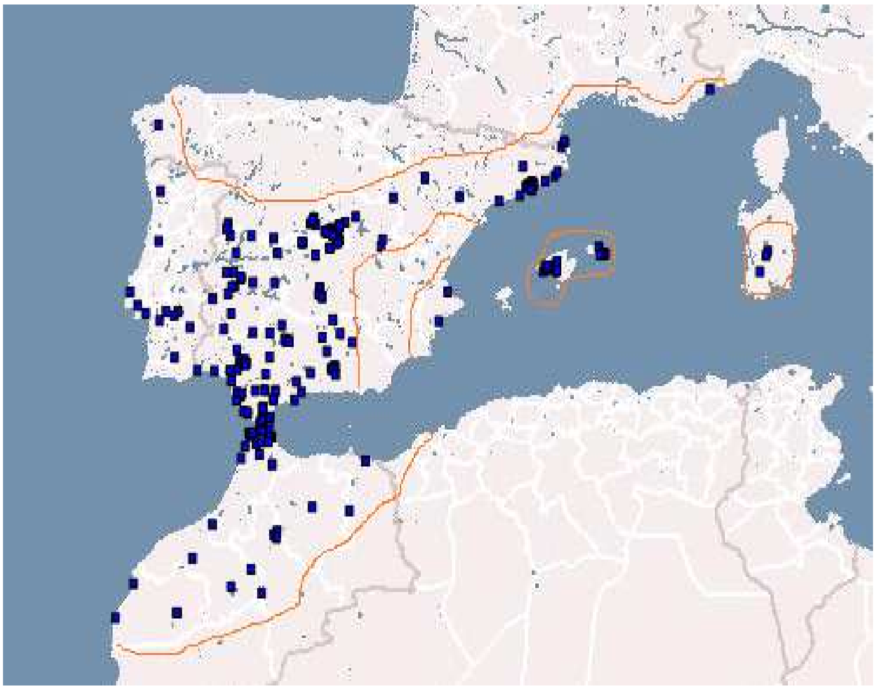

Figura 1.1 Distribución de *Aphaenogaster senilis*. Cada punto corresponde a una cita (304 citas). No están representadas las citas de Azores y Canarias. (Fuente: http//formicidae.org)

Hábitat y alimentación

Es particularmente abundante en el suroeste de la Península Ibérica, en diversos hábitats en relación a los suelos, vegetación, altitud y grado de perturbación humana (Tinaut 1989; Carpintero et al. 2000, 2001, 2007; Boulay et al. 2007a). Se alimenta de una gran variedad de presas, mayoritariamente cadáveres de artrópodos y en menor medida restos vegetales (Riasol, 1981; Riasol et al. 1986; Cerdá et al. 1988).

Dispersión de semillas

A. senilis es un eficaz dispersor de algunas plantas mirmecócoras (Gómez & Espadaler 1998; Manzaneda et al. 2007; Bas et al. 2009; Boulay et al. 2007a, 2009a,). En base a los resultados obtenidos por los autores anteriores, *A. senilis* puede ser considerada como una especie dispersora clave “keystone disperser” en el sentido que le dan Gove et al. (2007).

Comportamiento subordinado y tolerancia térmica

Es una especie de comportamiento subordinado respecto a otras especies de los géneros *Tapinoma*, *Tetramorium* y *Lasius* con las que a menudo coexiste. Éstas realizan reclutamientos en masa y llegan a dominar las presas después de un cierto tiempo. La estrategia de *A. senilis* para conseguir una elevada eficacia recolectora resulta de la combinación entre la rapidez para encontrar y recolectar presas, y la resistencia térmica (Cerdá et al. 2009). La eficacia recolectora de *A. senilis* se debe a que:

1. Hay muchas exploradoras y a que son hormigas grandes con mayor movilidad que las especies dominantes.
2. Transportan rápidamente el alimento encontrado al nido, bien de forma individual, o bien colectivamente en grupo de hasta unos 50 individuos (grupos de reclutamiento).
3. Pueden forrajear a temperaturas del suelo relativamente altas (hasta unos 45 ºC), muy cercanas a su tolerancia fisiológica, a las cuales las especies dominantes no tienen actividad recolectora.

Reclutamiento en grupo

El reclutamiento en grupo es un aspecto muy importante en el comportamiento de *A. senilis*, no solo en relación a la recolección de alimento, sino también a las migraciones y a la fisión colonial. Depende de decisiones individuales de las exploradoras y de la retroalimentación del proceso en el nivel de la colonia. En el caso del alimento, las obreras pueden "medir" la calidad, cantidad y transportabilidad del alimento (Cerdá et al. 2009). La obrera reclutadora deja un rastro químico al regresar al nido desde la fuente de alimento encontrada (van Oudenhove et al. 2012). Después retorna a la fuente de alimento guiando a un pequeño grupo (de 1 a 27 obreras -1 a 10 en más del 70 % de los casos-) (Cerdá et al. 2009). Sorprendentemente, la composición química de la feromona de pista en *A. senilis* difiere notablemente de la de una especie emparentada con ella, *A. iberica* (Lenoir et al. 2011).

Reconocimiento colonial

Como en otras especies que se reproducen por fisión, las obreras de *A. senilis* muestran poca agresividad con otras obreras de colonias cercanas de la misma especie y no defienden claramente un territorio (Ichinose et al. 2002). Esto puede estar relacionado con su comportamiento subordinado respecto a otras especies. Sin embargo, los individuos de una colonia son capaces de reconocerse. El reconocimiento se produce por un olor único y uniforme característico de la colonia que es resultado de la mezcla de olores de los individuos (Gestalt). Es producido por los hidrocarburos de la cutícula y de la glándula postfaríngea. El olor común es transmitido por el comportamiento de acicalamiento mutuo entre los individuos de la colonia, ya que no pueden hacer trofalaxia. Cuando los individuos son aislados durante unos días, su reincorporación desencadena un comportamiento de acicalamiento más intenso y si el aislamiento se prolonga por más de 20 días, la reincorporación produce una respuesta de agresión semejante a la que se da para individuos de otras colonias (Lenoir et al. 2001).

Ciclo colonial y reproducción

Las colonias de *A. senilis* son estrictamente monogínicas y monoándricas (Chéron et al. 2009) y contienen entre 200 y 3000 obreras monomórficas (media±SE: 1260 ±69; Boulay et al. 2007c), de longitud 6-10 mm y peso 5-8 mg (Boulay et al. 2009b, 2010). Las princesas (hembras sexuadas) (**Fig. 1.2a**), tienen alas cortas que no permiten el vuelo (braquípteras), por lo que de hacerlo, se dispersarían a distancias muy reducidas. Los machos (**Fig. 1.2b**) vuelan y se dispersan a distancias relativamente amplias. Las obreras (**Fig. 1.2d**) pueden dividirse en dos grupos: las que realizan tareas en el interior del nido, que son las más jóvenes, y las que salen al exterior a recolectar el alimento. Estas últimas son minoría y suponen aproximadamente la cuarta parte del total de obreras (Avargues-Weber & Monnin 2009).

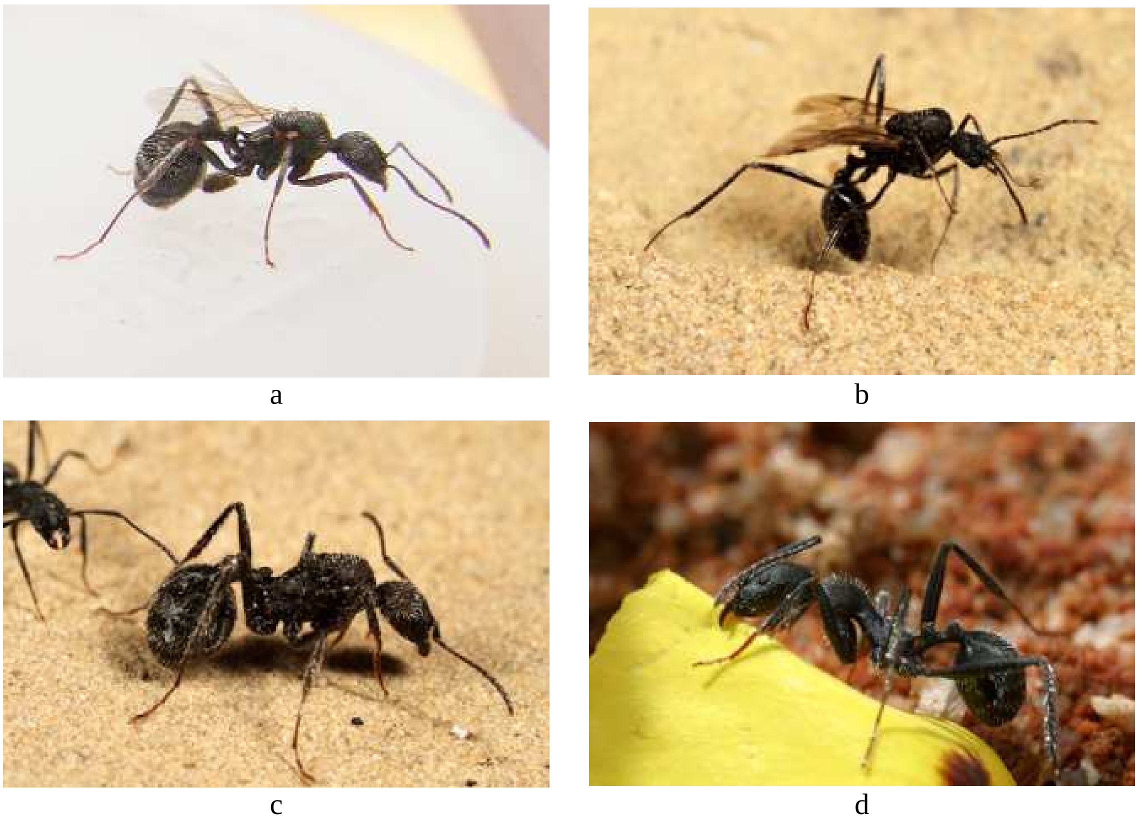
a b c d

Figura 1.2 Adultos en *A. senilis*: a) princesa, b) macho, c) reina, d) obrera. Las imágenes no están a la misma escala. La reina es ligeramente mayor que una obrera y pesa el doble que ésta. Los machos miden un poco más de la mitad que una obrera.(fuente: a: ixdeenero, www.lamarabunta.org, b-d: F. Amor)

Los estadios por los que pasan los individuos son: huevos, tres fases larvarias (LI, LII y LIII), preninfas, ninfas y adultos (**Fig. 1.3**)

Figura 1.3 Los seis estados larvarios en *A. senilis*. Los huevos y las larvas LI forman paquetes. (fuente: Boulay et al. 2009b)

El periodo de actividad de recolección de alimento va de febrero a noviembre. En el Sur de la Península no hay un periodo absoluto de hibernación aunque la actividad es muy pequeña en diciembre y enero. Como se verá con detalle más adelante, la actividad es máxima en los meses de abril y mayo y se reduce mucho en verano y otoño. Algunas colonias llegan a suspender casi totalmente su actividad en agosto y septiembre.

Durante el invierno (diciembre-febrero), la mayor parte de la nidada está compuesta por huevos y larvas L1 que se encuentran agrupadas en las cámaras más profundas. La mayoría de las crías son producidas en primavera (marzo-junio), lo cual da lugar a un incremento significativo de la población de la colonia en julio. La reproducción es por fisión colonial (Ledoux 1971, 1976; Boulay et al. 2007c, 2009b) y ocurre en la segunda parte del verano dando lugar a una reducción en el tamaño medio de las colonias. Después de eso, la población de obreras permanece constante durante el otoño e invierno para declinar en abril, probablemente por la muerte de las obreras nacidas durante el año anterior (Boulay et al. 2009b).

La reina inhibe la producción de sexuados mediante una feromona no volátil (Boulay et al. 2007c). Cuando las colonias son muy grandes, lo que ocurre en algunas de ellas al principio del verano, es probable que la dilución de la feromona real induzca la producción de sexuados: muchos más machos que hembras (proporción 172:1). Las larvas diploides son bipotentes hasta el segundo estadío larvario (Boulay et al. 2009b). Asimismo, cuando desaparece la reina, las colonias producen sexuados a partir de los huevos o larvas de la reina anterior, y de los huevos puestos por las obreras (de los que salen únicamente machos). Experimentos de laboratorio indican que el número de obreras limita las decisiones reproductoras de la colonia (Ruel et al. in press). Existe un número crítico de obreras (50-200), por debajo del cual la sustitución de la reina (si ésta muere) es menos probable. La asignación de tareas entre los miembros de la colonia es un fenómeno plástico que varía en función del número de obreras y el tiempo. Así, los grupos pequeños tienden a dar mayor prioridad al cuidado de las larvas en relación al esfuerzo de forrajeo. Esta plasticidad consigue mantener la capacidad para la cría de las larvas, pero a costa de renunciar a la producción de princesas (Ruel et al. in press).

Migraciones coloniales

Las colonias de *A. senilis* cambian de nido con frecuencia (migraciones). En el género *Aphaenogaster* esto se ha estudiado en *A. rudis*, *A. araneoides* y *A. senilis* (Smallwood 1982; McGlynn et al. 2004; Boulay et al. 2010; Galarza et al. 2012). La migración propiamente dicha dura alrededor de 2 horas. La mayor parte de las obreras se desplazan por sí mismas, a diferencia de lo que ocurre en *Temmothorax* (Avargues-Weber & Monnin 2009; Ángel Barroso, observaciones personales). El transporte de las crías y el viaje de la reina se producen en el periodo central de la migración. La preparación de la migración es realizada por las obreras recolectoras. Éstas buscan un nuevo nido, lo exploran, y si cumple ciertas condiciones (no bien conocidas) reclutan a sus compañeras de manera similar a los reclutamientos hacia una fuente de alimento. El reclutamiento es un proceso que se retroalimenta y llega a desencadenar la migración y el viaje de las obreras internas.

1.3 OBJETIVOS E HIPÓTESIS DE LA TESIS

El objetivo general de esta tesis es estudiar el ciclo anual de la especie *Aphaenogaster senilis* en un hábitat concreto, integrando distintos aspectos sobre el ciclo colonial anual con su ecología: alimentación, interacciones con plantas, distribución espacial y migraciones coloniales.

Los objetivos concretos son:

1. **Analizar las variaciones estacionales en el uso de los recursos alimentarios (dieta y comportamiento de aprovisionamiento de alimento) relacionándolas con el ciclo colonial y la disponibilidad de alimento.**

   La dieta y el comportamiento de recolección (*foraging ecology*) son un aspecto de gran importancia en la ecología de una especie. Los datos ya conocidos para *A. senilis* corresponden a la localidad de Canet de Mar, cerca de Barcelona y son datos globales (Cerdá et al. 1988). Queremos profundizar en el conocimiento de la dieta, el comportamiento recolector y su variación a lo largo del año realizando un muestreo más intenso en nuestra principal localidad de estudio. Éste es un paso previo para estudiar las interacciones con plantas. Particularmente, queremos probar la hipótesis de que *A. senilis* es, desde el punto de vista trófico, una especie oportunista, es decir que su dieta es amplia y se adapta a la disponibilidad de alimento.

2. **Probar la importancia de *A. senilis* como dispersora de semillas en un ecosistema mediterráneo, y analizar qué grado dependencia mutua existe entre las plantas dispersadas y la hormiga.**

   Ya hemos comentado que *A. senilis* es una eficaz dispersora de algunas plantas mirmecócoras, y que en el Mediterráneo se conocen pocas especies de plantas dispersadas por hormigas. Sin embargo, existen indicios de que, en la cuenca mediterránea, las hormigas podrían tener un papel como dispersantes más importante de lo considerado hasta ahora (Aronne & Wilcock 1994). Para analizar esta cuestión, hemos partido de los datos recogidos en el estudio de la dieta a lo largo del ciclo anual. A continuación hemos estudiado la dispersión de las especies más frecuentemente recolectadas por *A. senilis* y los beneficios que puede reportar esta relación mutualista: planta-hormiga, para ambos organismos.

3. **Entender el papel de las migraciones coloniales en la estrategia de dispersión en *A. senilis***

   Los efectos de las migraciones son muy poco conocidos, particularmente en las especies que se reproducen por fisión. Nos preguntamos si las migraciones puede servir para la dispersión de las colonias y cómo afectan los movimientos de éstas a la estructura genética de la población y al parentesco genético entre colonias vecinas (viscosidad de la población). Especialmente queremos probar si las migraciones producen una dispersión efectiva de las colonias.

4. **Describir la dinámica de la distribución espacial de las colonias a lo largo del ciclo anual y probar la hipótesis de su relación con el grado de cobertura vegetal**.

   Las colonias de *A. senilis* cambian de nido con frecuencia (migraciones). Esto supone que el patrón de distribución espacial de las colonias puede cambiar con el tiempo. Como se ha comentado anteriormente, en la mayor parte de las especies, se conoce poco sobre las causas que originan las migraciones y sobre los efectos en la población. El estudio pionero de J. Smallwood (1982) demostró que en *A. rudis* las variaciones en la exposición al sol son una causa importante de las migraciones. En *A. araneoides* la migración puede ser una respuesta al parasitismo o la depredación (McGlynn et al. 2004). Uno de nuestros objetivos es estudiar la causa de las migraciones en *A. senilis*, y concretamente someter a comprobación la hipótesis de que los cambios en la insolación desencadenan la migración.

1.4 DESCRIPCIÓN DE LAS ZONAS DE ESTUDIO

Los trabajos de campo se han realizado en el suroeste de la Península Ibérica, en dos zonas separadas entre sí unos 20 km: la Reserva Biológica de Doñana (RBD) y La Algaida (cercana a la ciudad de Sanlúcar de Barrameda) (**Fig. 1.4**). En esta última se ha desarrollado la mayor parte del trabajo. El clima es mediterráneo con influencia oceánica. La precipitación anual está en torno a 500 mm y es bastante variable entre unos años y otros. Los veranos son secos y calurosos, casi sin precipitaciones entre de junio y agosto y con una temperatura media diaria superior a 25 ºC (la media de la temperatura máxima diaria supera los 35 ºC en julio). Los inviernos son relativamente templados y húmedos, con precipitaciones concentradas entre octubre y abril. Los datos climáticos recogidos en aeropuerto de Jerez de la Frontera (a 21 km de La Algaida) indican que el período de 2003-2006 fue relativamente seco en comparación con el promedio, mientras que los últimos tres años de estudio han sido más húmedos. (**Fig. 1.5**)

Figura 1.4 Localización en Google Earth de las dos zonas de estudio y de los sitios incluidos en ellas: Reserva Biológica de Doñana, con 4 sitios: Comedero, Beles, Jaulón y Visita, y La Algaida (Sanlúcar de Barrameda) con un sitio: Puntal de Boza (fuente Google Earth).

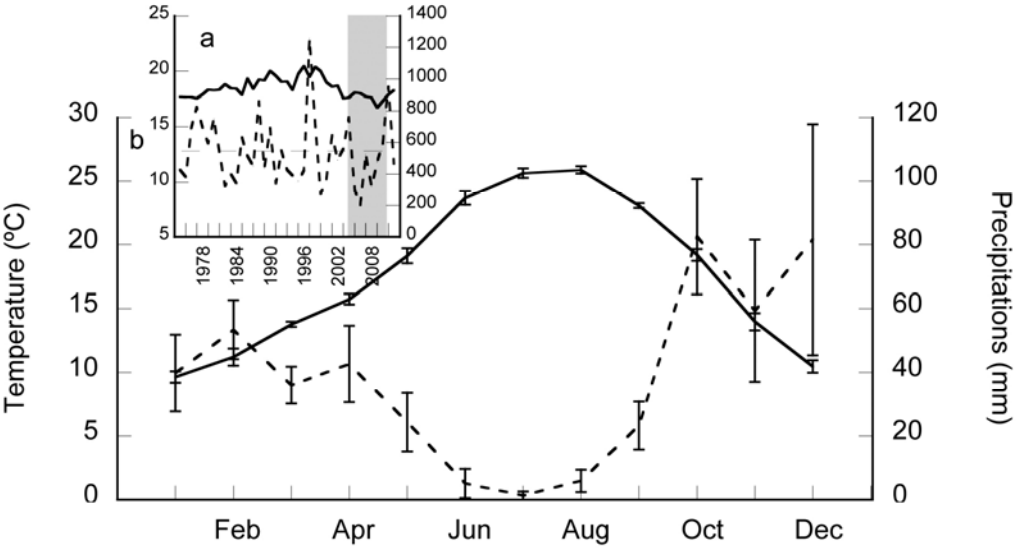

Figura 1.5 Datos climáticos (precipitaciones y temperatura) tomados en el aeropuerto de Jerez de la Frontera (a 21 km de distancia de la Algaida) durante: a) el periodo interanual 1976-2010, y b) el año 2010. Línea continua: temperatura; línea discontinua: precipitaciones. La franja gris corresponde al periodo de toma de datos de la tesis. Todos los valores son medias ± SE.

1.4.1 Reserva Biológica de Doñana

La RBD está incluida en el Parque Nacional de Doñana. En ella hemos trabajado en 4 sitios que denominaremos: Comedero (36º 59´ N, 6º 30´ W), Beles (36º 58´ N, 6º 28´ W), Jaulón (36º 59´ N, 6º 26´ W) y Visita (36º 58´ N 6º 28´ W) **(Fig. 1.6**), separados entre sí 4 - 6 km y próximos a la línea de costa (4-5 km) pero a los que no llega la influencia del viento marino (**Fig. 1.4**). Geomorfológicamente la zona es un manto eólico de dunas fitoestables. (Montes et al. 1998). El suelo es arenoso con poca materia orgánica y escasez de cationes de cambio. La capa freática se halla generalmente a más de 2 metros de profundidad.

Comedero Visita

El Jaulón Las Beles

Figura 1.6 Los cuatro sitios de estudio localizados en la RBD

La vegetación es similar en los cuatro sitios. Se denomina "Monte Blanco" (Asociación fitosociológica: *Halimio halimifolii-Stauracanthetum genistoides,* Rivas Martínez et al. 1980) y consiste en un matorral abierto formado mayoritariamente por Jaguarzo (*Halimium halimifolium), Halimium conmutatum*, Aulaga (*Stauracanthus genistoides*), Alucema (*Lavandula stoechas*), y Romero (*Rosmarinus officinalis*).

Comedero presenta las mismas especies de arbustos, junto a Pinos Piñoneros (*Pinus pinea*) y Sabinas (*Juniperus phoenicia*) dispersos. Dado su carácter xérico, la cobertura de herbáceas varía entre años dependiendo de las precipitaciones (López Albacete 2009). La vegetación está descrita en detalle en Rivas Martínez et al. 1980, y López Albacete 2009. En la RBD se ha estudiado la distribución espacial de los nidos (Las Beles), las migraciones coloniales (Las Beles y Jaulón) y la estructura genética de la población (Comedero, Visita y las Beles).

1.4.2 La Algaida

La Algaida (36º 51´ N, 6º 19´ W) es un pinar-sabinar incluido en el sector Sur del Parque Natural de Doñana, en Sanlúcar de Barrameda (Cádiz), próximo a la margen izquierda del río Guadalquivir (2,5 km) y a unos 8 km del mar. El pinar-sabinar de La Algaida está situado sobre el segmento norte de un cordón dunar de unos 7 km de dirección SSO-NNE., está rodeado por marismas transformadas y limita al sur con un núcleo urbano. El suelo es arenoso, la topografía, ondulada de origen eólico, con un desnivel máximo de unos 10 m y la capa freática oscila entre menos de 1 y cerca de 10 m de profundidad.

Hemos distinguido 4 hábitats en el pinar:

1) Pinar-sabinar
2) Jaguarzal monoespecífico de zonas incendiadas
3) Matorral esclerófilo de zonas bajas y transición a la marisma
4) Pastizal-juncal de transición a la marisma.

Inicialmente (durante el verano de 2002) se hizo una prospección de las especies de hormigas en los diferentes hábitats, utilizando trampas de caída y observación directa. Se excluyó el jaguarzal, muy degradado y en el que solo está presente *Tapinoma nigerrimum*. Se eligieron 5 lugares de muestreo. En cada uno se dispusieron dos unidades de trampas, separadas 10 m, durante 24 h. Cada unidad de trampeo se componía de 7 trampas de caída (vasos de 4,5 cm de diámetro) llenas de agua hasta la mitad y con unas gotas de detergente. Los vasos se dispusieron formando dos rombos (5 m de diagonal) unidos por uno de sus vértices. Los resultados de presencia y abundancia se indican en la **Tabla 1.1**.

Aphaenogaster senilis está presente en los tres hábitat pero es más abundante en los pastizales situados en el límite entre el pinar y la marisma. Con estos datos, seleccionamos como zona de estudio un lugar denominado “Puntal de Boza” (hábitat de pastizal), de fácil acceso, poco frecuentada y donde *A. senilis* es abundante (área de estudio: 1,5 ha). Está situada entre el Pinar de la Algaida y la Marisma de Henares, prácticamente al nivel del mar. Se trata de un ecotono entre el pinar-sabinar sobre sustrato arenoso y una marisma transformada con vegetación de almajos. En esta transición se suceden: una orla de vegetación arbustiva muy densa y de gran porte (lentiscal) y pastizales con juncos, salpicados de manchas pequeñas de matorral (**Fig. 1.7, 1.8)**.

Tabla 1.1 Abundancia (%) de cada especie de hormigas en los diferentes hábitat del pinar de La Algaida estimada a partir de trampas de caída (14 trampas/ 24 h). Se indican, para cada hábitat, los porcentajes de hormigas, el número total de individuos y la riqueza. Matorral 1: camino cabañas; matorral 2 Puntal de Boza; pastizal 1: Los Prados; pastizal 2: Puntal de Boza. Fechas de muestreo: 13 julio y 11 agosto 2002.

Especies	Pinar /sabinar	Matorral 1	Matorral 2	Pastizal 1	Pastizal 2
Aphaenogaster senilis	16,7	2,1	0,0	36,1	12,7
Cataglyphis rosenhaueri	0,0	0,0	0,0	34,2	54,7
Camponotus pilicornis	0,0	0,0	0,0	0,6	0,7
Crematogaster auberti	12,1	6,4	21,9	0,0	0,0
Lasius grandis	0,0	0,0	31,5	0,0	0,0
Messor barbarus	0,0	0,0	12,3	4,4	2,2
Messor marocanus	12,1	43,6	15,1	3,2	1,4
Pheidole pallidula	4,5	4,3	2,7	0,0	0,7
Plagiolepis pigmaea	0,0	0,5	0,0	0,0	0,0
Tapinoma nigerrimum	7,6	38.3	9,6	0,0	23,9
Tapinoma madeirense	0,0	0	2,7	1,3	1,4
Tetramorium caespitum	0,0	1,1	0,0	0,0	0,0
Tetramorium forte	0,0	0,5	0,0	20,3	2,2
Tetramorium semilaeve	47,0	3,2	4,1	0,0	0,0
Nº total obreras capturadas	66	188	73	158	276
Riqueza de especies	6	9	9	7	9

La topografía es plana con diferencias decimétricas. El suelo es de características intermedias y cierta heterogeneidad en cuanto a la textura, salinidad y encharcamiento (gradientes arenoso-arcilloso y salino). El horizonte superficial (30-40 cm) suele ser arenoso-limoso y oscuro, mientras el horizonte subyacente es siempre más claro y arenoso. La capa freática es somera, y se encuentra aproximadamente a un metro de profundidad, con oscilaciones estacionales. A unos 50 ó 60 cm de profundidad el suelo suele estar húmedo incluso en verano. Las partes arcillosas llegan a encharcarse en los años de abundante precipitación.

Figura 1.7 Vista de la zona de estudio en la Algaida tomada con Google Earth (Puntal de Boza). Ecotono entre pinar y marisma. Cuadrado rojo: zona de mapas de nidos de *A. senilis*. Área verde: zona de estudio de dispersión de *Arum italicum, Phillyrea angustifolia* y *Pistacea lentiscus*. Área azul: zona estudio dispersión de *Ornithogalum ortophylum*. Área amarilla: zona de estudio de migración de colonias de *A. senilis*. Área total 1,5 ha. (Fuente: Google Earth).

La comunidad de las manchas arbustivas está dominada por el Lentisco (*Pistacea lentiscus*) acompañada habitualmente por Olivilla o Labiérnago (*Phillyrea angustifolia*) y Sabina (*Juniperus sabina*). Sobre ellas aparecen frecuentemente varios arbustos trepadores y en su interior es abundante *Arum italicum.* Los juncos merinos (*Juncus acutus*) son muy abundantes. Forman macoyas (matas densas y circulares) de tamaño mediano o grande que se concentran en las partes algo más bajas.

El pastizal presenta una gran diversidad de especies anuales y vivaces (unas 50 especies) que se suceden a lo largo del ciclo anual y se distribuyen según los gradientes físico-químicos. Este hábitat es semejante a “la vera” del Parque Nacional, pero se seca mucho más en verano debido a que las marismas de esta zona fueron drenadas y desecadas en los años 60 del siglo XX. Como consecuencia, en verano el pastizal queda arrasado por la sequía (**Fig. 1. 8**). De forma semejante a lo que comentamos para la EBD, la cobertura y abundancia de las diferentes especies herbáceas varían de unos años a otros dependiendo de las precipitaciones.

Figura 1.8. La Algaida (Puntal de Boza) en diferentes épocas del año. Arriba, aspecto en primavera. Abajo, aspecto en verano.

Las especies vegetales más abundantes son:

- Febrero-marzo: *Narcisus papiraceus, Bellis annua, Miosotis sp.* y *Anagallis arvensis.*
- Marzo-mayo: años húmedos: *Trifolium sp.* y *Medicago sp.*; años secos: *Echium plantagineum* y *Neatostema apulum*.
- Junio-julio: *Pulicaria paludosa*. Fructificación de *Arum italicum* (dentro de las matas de lentiscos)
- Octubre -noviembre: *Heliotropium europaeum* y *Narcisus serotinus*.

En los lugares más bajos y arcillosos predomina una especie perenne, *Frankenia laevis* que florece en mayo. La vegetación de una zona muy similar (a unos tres km de distancia), está descrita en Gallego et al. (1998). Hay una presión importante de ganado vacuno que da carácter nitrófilo al área. También como consecuencia del pisoteo, el suelo está bastante compactado en los primeros centímetros.

La comunidad de hormigas está formada por 21 especies (Tabla 1.2). Las especies más abundantes son: *Tetramorium forte, Aphaenogaster senilis, Cataglyphis rosenhaueri, Messor marocanus, Messor barbarus* y *Crematogaster auberti*. *Tetramorium forte* es muy abundante, realiza reclutamiento en masa y tiene una alimentación omnívora semejante a la de *A. senilis*. Es la especie dominante en la comunidad. *Cataglyphis rosenhaueri* también tiene una alimentación similar. Recolecta sus presas individualmente y es muy termófila. *Messor marocanus* y *M. barbarus* son fundamentalmente granívoras. *Crematogaster auberti* está asociada a la vegetación arbustiva.

Tabla 1.2 Lista y abundancia relativa de especies de la zona de estudio “Puntal de Boza” en La Algaida: muy abundante (***), frecuente o localizada (**), escasa o rara(*)

	Especie	Abundancia
1	*Aphaenogaster senilis*	***
2	*Camponotus pilicornis*	*
3	*Cataglyphis rosenhaueri*	***
4	*Crematogaster auberti*	**
5	*Crematogaster scutelaris*	*
6	*Hipoponera sp.*	*
7	*Messor barbarus*	***
8	*Messor marocanus*	***
9	*Messor bouvieri*	*
10	*Messor lusitanicus*	*
11	*Messor hispanicus*	*
12	*Pheidole pallidula*	*
13	*Plagiolepis pygmaea*	*
14	*Plagiolepis schmitzii*	*
15	*Solenopsis sp.*	*
16	*Tapinoma erraticum*	*
17	*Tapinoma madeirense*	*
18	*Tapinoma nigerrimum*	**
19	*Tapinoma simrothi*	*
20	*Tetramorium forte*	***
21	*Tetramorium semilaeve*	*

CHAPTER 2

A year in ant life: opportunism and seasonal variations in the foraging ecology of *Aphaenogaster senilis*[1]

ABSTRACT / RESUMEN

Ants are important consumers in most terrestrial ecosystems. They show a great diversity of diets and foraging strategies. Here, we analysed how circannual variations in resource use by the mediterranean species *Aphaenogaster senilis* is related to colony life cycle and resource availability. In southwestern Spain, this species is active almost all year round but foraging intensity decreases by 10 folds between March and November following larval production. In summer, ants refrain from foraging at midday to escape from high temperatures. The proportion of plant/animal-derived items collected by foragers did not vary significantly from March to November. However, isotopic analyses indicate a decrease of *A. senilis* trophic level between June and September, suggesting all collected material is not eaten. Interestingly, most animal preys were collected by individual ants, and many of them were retrieved alive. Therefore *A. senilis* is not only a scavenger but also is a non-negligible predator, particularly for aphids. The abundance of the major

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[1] Ángel Barroso, Shephane Caut, Xim Cerdá, Fernando Amor and Raphaël Boulay. Submitted Ecoscience

animal-derived items in the diet was proportional to their abundance in the study area. We conclude that *A. senilis* is an opportunistic species able to feed on a variety of resources which may be a key its ecological success.

Las hormigas son importantes consumidores en la mayoría de los ecosistemas terrestres. Muestran una gran diversidad de dietas y estrategias de aprovisionamiento (búsqueda y recolección de alimento). Aquí analizamos, para la hormiga mediterránea *Aphaenogaster senilis,* la relación entre las variaciones estacionales (circanuales) en el uso de los recursos y, la disponibilidad de los mismos y el ciclo anual de la colonia. En el Suroeste de España *A. senilis* está activa casi todo el año, pero la intensidad de (forrajeo) recolección de alimento se reduce aproximadamente a la décima parte entre marzo y noviembre, siguiendo a la reducción en la producción de larvas. En verano, las hormigas cesan la actividad recolectora en las horas centrales del día para evitar las altas temperaturas del suelo. La proporción entre el número de ítems de animales y plantas en el alimento recolectado no varía significativamente entre marzo y noviembre. Sin embargo, los análisis de isótopos estables indican un descenso en el nivel trófico entre junio y septiembre, sugiriendo que no todo el material recolectado es finalmente comido. Curiosamente, la mayoría de las presas animales fueron recolectadas de forma individual y muchas de ellas, vivas. Por lo tanto, *A. senilis* no es solo una hormiga necrófaga, sino también una predadora no despreciable, particularmente en el caso de los áfidos. La abundancia en la dieta de los principales grupos animales (órdenes de insectos) es proporcional a su abundancia en el área de estudio. Concluimos que *A. senilis* es una especie oportunista capaz de alimentarse de una amplia variedad de recursos lo cual puede ser una clave de su éxito ecológico.

2.1 INTRODUCTION

Gathering food for survival and reproduction is an essential activity for most animals. Foraging is costly because it requires time and energy that cannot be allocated to other activities and because it increases the risk of predation and parasite exposure. As a consequence, animals face behavioural trade-offs to which they are expected to respond by optimizing when, where and on what kind of food they forage (Stephens & Krebs 1986). In the past decades numerous experimental and theoretical studies have solved important questions on the evolution of animal foraging behaviour (Stephens et al. 2007). Yet, basic studies on wild animal feeding habits are still needed particularly for non-specialist consumers that may vary their food intake in function of environmental conditions.

Ants show a great diversity of foraging strategies, including different types of consumers and levels of cooperation during food recollection (Hölldobler & Wilson 1990). Because of their abundance in most terrestrial habitats, their foraging decisions may have important consequences at the ecosystem level. Ant foraging activity patterns may change both on spatial and temporal (seasonal and daily) scales, depending on environmental conditions (Cook et al. 2011). For example, temperature, humidity and light intensity are abiotic factors that limit or trigger ant foraging activity (Amor et al. 2011; Cerdá et al. 1998; Chong & Lee 2009; Narendra et al. 2010; van Oudenhove et al. 2012). Moreover, biotic factors such as colony composition (Judd 2005; Abril et al. 2007; Dussutour & Simpson 2009), interspecific competition (Carroll & Janzen 1973), resource availability (Briese & Macauley 1980) and the presence of predators and parasites (Orr & Seike 1998) determine foraging behaviour as well as the quality of collected resources.

So far, many studies have analysed ant diet by direct observations of retrieved food items. Some species show a clear consistency in their food choice, independently of temporal and spatial variations of food source abundance. This is the case of many specialist hunters that are behaviourally and morphologically adapted to capture a certain kind of prey (e.g. Hölldobler 1982; Dejean et al. 1999). Other species, by contrast, show a remarkable variability of food source utilisation. Temporal diet changes may result from important plasticity allowing opportunistic ants to adjust their feeding choice to the most abundant and/or profitable items (Mooney & Tillberg 2005). However, they can also be due to variations in the internal demand as a consequence of circannual demographic changes. For example, in temperate habitats, between-season variations in the number of larvae may affect the relative needs for proteins and carbohydrates (Cassill & Tschinkel 1999; Judd 2005; Abril et al. 2007; Dussutour & Simpson 2009; Cook et al. 2011). In addition, the abundance of one type of food may switch forager preference for a rare alternative resource in order to maintain a balanced diet (Edwards & Abraham 1990).

The study of ant trophic ecology is complicated by several aspects of their life history, including their social life and caste system. Moreover, only a fraction of the items retrieved to the nest may be consumed and assimilated while the remaining may be used for other functions like nest construction. Stable isotopes analysis offers a powerful complementary approach to traditional observational analyses, in order to elucidate diet temporal and spatial variations (Kelly 2000; Caut et al. 2009). This method is based on the fact that an organism's nitrogen and carbon isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) reflect assimilated resources (see Post 2002 for review). The former typically increases by 3–4‰ at each trophic level while the latter is useful to distinguish between different sources of carbon (e.g. C_3 vs C_4 plants). Yet, compared to other organisms, the use of stable isotopes in ant trophic ecology is still in its outset (Feldhaar et al. 2010). They proved to be useful for identifying spatial and temporal variations of ant trophic position (Blüthgen et al. 2003; Mooney & Tillberg 2005; Menke et al. 2010; Gibb & Cunningham 2011) and to highlight diet differences among nest mates (Smith et al. 2008; Smith & Suarez 2010). To our knowledge no study has compared the results of food retrieval observations with ant stable isotope analysis.

In the present study, we analysed the circannual variations in the foraging behaviour and diet of *Aphaenogaster senilis.*

- First, we analysed circannual variations in the foraging rate and daily pattern of activity in relation with larval production and environmental conditions.
- Second, we analysed the retrieved food items and compared the abundance of different insect preys with their abundance in the field throughout the year. We expected *A. senilis* trophic opportunism to be evidenced by a good correlation between prey abundance and retrieval.
- Finally, we analysed worker isotopic ratios in order to detect circannual variations in nutrient intake.

2.2 MATERIALS AND METHODS

2.2.1 Study site

The study was conducted at La Algaida. Data were collected over a period of 7 years, from December 2003 to November 2009.

2.2.2 Circannual variations of foraging activity and colony productivity

To assess seasonal variations in colony productivity, a total of 65 nests were excavated (**Fig. 2.1**) between December 2002 and November 2009 (range 2-18 nests per months of the year, median = 3). Each colony was brought to the laboratory to count the workers and weight the total larval fresh biomass.

Figure 2.1 Picture of the inside of *Aphaenogaster senilis* nest during the course of an excavation and showing empty chambers

Aphaenogaster senilis foraging activity was estimated by observing focal nests during sessions of 10 min every hour on 3-4 nests per day, from sunrise to sun set. During each session, all out and incoming ants were recorded. Among incoming ants, we also differentiated between those carrying a food item and those returning without food. Measurements of foraging activity were conducted on 23 days and 50 different focal nests between March 2003 and November 2009. Each nest was observed on 1 to 8 different days with at least one month between two consecutive observations.

2.2.3 Analysis of retrieved items

Circannual variations in the composition of items retrieved by *A. senilis* workers were analysed using data collected on 10 days in April 2006 and February, March and May to November 2009. All items transported by the ants were collected while they were returning to their nest during sessions of 10 min per hour on 3-4 nests per day. Each item was conserved in alcohol until identification in the laboratory. Plant material was identified to the genus or species level. Animal material was identified to the order level. Animal prey length was measured to the nearest millimetre.

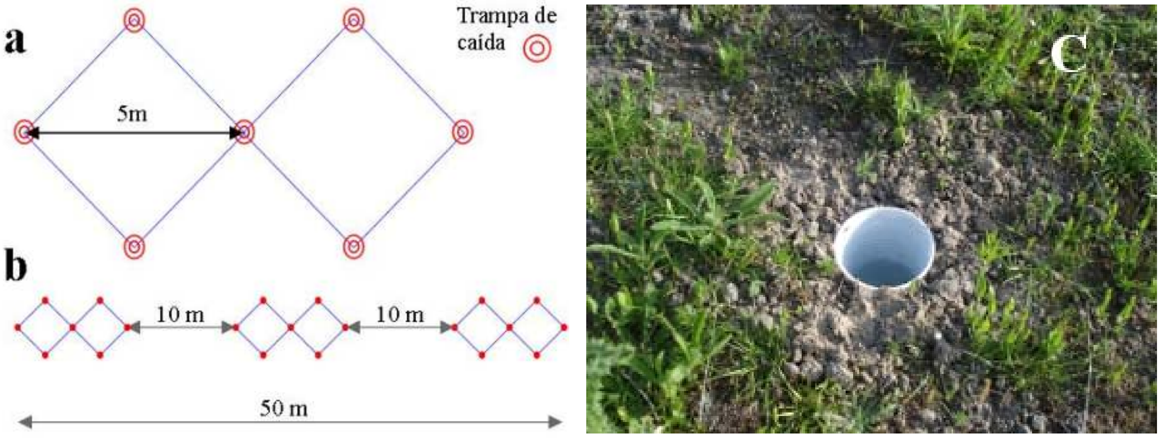


Figure 2.2 Sampling system pitfall a) Disposition of each sampling unit of pitfall traps formed by 7 plastic cups placed at the vertices of two squares aligned that share a vertex. b) Sampling transect 50 m long consists of 3 sample units aligned. c) Pitfall trap installed

In order to estimate variations in the availability of animal preys, 3 sets of 7 pitfall traps (4 cm in diameter, 7 cm deep plastic cup filled with water and soap) were installed along a 50 m transect during 24h (**Fig. 2.2**). Within sets, pitfall traps were arranged in two rhombuses (5m diagonal) united by one of their corners. The biological material was pooled by sets of 7 traps and kept in 70% alcohol for further identification and isotopic analyses. Pitfall traps were installed soon after prey collection on a day with similar weather.

2.2.4 Isotopic analyses

Isotopic analyses were conducted on 24 samples of *A. senilis* workers collected in pitfall traps throughout the year (April 2006 and February, March, May to November 2009). We also analyzed items retrieved by the ants (n = 54). All samples were dried at 60°C for 48 h, ground to a fine powder, weighed in tin capsules and stored in a dessicator until isotope measurement. For adult ants, each sample consisted of thoraces and legs to provide sufficient mass (ca. 1 mg) for accurate determination of isotope ratios. Abdomens were excluded to prevent the contamination by recent food residuals. Comparisons of stable isotope values between heads and thoraxes have indicated no significant differences between these tissues (Tillberg et al. 2006). Collected items were processed individually or pooled to obtain sufficient material.

Isotopic analyses were performed using a mass spectrometer (Optima, Micromass, UK) coupled to a C-N-S elemental analyser (Carlo Erba, Italy). δ^{13}C values were not used because all the plants present in the area use the same mode of carbon fixation. δ^{15}N values (‰) were expressed relative to atmospheric N2: δ^{15}N= [(Rsample/Rstandard)-1]x1000, where R is 15N/14N. A reference material was IAEA-N1 (+ 0.4‰). One hundred replicate assays of internal laboratory standards indicate measurement maximum errors (SD) of ± 0.15‰ for nitrogen isotope measurements.

2.2.5 Data analyses

Data were analysed using the R software (R Development Core team 2010). Seasonal variations of larval fresh weight (log-transformed) were analysed by fitting a general linear model (GLM) using the nlme package. The month and year of excavation were considered fixed and random effects, respectively. A bayesian approach was then used to reduce the number of non-significant levels within the explanatory variable. To that end, the two consecutive months with the most similar average larval weights were merged to a unique new factor level. A second model was then fitted and compared to the initial full model using the anova command based on the Bayesian Information Criteria (Schwartz, 1978). This operation was repeated several times until all consecutive factor levels were significant.

To assess seasonal variations in the foraging activity, square-root transformed numbers of incoming ants per day (the sum of all 10-min sessions multiplied by 6) were compared between months using a GLM in which the year and the colony were considered random factors (nlme package for R). The number of factors within the variable Month was reduced using a Bayesian approach as explained before.

Variations in the number of collected items per day were assessed by the same procedure using the number of loaded incoming ants as the response variable.

In order to determine changes in the daily pattern of activity throughout the year, we first normalized number of incoming ants per hour. To that end, the number of outcomes observed at each observation session was divided by its maximum during the same day and nest. We then tested the correlation between the average normalized number of incoming ants per hour during one month and the next one. If the correlation was significant, the average was recalculated over months and the operation repeated with next month. However, the lack of significant correlation between consecutive periods would indicate a change in the daily pattern of activity.

Seasonal variations in the proportion of animal-derived collected items were assessed by fitting a linear model (LM) with the month included as a fixed factor. Variations in the proportion of the four main insect orders in the diet were tested in a similar way. We used a Bonferroni procedure to reduce α threshold and cope with non-independence of these percentage data. A Pearson correlation test was employed to test the relation between the average abundance of an animal order in the study area (obtained from the pitfall traps) with its average representation in ant diet (obtained from collected items).

LMs were fitted to test seasonal variations in *A. senilis* $\delta^{15}N$ values. Finally, *A. senilis* $\delta^{15}N$ values were compared to those of the main collected items by means of GLMs in which the month of collection was included as a random factor.

2.3 RESULTS

2.3.1 Larval production and foraging activity

Colonies of *A. senilis* collected at La Algaida contained larvae throughout the year. However, larval biomass varied greatly between months, showing a clear annual cycle with two main seasons. Larval biomass was relatively high in winter-spring (December-May) and significantly lowers in summer-fall (June-November; **Fig. 2.3A**; GLM: $F_{1, 58} = 43.60$, $P < 0.0001$).

Seasonal variations of foraging activity followed a similar pattern, being elevated in spring (**Fig. 2.3A**; March to June; 767±61 trips.d^{-1}, Mean±SE) and decreasing significantly in summer, fall and winter (July to November, and February: 237±22 trips.d^{-1}, Mean±SE; GLM: $F_{1, 35} = 88.04$, $P < 0.0001$). However, in contrast to larval biomass which started to increase in winter, foraging remained very low from December to February.

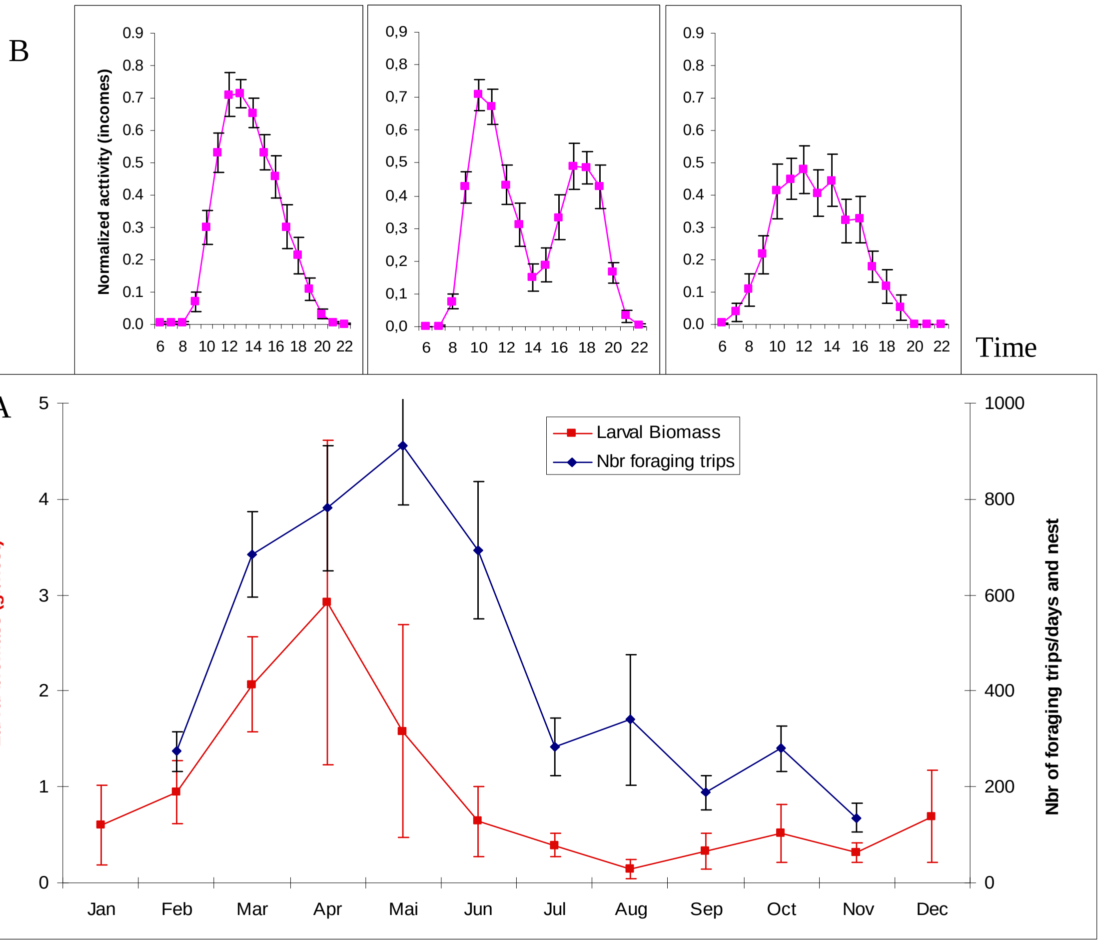


Figure 2.3 Larval production and foraging activity. (A) Circannual variations in the foraging activity (black line, n= 88 nest) and larval biomass (red line, n = 65 nest). (B) Variations of the daily rhythm of foraging activity between spring (Feb, Mar, Apr), summer (Mai, Jun, Jul, Aug) and fall (Sep, Oct, Nov) n=1462. All values are means ± SE

The number of preys retrieved per day also followed the same trend (GLM: $F_{1,35}=90.50$, $P < 0.0001$). It decreased from 548±47 items.d^{-1} (Mean±SE) between March and June to 133±15 items.d^{-1} in July-November and February (Mean±SE).

The daily pattern of foraging activity also varied greatly throughout the year. Pairwise correlations between the normalized activity in consecutive months revealed three periods. In March and April (**Fig. 2.3B**), the activity followed an unimodal pattern with a peak at midday. Similarly the hourly activity in September, October and November (**Fig. 2.3B**) was unimodal. By contrast, in summer (**Fig. 2.3B**; May-August), the ants forgo foraging at the central hours of the day (14:00) giving rise to a bimodal pattern of activity with two peaks at 10:00 and 17:00. The drastic changes of rhythm between spring and summer and between summer and fall were evidenced by the lack of correlation between normalized activity at the same hour of the day (Pearson correlation: r=0.01, t=0.01, P=0.99 and r= 0.25, t=0.88, P=0.39) respectively

2.3.2 Retrieved items

A total of 1349 prey items were collected and identified, 62±4% of which were of animal origin while the remaining were plants. The proportion of animal items varied significantly throughout the year (LM: $F_{9,\ 27}$=2.72, P=0.021) because of a higher proportion of animals in February than during the remaining of the year (**Fig. 2.5A**; 88±4% vs 58±3%, Mean±SE for February vs the rest year; t=-3.853; P<0.001).

Most retrieved items were small (< 3mm) and were brought to the nest by individual ants (**Fig. 2.6**). Six percent of the animals items exceed 8 mm and are usually brought cooperatively (**Fig. 2.4**) Also, a few unusually large preys (e.g. an earthworm of 45 mm length) were retrieved cooperatively too. Overall, the number of items of one size class in the diet was proportional to the number of captures in the pitfall traps (Pearson χ^2_{64}=72, P=0.2303).

Figure 2.4 A bee is transported cooperatively (photo:F. Amor)

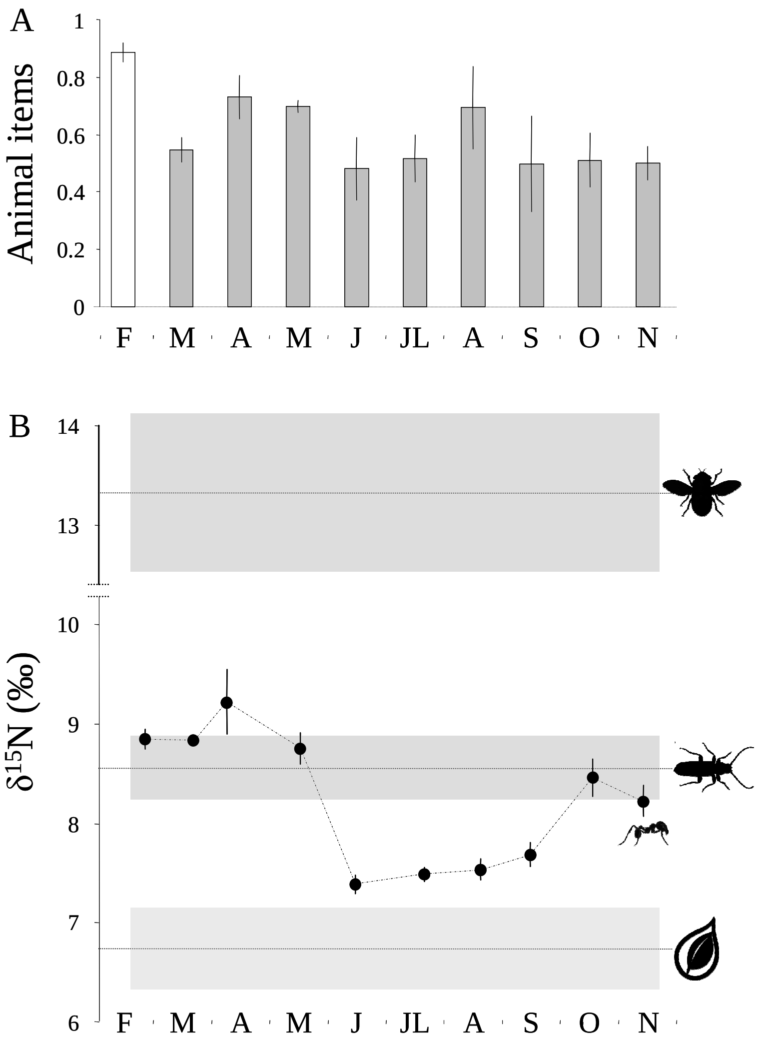


Figure 2.5 Proportion animal / plant item collected and worker nitrogen isotopic variation. (A) Proportion of animal items collected by *Aphaenogaster senilis* along the year. (B) Variations of worker nitrogen isotopic values along the year. Horizontal lines and grey polygones indicate the values of the three main resource categories (Plants, Dipterans and other insects (Coleopterans, Hemipterans and Hymenopterans)). Nitrogen isotopic values were corrected with a discrimination factor of 3‰ (Feldhaar et al. 2010). All values are means + SE;

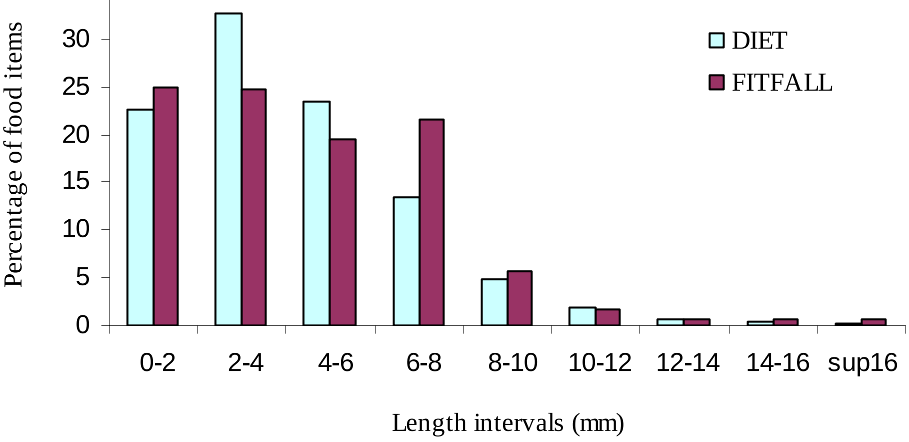


Figure 2.6 Percentage of class size (mm) of animal items in ant diet (black, n=884) and in pitfall traps (white, n=4929).

Plant items were mostly composed of fruits (29%), flowers or petals (25%) and seeds (17%) of several species, including *Phyllirea angustifolia*, *Pistacea lentiscus* and *Arum italicum* (**Fig. 2.7, 2.8**). Twenty-four invertebrate orders composed animal-derived items. The majority were dipterans (mostly mosquitoes), hemipterans (mostly aphids), coleopterans and hymenopterans. The proportion of dipterans, hemipterans and coleopterans did not vary significantly during the year (23±8%, LM: $F_{9,\ 27}$=2.05, P=0.05; 14±4%, LM: $F_{9,\ 27}$=2.24, P=0.07; 10±2%, LM: $F_{9,\ 27}$=0.45, P=0.89, respectively; 〈=0.015 after Bonferroni correction, **Fig. 2.9**). The proportion of hymenopterans (mostly other ants) was significantly higher in June-July (74±30%, Mean±SE) than during the rest of year from 14±2% (LM: $F_{1,\ 35}$=72.2, P<0.001). Although many retrieved animal items were captured dead or dying, aphids, mosquitoes, small insect larvae and some small coleopterans were clearly captured alive. In some occasions, even large living animals (caterpillars, earthworms and large coleopterans) were transported cooperatively.

There was a significant correlation between the abundance of an animal order in the area of study and its representation in *A. senilis* diet (**Fig. 2.9** y **2.10**; Pearson correlation: t=6.19, P<0.0001, R^2=0.62). Yet, hemipterans appeared as an outlier mostly because pitfall traps are not suited for estimating their abundance. Hence, removing them from the analysis enhanced R^2 to 0.74.

Figure 2.7 Plant items: Left, Petal of *Cistus salvifolius*. Right, Fruit of *Arum italicum* (photo:F. Amor)

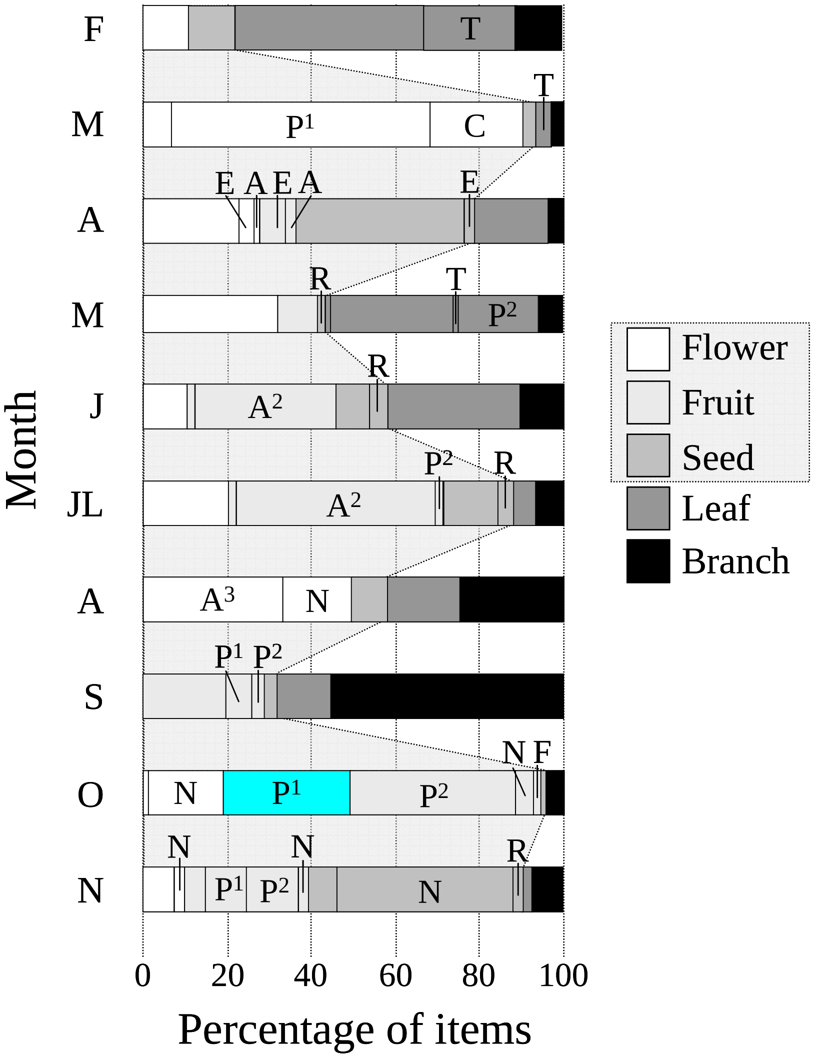


Figure 2.8 Relative percentage of different plant parts (flower, fruit, seed, leaf and twigs). The proportions of the identified species are represented by letters (T, *Trifolium sp*; P1, *Phillyrea angustifolia* ; C, *Cistus salvifolius* ; E, *Erodium sp.*; A, *Anagallis sp.*; R, *Rhamnus alaternus* ; P2, *Pistacea lentiscus* ; A2, *Arum italicum* ; A3, *Asparagus sp.*; N, *Narcissus serotinus* ; F, *Frankenia laevis*). Gray polygon connecting the different months is the consomable part for this species (flower, fruit, and seed).

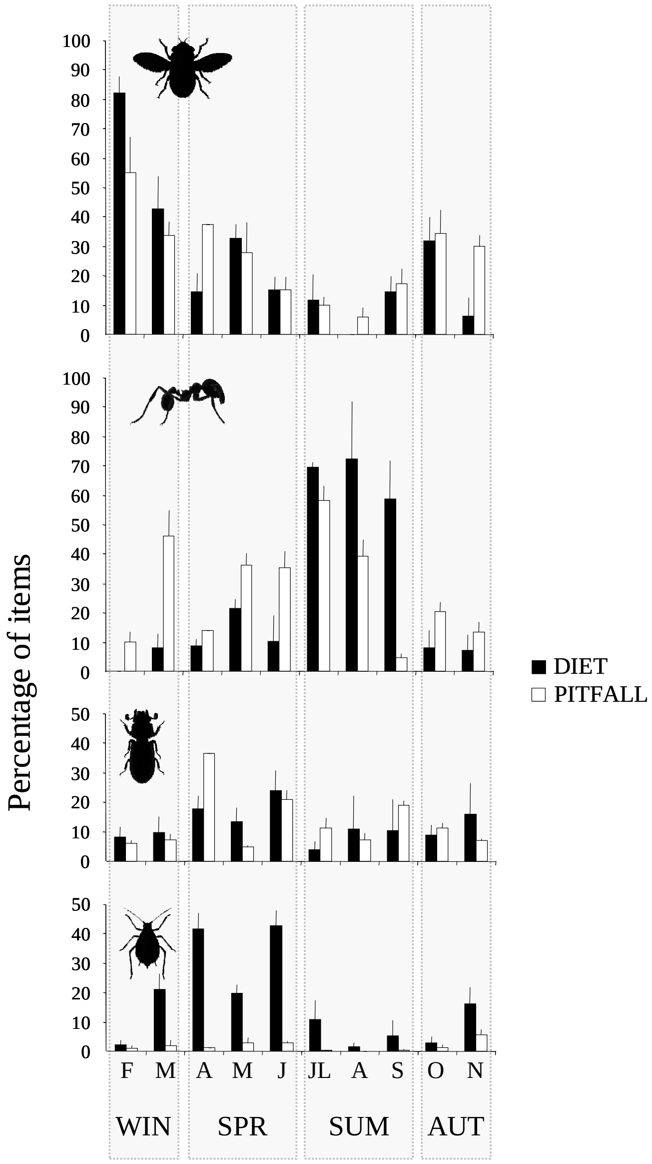
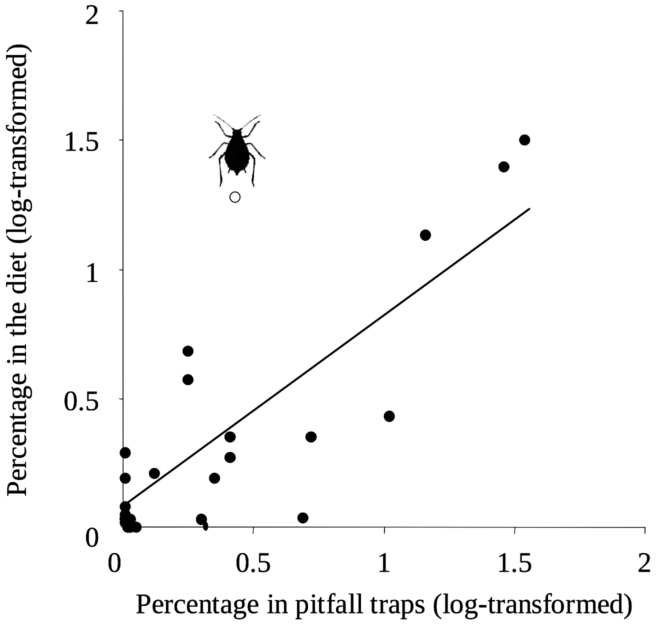


Figure 2.9 Insects collected by ants and captured in the pitfall. Percentage (mean + SE) of the four more important insect taxonomic groups (Dipterans, Fourmicidae, Coleopterans and Hemipteranss) collected by ants (black bars) and captured in the pitfall traps (white bars).

Figure 2.10 Regression between the abundance of invertebrate order in the area of study and its representation in *Aphaenogaster senilis* diet. All values were log-transformed. The regression does not account for aphids.



2.3.3 Isotopic analyses

A. senilis workers $\delta^{15}N$ values varied significantly throughout the year (LM: $F_{1, 20}$=19.6, P<0.0001) with clear distinction between seasons **(Fig. 2.5B**). Hence, $\delta^{15}N$ values decreased significantly between the spring (February-May; Mean±SE: 8.92±0.1) and summer (June-September; Mean±SE: 7.52±0.05; $t_{(spring\text{-}summer)}$=-12.56, P<0.0001). In fall (October-November) $\delta^{15}N$ values raised again to an intermediate level between those of summer and spring (8.34±0.12; $t_{(spring\text{-}fall)}$=-4.23, P=0.0002 and $t_{(summer\text{-}fall)}$=6.25; P<0.0001).

Isotopic analyses of *A. senilis* main food resources indicated that coleopterans, hymenopterans and hemipterans formed an homogenous group with low $\delta^{15}N$ values. Their $\delta^{15}N$ values **(Fig. 2.5B**; Mean±SE: 5.56±0.35) were significantly higher than plants (3.74±0.46; t=8.08, P<0.0001) and significantly lower than dipterans (10.37±0.89, t=-4.99; P<0.0001). *A. senilis* $\delta^{15}N$ values were in between herbivores and dipterans ($t_{(A.\ senilis\text{-}herbivores)}$=-5.52, P<0.0001; $t_{(A.\ senilis\text{-}dipterans)}$=2.23, P=0.029).

2.4 DISCUSSION

In this study, we have documented important seasonal variations of *A. senilis* foraging rate, daily pattern of activity and diet in Southern Spain. Results show that colonies are active almost throughout the year but larval biomass production and foraging activity are prominent in spring. The daily pattern of activity changes greatly between seasons probably as a consequence of temperature constrains. Colonies are omnivorous, feeding on a wide array of food resources including insects (dead and alive) and plant materials. Direct observation of retrieved food items did not highlight a major shift in *A. senilis* alimentation throughout the year. However, worker isotopic analyses suggested a reduction of animal protein consumption in summer.

Like most animal species in temperate and mediterranean habitats (Wyndham, 1986; Levey & Stiles 1992), *A. senilis* displays a clear annual cycle of brood growth and foraging activity. Larvae mostly develop in spring which also corresponds to a major increase of the foraging activity. Although there is an evident relationship between colony-level food intake and larval growth, the causal chain relating both phenomena may be mediated by other factors. For example, larval growth and worker foraging behaviour may be constrained by the same environmental variables including temperature and humidity. In particular, cold and hot winter and summer temperatures, respectively may limit foraging and larval growth. By contrast, spring weather conditions may stimulate foraging and increase food income, thus favouring larval growth.

Another factor that may potentially stimulate foraging rate is colony members hunger state. Larvae hunger state is known to stimulate worker foraging in some ant species (Brian & Abbott 1977; Dussutour & Simpson 2009). It is therefore possible that the progressive increase of small larvae in February-March triggers adult foraging behaviour. However, in July, the number of *A. senilis* ant incomes per day was still relatively high while larval biomass was already close to zero suggesting larval presence was not the only stimulating factor of foraging. Young adult workers and sexuals hunger state may also affect ant foraging decision, as suggested by Cassill and Tschinkel (1999) and Judd (2005). Finally, demography and age-dependent division of labour may also influence seasonal variations of the foraging rate. Hence in spring, colonies are composed of relatively old workers that were born before the winter and that may be more prone to forage than younger ones (Robinson 1992; Musceder & Traniello 2012).

We found a major shift in the daily pattern of activity of *A. senilis* between spring, summer and fall. While colonies were mostly active at the central hours of the day in spring and fall, the activity decreased drastically at midday in summer probably to escape from extremely hot ground temperature. In effect, in the study area ground temperature may approach 70 ºC which is much above the lethal temperature of *A. senilis* (Critical Thermal Limit: 46°C, Lethal Temperature: 50 ºC (Cerdá et al. 1998)). In Mediterranean habitats, elevated ground temperature has been widely reported to constrain ant activity (Cros et al. 1997; van Oudenhove et al. 2011) and many ant species exhibit a shift of their daily rhythm between the hot and cold seasons (Amor et al. 2011; van Oudenhove et al. 2012). In addition to increasing the risk of mortality, hot ground surface may interfere in ant chemical communication system by reducing trail marks stability and preventing nest mate recruitment (Ruano et al. 2000; van Oudenhove et al. 2012).

Observations of retrieved items suggest *A. senilis* is an omnivorous species with low feeding specificity. Most food items were sufficiently small to be transported by individual ants. However, in some rare occasions, the ants retrieved extremely large preys, which required the cooperation of several individuals. The value of such rare large preys for the colony compared to the majority of small items is probably high. Cerdá et al. (1998) found that the 12% largest preys retrieved by *A. senilis* represented up to 72% of the transported biomass.

The proportion of insect taxa in *A. senilis* diet was well predicted by their abundance in the area of study measured with pitfall traps. This highlighted the relative opportunism of *A. senilis* and its capacity to use a large array of preys. Aphids represented an outlier in the relation between insect abundance in the field and their occurrence in *A. senilis* diet. This is because aphids are sessile animals that are not well sampled using pitfall traps. Like many ants in Mediterranean habitats (Fellers & Fellers, 1982; Retana et al. 1991; Angulo et al. 2012) *A. senilis* behaved as a scavenger that retrieved dead or moribund preys. It also predated on a number of living preys, including caterpillars, coleopterans and numerous aphids. However, in contrast to many species such as *Tapinoma* and *Lasius* that have a dual role (mutualistic and predator), on aphids *A. senilis* does not tend them in order to get liquid honeydew (Bristow, 1984; Matsuura & Yashiro, 2006). Therefore, *A. senilis* may have a net positive indirect effect on plants by reducing the pressure of herbivory.

In addition to insects, *A. senilis* retrieved a relatively large amount of seeds and fruits from several plant species. Previous studies have shown that in the south of the Iberian Peninsula *A. senilis* and the congeneric species *A. iberica* contribute disproportionately to the dispersal of various myrmecochores (Espadaler & Gómez 1997; Boulay et al. 2005, 2007a; Bas et al. 2009). Similarly, the genus *Aphaenogaster* represents a keystone disperser of myrmecochores of North American temperate woodlands (Ness et al. 2009). However, none of the diaspores removed by *A. senilis* at our study site had an elaiosome. *Phyllirea angustifolia*, *Pistacea lentiscus* and *Arum italicum* were among the most frequently removed plant species. The former two are bird-dispersed while the latter has no known legitimate disperser. Our results thus confirm the potential importance of *A. senilis* in the redistribution of numerous seeds adapted to other modes of dispersal (Traveset 1994; Hulme 1997). Further studies should nevertheless investigate the survival and germination rates of seeds transported by *Aphaenogaster*.

Direct observations of the retrieved food items showed a significant difference in the proportion of animal-derived items between February and the rest of the year. However, in February *A. senilis* has a very low activity and the result for this month should be taken cautiously. In contrast to direct observations, ant $\delta^{15}N$ values decreased between June and September suggesting an important diet shift towards a higher consumption of plant-derived materials. The apparent incongruity between isotopic and observational data may be due to high between-nest variability of observational data compared to isotopic data. Moreover, although the number of retrieved fruits and seeds did not change, their contribution to the diet in terms of biomass may be much higher in Summer than Spring. Large fruits like those of *A. italicum*, *P. angustifolia* and *P. lentiscus* were mostly collected from June to November. These results therefore highlight the interest of isotopic analyses to capture differences in ant diet that are difficult to observe from the analysis of retrieved items only.

In ants, larvae are the major consumers of proteins while adult workers rely on carbohydrates (Vinson 1984). The reduction of larval biomass between spring and summer could therefore provoke a reduction of the needs for insect preys in favour of plant-derived carbohydrates. Such seasonal change in food preference has been reported for example in *Solenopsis invicta* (Stein et al. 1990) and *Pheidole ceres* (Judd 2005) y *Linepithema humile (*Abril et al. 2007).

Overall, our results suggest that the foraging rate of *A. senilis* varies throughout the year in relation with larval biomass and environmental constrains. Foragers appeared opportunistic, transporting an important variety of food items to the nest, although a selection may occur within the colony in function of the internal demand. This apparent plasticity in the foraging activity and resource use may be a key of the ecological success of this otherwise behaviourally subordinate species. Further studies are needed to better understand ant feeding ecology in natural conditions in relation to colony life cycle. Observational data and isotopic analyses represent complementary tools to get a fine image of food selection at different scales.

CHAPTER 3

Various degrees of interdependence asymmetry between a “keystone disperser” ant and non-myrmecochorous Mediterranean plants[2]

ABSTRACT / RESUMEN

In contrast to other plant-animal mutualisms, seed dispersal interactions, and particularly seed dispersal by ants, are generally considered asymmetric, non-specialized relationships in which dispersers depend less on plants than vice versa. Although true myrmecochory is well understood in many terrestrial ecosystems, dispersal of non elaiosome-bearing seeds by ants has barely been studied outside the Neotropics. *Aphaenogaster senilis*, a common ant in Southern Spain, collects a great variety of non-myrmecochorous diaspores along with insect preys. At our study site, fleshy fruits of *Arum italicum*, *Phillyrea angustifolia* and *Pistacia lentiscus* represent up to one fourth of the items collected by *A. senilis* from June to November. However, they are mostly ignored by other ants. In the laboratory, the addition of *A. italicum* fruits to *A. senilis* insect-based diet increased male production and both worker and queen pupae size. Seeds were transported up to 8m away from the mother plant and deposited in a favorable habitat allowing germination of a relatively high proportion. Given important differences in total

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[2] Ángel Barroso, Fernando Amor, Xim Cerdá and Raphaël R Boulay, submitted

crop size between species, our data suggest that *A. senilis* removes virtually all seeds of *A. italicum,* but a negligible fraction of *P. lentiscus* seeds. We conclude that in contrast to the common view, dispersal of non-myrmecochorous Mediterranean plants by ants might be an important phenomenon. Keystone disperser ants like *A. senilis* probably obtain an important fitness advantage from non-myrmecochorous diaspore collection. However, plant benefit may vary greatly according to crop size and the existence of alternative dispersal agents.

En constraste con otros mutualismos planta-animal, las interacciones relacionadas con la dispersión de semillas, y particularmente la dispersión por hormigas, son consideradas relaciones asimétricas no especializadas, en las cuales los dispersores dependen menos de las plantas que viceversa. Aunque la verdadera mirmecocoría ha sido estudiada en muchos ecosistemas terrestres y en general es bien entendida, la dispersión por hormigas, de semillas no portadoras de elaiosoma apenas ha sido analizada fuera de América Tropical. *Aphaenogaster senilis,* una hormiga común en el sur de España, recolecta una gran variedad de diásporas no mirmecócoras junto con insectos. En nuestra área de estudio los frutos carnosos de *Arum italicum, Phillyrea angustifolia* y *Pistacea lentiscus* representan más de un cuarto de los items recolectados por *A. senilis* entre junio y noviembre. Sin embargo, son generalmente ignoradas por otras hormigas. En el laboratorio, la adición de frutos de *Arum italicum* a una dieta base de insectos incrementa la producción de machos y el tamaño de las pupas de obreras y reinas en *A. senilis.* Las semillas son transportadas hasta más de 8 metros desde las plantas madres y depositadas en hábitats favorables, permitiendo su germinación en una proporción relativamente alta. Se producen importantes diferencias entre especies en el total de producción de frutos; nuestros datos sugieren que *A. senilis* transporta virtualmente todas las semillas de *Arum italicum* pero una fracción insignificante de *Pistacea lentiscus*. Concluimos, en contraste con el punto de vista común, que la dispersión por hormigas de plantas mediterráneas no mirmecócoras puede ser un fenómeno importante. Las hormigas dispersoras claves “keystone disperser”, como *A. senilis,* probablemente obtienen un importante incremento en su eficacia biológica, de las diásporas no mirmecócoras. Sin embargo, el beneficio para las plantas puede variar mucho según la cantidad de semillas producidas y la existencia de agentes dispersores alternativos.

3.1 INTRODUCTION

Seed dispersal by animals is a popular topic in ecology and evolutionary biology (Snow 1971; McKey 1975; Janzen 1983; Herrera 1995, 2002). Many fruit and seed consumers were shown to positively affect plant recruitment and population dynamics, emphasizing the mutualistic nature of this kind of interaction (reviews by Wang & Smith 2002; Fenner & Thompson 2004).

However, the degree of specialization among partners and influence of current dispersers on the evolution of plant dispersal adaptations are still debated (Wheelwright & Orians 1982; Herrera 1995; Jordano 1995; Alcántara et al. 2007). Several lines of evidence suggest that, in contrast to other highly specialized, tightly coevolved plant-animal mutualisms (e.g. pollination), seed dispersal may consist of asymmetric interactions evolving through diffuse selection. First, plant-disperser pairwise interactions are relatively rare. On the contrary, plants' diaspores attract phylogenetically diverse animal guilds that change over time and space. Second, while plants often rely entirely on animals for seed-dispersal, the reverse is not necessarily true because animals can feed on a large variety of resources, creating asymmetric partner interdependence. Third, animals can have contrasting qualitative and quantitative effects on seed dispersal. They include dispersers, seed predators and exploitative parasites (cheaters) that use plant rewards without contributing to effective dispersal (Herrera 1984; Horvitz & Schemske 1986; Bronstein 1994; Boulay et al. 2007a; Manzaneda et al. 2007).

Ants constitute the most abundant animal fauna of many terrestrial habitats. They are also among the main seed dispersers. So far, studies on seed dispersal by ants have paid much attention to true myrmecochory. Myrmecochores' seeds bear a lipid-rich elaiosome that triggers diaspore transport to ant nest (Marshall et al. 1979; Brew et al. 1989; Hughes et al. 1994; Boulay et al. 2006). After the elaiosome has been consumed, the ants discard the intact seed with other food remains. By transporting the vulnerable seeds soon after their release, ants allow the colonization of new habitats (Gorb & Gorb 2003) and reduce intraspecific competition and mortality by predation and fire (Heithaus 1981; Bond & Slingsby 1984; Hughes & Westoby 1992; Manzaneda et al. 2005; Boulay et al. 2007a, 2009a).

However, ant service may differ greatly between species (Giladi 2006; Gove et al. 2007) coined the term “keystone dispersers” for ant species that contribute disproportionately to seed dispersal. On the contrary, it was shown that, locally, many ants parasite mutualisms by detaching elaiosomes *in situ* without transporting the seeds (Zelikova et al. 2008; Ness et al. 2009; Boulay et al. 2007a, b; Manzaneda et al. 2007; Aranda-Rickert & Fracchia 2010).

Ants not only disperse elaiosome-bearing seeds. For example, removal of fleshy fruits by ants has been widely documented in the tropics, particularly in the New World, where true myrmecochory is rare (Roberts and Heithaus 1986; Böhning-Gaese et al. 1999; Pizo & Oliveira 2000; Christianini et al. 2007; Christianini & Oliveira 2009, 2010). Most of these plants are primary bird-dispersed shrubs or trees. Ants attracted by pulp remains remove them either from bird droppings or after their natural fall from the plant.

In Mediterranean scrublands, the role of ants in fleshy fruits dispersal remains controversial (Herrera 2001). The harvester ant *Messor minor* was suggested to disperse several garrigue bird-dispersed plants in Southern Italy (Aronne & Wilcock 1994). However, seed survival and germination after being manipulated by this granivore ant was not tested. Moreover, the seeds of two of the most frequently removed plants in this study, *Rhamnus alaternus* and *Myrtus communis*, do have an elaiosome and might therefore be classified among true myrmecochores (Bas et al. 2009). Although fleshy fruits transport by *Aphaenogaster* and *Cataglyphis* ants was also observed in Spain, the importance of this phenomenon is unknown (Traveset 1994; Hulme 1997; Bas et al. 2009).

Seed dispersal by ants has long been considered as a typical example of non-specialized interaction potentially involving many ant species with no feeding specificity (Beattie 1985; Beattie & Hughes 2002). At first glance, food rewards offered by ant-dispersed plants show little morphological and chemical specificity, suggesting that they can attract a large array of omnivorous ants. In contrast to lipids contained in seeds, those composing elaiosomes are similar to those of insect corpses (Hughes et al. 1994). It was therefore proposed that myrmecochores' seed dispersers were scavenger ants that rarely collected plant material other than elaiosomes (Hughes et al. 1994).

However, in spite of a few recent studies (e.g. Lubertazzi et al. 2010), the diet of seed disperser ants has not been investigated in great detail, which limits our understanding of their degree of dependence on plants. Moreover, although ant dependence on myrmecochorous seeds has been tested experimentally, the results of independent studies are rather inconsistent. In the field, Morales & Heithaus (1998) showed that increasing the availability of myrmecochorous seeds for *Aphaenogaster rudis* colonies enhanced queen production, resulting in a less male-biased sex ratio. Other studies conducted in the laboratory showed an increase of worker but not sexual production in *Myrmica ruginodis* and *M. rubra* (Gammans et al. 2005; Fokuhl et al. 2007). In *Temnothorax crassispinosus* it increased female weight and decreased male weight (Fokuhl et al. 2012). Adding *Datura* elaiosome-bearing seeds to a standard diet had no effect on queen survival or brood production in *Pogonomyrmex californicus* (Marussich 2006).

To our knowledge, ant dependence on fleshy fruits has not yet been investigated. Nevertheless, in the tropics, Ponerine ants that are morphologically and behaviorally adapted to predate on living insects are among the main secondary dispersers of fleshy fruits (Pizo & Oliveira 1998; Fourcassié & Oliveira 2002; Passos & Oliveira 2004). In this case, plant rewards do not mimic these ants' preys. It might therefore be hypothesized that these ants may obtain a specific advantage by feeding on fruit pulp. For example, they may find nutrients lacking in insect preys.

In the present study, we questioned asymmetric interdependence in mutualisms between ants and non-myrmecochorous plants in Mediterranean habitats. Our aim was to test the general hypotheses that:

1) A limited number of ant species both depend on and provide dispersal service to many non-myrmecochores and
2) The benefit obtained by plants varies between species depending on crop size.

We assessed the interdependence between *A. senilis* ants and non-myrmecochorous diaspores by estimating both the proportion and number of fruits and seeds in their diet throughout their period of activity in an experimental plot located in Southern Spain. We reasoned that *A. senilis* dependence on plant would be a function of the proportion of diaspores in their diet, while plant dependence on *A. senilis* would be a function of the number of retrieved diaspores compared to species-specific crop size. We also tested experimentally whether diet supplementation with *Arum italicum* fruits affected larval fate and pupae size in the laboratory. Finally, we compared *A. Senilis* diaspore removal behavior with that of other ants present in the same community and measured seed survival, germination rates and dispersal distances.

3.2 MATERIALS AND METHOD

3.2.1 Study system

The study was conducted at *La Algaida*, (Puntal de Boza) (**Fig. 1.7, 1.8**). The plant species studied are *Pistacia lentiscus* (Anacardiaceae), *Phillyrea angustifolia* (Oleaceae) (**Fig. 3.2a, b**), *Arum italicum* (**Fig. 3.1**) and *Ornithogalum orthophyllum* (**Fig. 3.2c**). The former two are shrubs. Both produce large crops of small bird-dispersed drupes that can exceed 10000 fruits in the case of *P. lentiscus* (Jordano 1989). *Arum italicum* (*Araceae*) and *Ornithogalum orthophyllum* (*Lilliaceae*) are herbs. *A. italicum* is a perennial shade-tolerant herb that grows preferentially below *P. lentiscus.* Each plant is composed of 1-3 30 cm-high spadices that produce up to 100 red fleshy fruits that mature in summer (Méndez & Díaz 2001). The fruits are toxic to vertebrates and to our knowledge no legitimate disperser has been reported (Herrera 1989). *O. orthophyllum* is also a perennial herb that grows in patches. Each 5-10 cm stalk produces a few capsules that often lie directly on the ground (**Fig. 3.2c**). No myrmecochore is present in the study area, but a few plants of *Rhamnus alaternus* grow about 1 km away.

Figure 3.1 Life cycle of *Arum italicum* in the study area: a, b) Phase leaves only (October-March), c) Flowering (April-May), d) Fruits (June-August).

a b

c d

Figure 3.2 Plant species which diasporas are collected by *Aphaenogaster senilis* in the study area: a) *Phillyrea angustifolia* (Olivilla or Labiérnago), b) *Pistacea lentiscus* (Mastic), c) *Ornithogalum orthopyllum*, d) *Narcissus serotinus* (Narcissus fall).

3.2.2 Interdependence between *A. senilis* and non-myrmecochorous diaspores: diet analysis

The proportion and number of diaspores in *A. senilis* diet were estimated in two steps. First, the proportion of diaspores in the diet was estimated for a total of 57 nests and 16 sampling days between June 2003 and November 2009 by collecting foragers' load on their way back to the nest. All food items were kept in 70% alcohol and identified in the laboratory. In a second step, the number of loaded workers returning to their nest was recorded on 86 nests and 19 sampling days between April 2004 and November 2009 during 10 min sessions every hour, from the beginning to the end of the daily foraging activity. Here, in order to limit interference caused by observers on ant foraging activity, foragers were not collected. For each nest, the estimated number of retrieved items was then multiplied by the proportion of diaspore in the diet during the same month of the year (obtained from step 1). Between months differences in diaspore proportions and numbers were compared by ANOVA (R Development Core Team, 2010).

Figure 3.3 A worker of *Aphaenogaster senilis* carrying a fruit of *Arum italicum* (photo:F. Amor)

3.2.3 *A. senilis* dependence on non-myrmecochorous diaspores: diet supplementation

Ten colonies were collected in June 2011 near the study site. They were maintained in the laboratory in 26x17x12 cm (length x width x height) plastic containers, the inner wall of which was coated with Fluon®. Colonies were fed with *Tenebrio molitor* mealworms provided *ad libitum* 3 times a week, and maintained at 28 ºC in darkness. The ants could shelter in four 2 x 20 cm test tubes half filled with water maintained with a cotton plug.

After three months in control conditions, two queenless groups of 200 workers were prepared from each mother colony. Each experimental group also received 20 1st instar larvae. From that time on, each group was fed either with mealworm or with mealworm plus 6 *A. italicum* fruits per week. The fruits were frozen at -20 ºC just after collection in the field. The production of worker, queen and male pupae was checked 3 times a week during 3 months. Pupae length was then measured on a Leica® stereomicroscope equipped with a digital camera. Worker mortality rate (percentage of death.d^{-1}) was estimated by counting the number of living workers after 42 and 84 days and averaging mortality rates over both periods. Linear mixed models were used to test differences in the production and size of worker, queen and male and in worker mortality rates. The colony of origin was included as a random factor, while diet was a fixed factor. Holm's sequential Bonferroni procedure was used to control for the risk of accepting false negatives (R Development Core Team, 2010).

3.2.4 Dependence of plants on *A. senilis*: relative specificity of diaspore-removal behavior

The relative importance of diaspore removal behavior by ants versus vertebrates was tested by setting up fruit depots of three plant species (*A. italicum*, *P. angustifolia* and *P. lentiscus*) on the ground during their respective fructification periods. At 8:00 a.m., 15 pairs of depots containing 10 fruits each were set up near the plants. One depot of each pair was open to all animals, while the other was covered with a vertebrate excluder that allowed ant passage only (**Fig. 3.4**). A similar experiment was conducted for *O. orthophyllum* in more natural conditions. Preliminary observations indicated that ants cut the peduncle of the fruit and the stalk in order to remove the fruit. Therefore, we selected 15 pairs of plants, counted the number of capsules at 8:00 a.m. and covered half of them with a vertebrate excluder, while the other half remained accessible to all animals. Non-removed diaspores were counted after 24 hours.

Figure 3.4 Vertebrate excluder with a fruits of *Phillyrea angustifolia*

Linear mixed models were used to compare diaspore removal between control (open) and experimental (vertebrate excluder) depots, with the pair considered as a random factor. Plant species and Vertebrate excluder were fixed factors. The lme function from the R software was used to fit linear mixed models (R Development Core Team, 2010).

A comparison of diaspore removal behavior between ant species was also carried out in the field for *A. italicum*, *P. lentiscus*, *P. angustifolia* and *O. orthophyllum*. Depending on the plant species, 15 and 30 observation stations were set up in the morning between 8:00 and 10:00 a.m. close to fruiting plants. Each station was composed of a 60x60 cm quadrat at the center of which 5 to 20 fruits were deposited, depending on the plant species. Ant presence and behavior were recorded in the quadrats during 3 min every hour until 8:00 p.m. or until the fruits had been removed.

Ant behavior was classified as i) ignore, ii) antennate, explore or feed on the pulp and iii) remove diaspore. For each plant species, the frequency of each behavioral category was compared between ant species by means of Pearson χ^2 test (R Development Core Team, 2010). In addition, linear mixed models were fitted for each plant species with removal rate per hour as the response variable and the occurrence of each ant species in the quadrat during the previous hour as explanatory variable. The quadrats were included as random variables.

3.2.5 Dependence of plants on *A. senilis*: dispersal distance, seed survival and germination

Distance of diaspore dispersal by *A. senilis* was estimated for *A. italicum* only. Thirty plants were censored during periods of 3 min during 12 hours. When *A. senilis* workers were observed removing fruits, they were followed to their nest and the plant-nest distance was measured. Then an area represented by a 2 m radius quarter-circle centered on the nest was carefully checked to locate rejected *A. italicum* seeds. Lilliefors normality test was used to test whether dispersal distance followed a normal distribution (R Development Core Team, 2010).

The seeds *A. italicum*, *P. lentiscus*, *P. angustifolia* and *O. orthophyllum* were collected after they had been rejected in the vicinity of the nests (< 1m). They were brought to the lab and moistened in water during 24h. They were then cut longitudinally and placed in a 1% Tetrazolium (TZ) water solution during 24h. Living (respirating) embryos were expected to color red (Grooms 2006). Pearson chi-square test was used to compare seed survival between plant species.

Finally, two germination tests were conducted on *A. italicum* (**Fig. 3.5**). Fifty seeds collected outside *A. senilis* nests (<1m; dispersed) or on the plants (non-dispersed) were sowed individually in peat pots maintained in a green house at 25 ºC. Water was provided every 2-3 days. The proportion of germinated seeds in both groups was checked after 20 weeks and compared using Yates-corrected chi-square test. In addition, a germination test was conducted in the field using seeds collected in August 2009. Sixteen and six envelopes prepared with a mosquito net were filled with 12 dispersed and non-dispersed seeds, respectively. They were sowed in a favorable habitat, below *P. lenstisus*, in September 2009. Germination was checked after 27 weeks. The proportion of germinated seeds per envelope was compared between treatments by ANOVA (R Development Core Team, 2010).

a b

Figure 3.5 Germination tests in *Arum italicum*. a) In the laboratory, b) In the field, in the study area

3.3 RESULTS

3.3.1 Interdependence between *A. senilis* and non-myrmecochorous diaspores: diet analysis

A total of 2148 retrieved food items were sampled, out of which 334 (16%) were seeds or fruits. The remaining were mostly insect corpses, flower petals and bird feces. The proportion of diaspores in the diet was close to zero from February to May but increased significantly in the second part of the year (difference between months: ANOVA: $F_{9, 47}$ = 6.13, P < 0.0001). In October diaspores represented 46 % ± 10 % (mean ± SE) of all retrieved food items. Fruits of *A. italicum, P. lentiscus* and *P. angustifolia* were among the preferred diaspores (**Fig. 3.6**). In July, fruits of *A. italicum* accounted for almost one fourth of the retrieved items (22% ± 9 %, **Fig. 3.7a**). In October, those of *P. lentiscus* and

P. angustifolia also accounted for a major fraction of *A. senilis* diet (October: 12 % ± 8 %, and 25 % ± 13 %, respectively). Other seeds and fruits retrieved in lesser proportions belonged to *J. acutus* (*Juncaceae*), *N. serotinus* (*Amaryllidaceae*), *Rhamnus alaternus* (*Rhamnaceae*), *Anagalis sp.*(*Primulaceae*), *Erodium sp.*(*Geraniaceae*), *Medicago sp.*(*Fabaceae*), *Frankenia laevis* (*Frankeniaceae*) and various species of *Poaceae* and *Asteraceae* that could not be identified.

a b

c d

Figure 3.6 *Aphaenogaster senilis* carrying different fruits; a) *Arum italicum*; b) *Pistacea lentiscus*; c) *Phillyrea angustifolia*; d) *Ornithogalum orthopyllum* (photo:F. Amor)

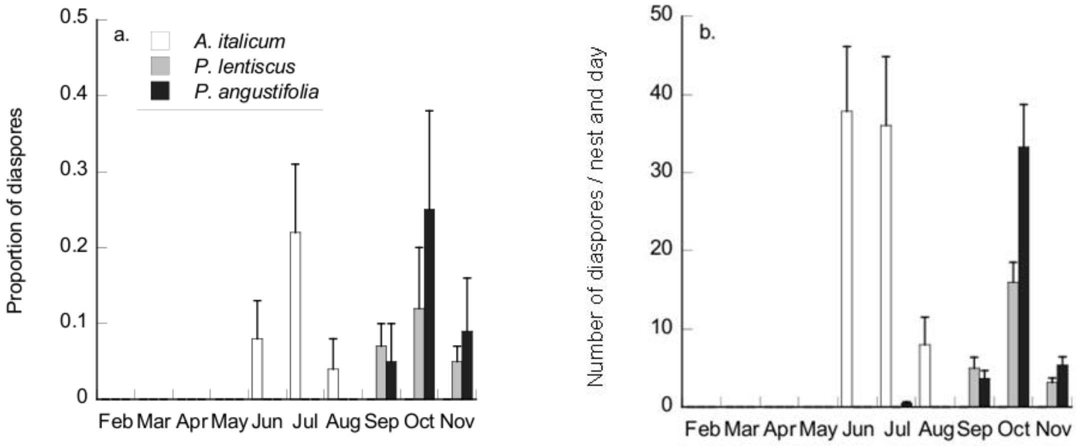

Figure 3.7 Annual variations in the proportion (a) and number (b) of diaspores in the diet of *A. senilis.* Values are means ± SE

The foraging activity of *A. senilis* showed a marked annual pattern. The number of retrieved food items was the highest in May, ranging between 336 and 1242 items.nest^{-1}.d^{-1}, and the lowest in November, ranging between 6 and 96 items.nest^{-1}.d^{-1}. The combination between the total number of retrieved items per nest and day and the proportion of diaspores indicated that the estimated number of retrieved diaspores increased significantly between June and November compared to the previous months (**Fig. 3.7b**; ANOVA: $F_{9, 76} = 11.58$, $P < 0.0001$). In June, colonies of *A. senilis* retrieved up to 37.9 ± 8.3 (mean ± SE) fruits of *A. italicum* per day and in October they collected 16.0 ± 2.6 and 32.3 ± 5.4 fruits of *P. lentiscus* and *P. angustifolia,* respectively.

3.3.2 Dependence of *A. senilis* on non-myrmecochorous diaspores: diet supplementation

The addition of *A. italicum* fruits to a normal mealworm-based diet had two major consequences. It increased diploid larvae size and workers' male offspring number. Although the number of 1st instar diploid larvae developing into workers and queens did not differ significantly between the two diets, both female castes were significantly larger when *A. italicum* fruits were provided (**Table 3.1**). Queen and worker pupae were 10% and 9% longer when *A. italicum* fruits were provided.

Table 3.1 Effect of diet supplementation with *A. italicum* fruits on the number and size of worker, male and queen pupae and worker survival rate. All values are means ± SE. Bold values denote significant differences after Holms' sequential Bonferroni correction. MW: mealworm diet; MW/Ar: mealworm diet supplemented with *A. italicum* fruits.

	MW	MW/Ar	Df (Num, Den)	F	P
Number of new queens	3.4 ± 0.37	2.5 ± 0.52	1, 9	2.93	0.1212
Number of new workers	9.4 ± 1.49	11.2 ± 1.22	1, 9	3.57	0.0913
Number of workers' male offspring	**2.8 ± 1.06**	**6.0 ± 1.17**	**1, 9**	**11.29**	**0.0084**
Queen size (mm)	**5.21 ± 0.07**	**5.74 ± 0.11**	**1, 44**	**20.70**	**0.0001**
Worker size (mm)	**4.18 ± 0.05**	**4.57 ± 0.05**	**1, 136**	**57.23**	**0.0001**
Male size (mm)	4.35 ± 0.04	4.44 ± 0.05	1, 44	3.02	0.0891
Worker mortality rate (% of death.d^{-1})	1.01 ± 0.07	0.97 ± 0.07	1, 9	0.09	0.7669

Only five male pupae were produced in total during the first three weeks of the experiment. Since orphan workers start egg laying at about 10 days, these males were in all likelihood queen's male offspring. They were removed from the analysis. Workers' male offspring pupae appeared after 50 days. They were 2.1 times more numerous when *A. italicum* fruits were provided (**Table 3.1**). Males were only 2% longer with *A. italicum* fruits, which was not significantly different. On average, 1.39±0.07 workers died every day in both treatments, which represented a mortality rate of 0.94 ± 0.10% dead workers.d^{-1}. Mortality rate did not differ according to the diet.

3.3.3 Dependence of plants on *A. senilis*: relative specificity of diaspore-removal behavior

The proportion of seeds removed in 24h differed significantly between plant species (lme: $F_{3,\ 59}$ = 12.79, P < 0001). *A. italicum* and *P. angustifolia* were the most rapidly removed fruits, while the removal rates of both *P. lentiscus* and *O. orthophyllum* were lower. The presence/absence of a vertebrate excluder did not significantly affect removal rate (**Table 3.2**; lme: $F_{1,\ 59}$ = 0.04, P = 0.84), nor did the Vertebrate excluder effect interact significantly with that of Plant species (lme: $F_{3,\ 59}$ = 0.33, P < 0.80). This suggests that once they are on the ground, diaspores of the four plant species are mostly ant removed and that vertebrates have little or no impact on removal.

Table 3.2 Difference of diaspore removal rate in 24h between four plant species with and without vertebrate excluders. Different letters between parentheses denote significant differences between plant species. There was no significant difference between open and vertebrate excluded depots. N=15 depots per species and treatment.

	Open depots	With vertebrate excluder
A. italicum (a)	0.97 ± 0.02	0.99 ± 0.01
O. orthophyllum (b)	0.46 ± 0.12	0.41 ± 0.12
P. angustifolia (a)	0.85 ± 0.08	0.83 ± 0.07
P. lentiscus (b)	0.49 ± 0.11	0.58 ± 0.11

A total of seven ant species were monitored around the studied plants. *A. senilis* (Asen) and *Tetramorium fortis* (Tfor) were active from February to November and were frequently observed in the square near the fruiting plants. By contrast, *Cataglyphis rosenhaueri* (Cros) and *Crematogaster auberti* (Caub) were only active in summer, during the fructification of *A. italicum*. The frequency of diaspore removal behavior was extremely biased towards *A. senilis.* With the exception of *M. barbarus* (Mbar), that was seen transporting one drupe of *P. angustifolia* and one drupe of *P. lentiscus*, *A. senilis* was the only species that removed diaspores (**Fig. 3.8**). The other ant species either ignored them or explored them with their antenna. The proportion of removal behaviors by *A. senilis* versus both other behavioral classes (ignore and explore) did not vary significantly between plant species (χ^2 = 3.83, d.f. = 3, P = 0.2803), suggesting that *A. senilis* has no marked preference for any of them.

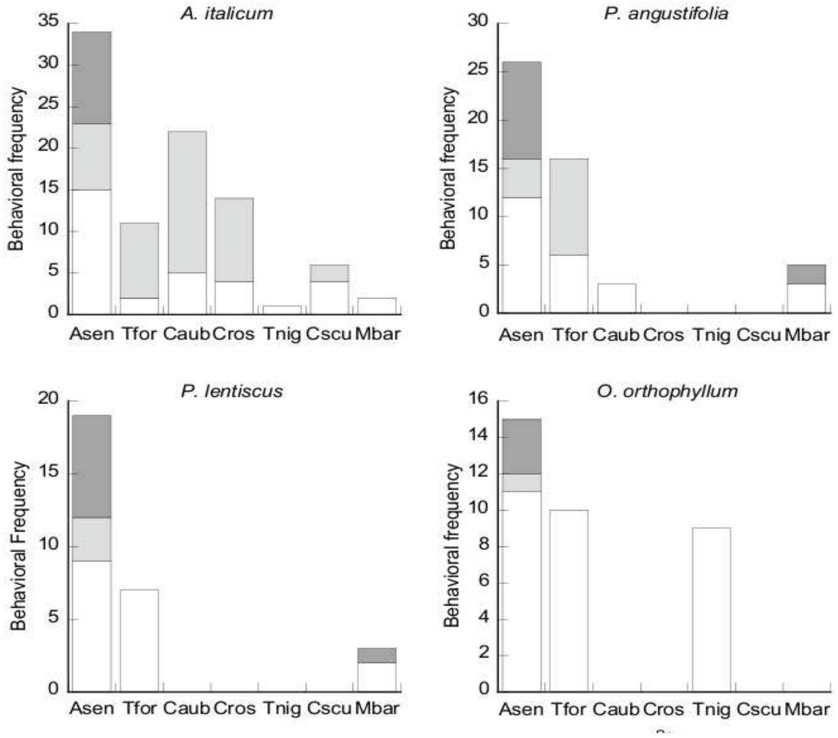

Figure 3.8 Relative frequency of behavioral interactions between the most abundant ant species and the diaspores of four plant species. White: ignore; light grey: antennate/explore; dark grey: remove. Ant species: Asen: *Aphaenogaster senilis*; Tfor: *Tetramorium forte*; Caub: *Crematogaster auberti*; Cros: *Cataglyphis rosenhaueri*; Tnig: *Tapinoma nigerrimum*; Cscu: *Crematogaster scutellaris*; Mbar: *Messor barbarus*.

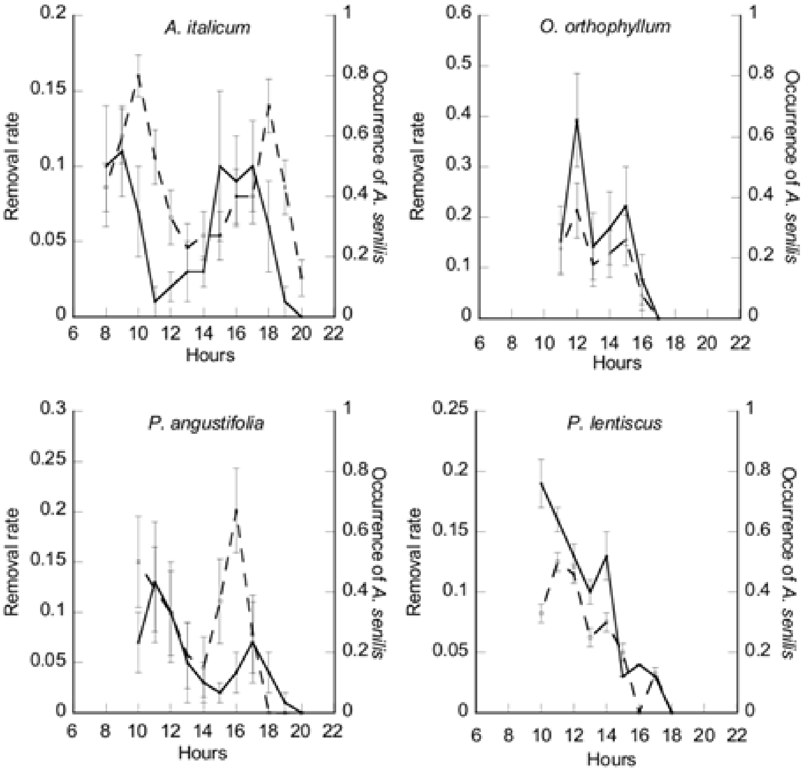

Figure 3.9 Relation between the occurrence of *A. senilis* in the quadrats (dashed line) and diaspore removal rate during the following hour (plain line). Values are means ± SE.

The previous results were corroborated by the fact that the proportion of removed diaspores in one hour correlated significantly with the occurrence of *A. senilis* during the previous hour, but not with that of other ant species (**Fig. 3.9; Table 3.3**).

Table 3.3 Results of the four linear mixed models testing the relation between the occurrence of ant species in a quadrat and diaspore removal rate during the following hour. Results in bold denote significant correlation between species occurrence and removal rate.

	A. italicum		*O. orthophyllum*		*P. angustifolia*		*P. lentiscus*	
	$F_{1,202}$	P	$F_{1,147}$	P	$F_{1,236}$	P	$F_{1,117}$	P
A. senilis	**9.14**	**0.003**	**439.4**	**<0.001**	**33.42**	**<0.001**	**25.10**	**<0.001**
T. fortis	3.14	0.078	0.07	0.1531	0.22	0.637	1.04	0.310
C. auberti	0.02	0.883	-	-	1.50	0.223	-	-
C. rosenhaueri	1.05	0.306	-	-	-	-	-	-
T. nigerrimum	2.92	0.089	2.06	0.7851	-	-	-	-
C. scutellaris	1.63	0.203	-	-	-	-	-	-
M. barbarus	2.55	0.115	-	-	3.03	0.083	0.59	0.442

3.3.4 Dependence of plants on *A. senilis*: Seed dispersal, survival and germination

Dispersal distance was only measured for *A. italicum*. The first seed movement occurred when the fruits were transported from the mother plant to an *A. senilis* nest. Of the 30 plants that were monitored, 28 were visited by one to three *A. senilis* colonies. The distribution of plant-to-nest distances differed significantly from the Normal distribution (**Fig. 3.10**; Lilliefors normality test: D = 0.1429, n=39, P=0.0432). It showed that the majority of seeds (41%) were primary moved to a nest located two to four meters away from the mother plant. However, a few seeds could be transported up 8m away. A second movement occurred after the ants had consumed fruit pulp. The seeds were then. A total of 399 *A. italicum* seeds were discovered after they had been discarded from ant nests. As previously, the distribution of secondary dispersal distances differed significantly from the Normal distribution, with most seeds (48%) rejected between 25 and 50 cm away from the nests (**Fig. 3.10**; Lilliefors normality test: D = 0.1201, P<0.0001).

TZ test indicated that 92% (n=26 seeds) of *A. italicum* seeds rejected by *A. senilis* were still alive. This percentage was also high and not significantly different for the other plant species (*P. lentiscus* (80%, n=10), *P. angustifolia* (96%, n=28) and *O. orthophyllum* (100%, n=37; χ^2 = 8, df = 6, P = 0.2381).

Germination tests gave contrasting results in the lab and in the field. After 20 weeks in laboratory conditions, non-dispersed *A. italicum* seeds germinated in a significantly higher proportion than dispersed seeds (88 % vs 68%; Yates corrected χ^2 = 4.72; df= 1; P = 0.029; n= 50 seeds per treatment). However, in the field, after 27 weeks, the average proportion of seeds that germinated per envelope was not significantly different between dispersed and non-dispersed seeds (51±21% vs 39±7%, mean±SE, respectively; ANOVA: $F_{1, 21}$ =1.05, P = 0.3186).

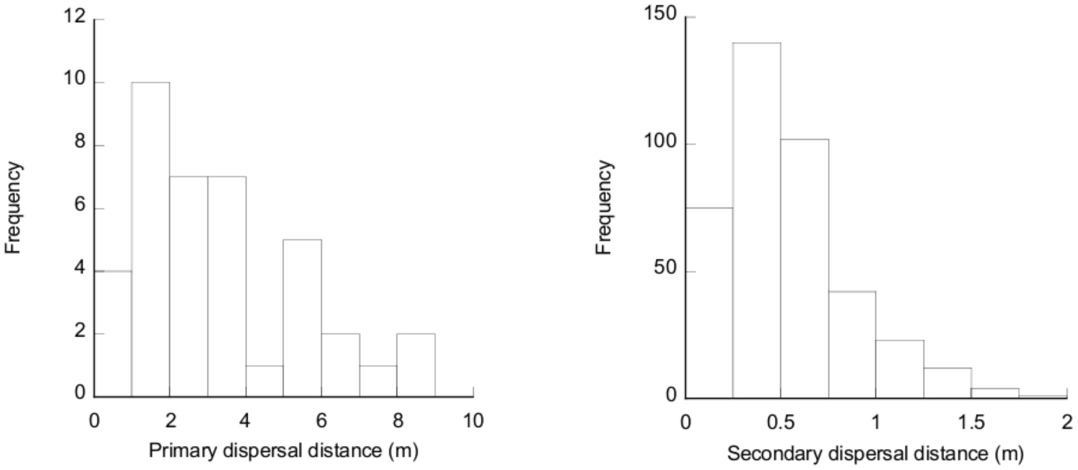

Figure 3.10 Distribution of primary and secondary dispersal distances of *A. italicum* fruits. Primary dispersal is from the plant to the nest. Secondary dispersal is from the nest to the final rejection location.

3.4 DISCUSSION

This study demonstrates the complexity and variable degree of interdependence asymmetry in ant-seed dispersal interactions. As hypothesized, detailed diet analysis showed that, depending on the season, non-myrmecochorous diaspores may account for a relatively high proportion of the food items retrieved by *A. senilis*, emphasizing their importance for ants. Moreover, the supplementation of a normal mealworm-based diet with *A. italicum* fruits modified worker, queen and male production in a manner that enhanced adult worker fitness. Our results also gave credence to our second hypothesis. *A. senilis* was the main transporter of *A. italicum* and *O. orthophyllum* and of the fallen fruits of *P. lentiscus* and *P. angustifolia*. Seeds were moved several meters away from the mother plant, and a high proportion survived and germinated. However, the estimated number of removed fruits per nest and day was low and suggested that, depending on the plant species, ants could either virtually retrieve the entire fruit crop or only a negligible portion.

So far, seed dispersal by ants has mostly been studied from the plant perspective by determining ants' potential effect on plant demography and reproduction. Yet, understanding the evolution of seed dispersal by ants also necessitates an insight into the effect of plant rewards on ants. Keystone seed dispersers diet is known for a few species only (Lubertazzi et al. 2010). At our study site *A. senilis* collected a large array of food items, which confirms their omnivorous regime. Particularly interesting are the relative importance and diversity of diaspores included in their diet. In summer and fall, fruits represented up to 1/4 of the retrieved items. These diaspores belonged to at least 12 plant families. *A. italicum*, *P. lentiscus* and *P. angustifolia* were among the preferred diaspores. A few seeds of *Rhamnus alaternus* were also collected, although this species does not grow in our study plot. This suggests that these seeds had been removed from bird droppings, as has been shown in other regions (Aronne & Wilcock 1994; Bas et al. 2009).

P. lentiscus and *P. angustifolia* are two bird-dispersed plants (Jordano 1989). The lack of difference in seed removal between open and vertebrate-excluded depots suggests that, during our study, birds or other vertebrates did not remove both shrubs' fallen fruits. To our knowledge, no legitimate disperser had been described for *A. italicum* and *O. orthophyllum*. Vertebrate excluders and the observation of removal behavior by ants suggest that *A. senilis* is the dispersal agent of both herbs. The fruits of *A. italicum* are toxic to vertebrates but not to *A. senilis*, which intensively removed them and consumed their pulp.

In the laboratory, the effect of adding *A. italicum* diaspores to an insect-based diet provoked different effects than those already reported with elaiosome-bearing seeds (Gammans et al. 2005; Fokuhl et al. 2007, 2012). *A. italicum* fruits did not alter orphan worker survival but increased their inclusive fitness by affecting offspring production. The absence of the queen does not represent a hopeless reproductive situation for *A. senilis* because workers can lay their own haploid male eggs and raise their sister larvae into replacement queens (Boulay et al. 2009b). Increasing male production by workers clearly enhances their direct fitness. Moreover, increasing both worker and queen pupae size probably increases their chance of success, and indirectly adult fitness. The mechanism by which the consumption of *A. italicum* fruits alters worker reproduction larval growth is beyond the scope of the present study. However, this effect is probably qualitative rather than quantitative, since mealworms were provided in large excess. The pulp of *A. italicum* contains a high concentration of carbohydrates and carotenoids (Debussche et al. 1987) and possibly many other nutrients that may affect larval development and worker egg-laying. In addition, the consumption of *A. italicum* pulp by adult workers may affect their physiology and behavior, and indirectly the way nurses rear the brood.

Removal of non-myrmecochorous diaspore is not a general behavior among ants. Although the offered fruits potentially attracted several ant species, most of them behaved as cheaters by feeding on the pulp without contributing effectively to dispersal. *A. senilis* was almost the only species that removed diaspores. Moreover, diaspore removal of the four studied plant species fitted the rhythm of *A. senilis* but not that of other ants. The genus *Aphaenogaster*, including *A. senilis*, was already considered as an important myrmecochore partner (Hughes et al. 1994; Boulay et al. 2005; Manzaneda et al. 2007; Boulay et al. 2007a, b; Espadaler & Gómez 1996; Zelikova et al. 2008; Ness et al. 2009). These results cast doubts on the hypothesis that seed-dispersal by ants is an unspecialized interaction. They also contradict the hypothesis according to which the main myrmecochorous seed dispersers are scavengers that rarely collect plant material other than elaiosomes (Hughes et al. 1994).

At least two non-exclusive hypotheses may explain the predominant role of *Aphaenogaster* species in fruits and seeds removal. First, as suggested by Boulay et al. (2007a), *Aphaenogaster* are generally subordinate species that are very quick to discover food items but are not efficient at defending them against small, mass recruiting dominant species like *Tetramorium forte* or *Tapinoma nigerrimum.* They may therefore prefer to remove food items rapidly to their nest to consume them away from the threat of competitors. A second hypothesis is that, contrarily to other ants, *Aphaenogaster* workers are not able of trophallaxis. They cannot fill their crop with liquid food in order to regurgitate it to their larvae. Instead, the brood is transported on the preys on which it feeds directly. Interestingly, in the tropics, Ponerine ants are among the main secondary dispersers of fleshy fruits (Pizo & Oliveira 1998; Fourcassié & Oliveira 2002; Passos & Oliveira 2004). Like *Aphaenogaster*, these species are not dominant and do not perform trophallaxis.

The benefit for plants of seed dispersal by ants depends on post-dispersal seed fate and on the number of seeds that are removed compared to annual crop size. Survival of ant-dispersed seeds was relatively high for the four plant species. Germination tests conducted on *A. italicum* gave a significant difference between dispersed and non-dispersed seeds in the laboratory but not in the field. This apparent discrepancy is difficult to explain and should be taken carefully, given the small sample size of non-dispersed seeds in the field. Although both tests suggested a lower germination rate for dispersed seeds of *A. italicum*, it was still relatively high (39 ± 7%). Such limited negative effect on germination rate may be compensated by advantages related to dispersal distance and deposition site. Primary dispersal allowed transporting most *A. italicum* fruits 2-4m away (and up to 8 m away) from the mother plant. This corresponds to the average foraging distance of *A. senilis* (Cerdá et al. 2009). This dispersal distance may be sufficient to promote the colonization of new habitats by small herbs like *A. italicum* but might not be relevant for shrubs whose fruits are transported a hundred meters away by birds (Traveset 1994; Hulme 1997). After they consumed the pulp, *A. senilis* scattered the seeds outside their nest, which probably contributed to reduce intraspecific competition among seeds. Moreover, at our study site, *A. italicum* only grows in the shade of shrubs separated by few meters-gaps of low vegetation. *A. senilis*, which in summer also nests in the shade of shrubs, may allow seeds to cross these gaps to reach favorable habitats.

From the ant perspective, the proportion of seeds in the diet was relatively high during the second half of the year. However, at that time ant foraging activity decreased dramatically and the absolute number of collected diaspores did not exceed 40 per day. This means that one nest could virtually remove all the fruits of a spadix (up to 100; Méndez & Díaz 2001) in less than three days. By contrast, for shrubs like *P. lentsicus*, ant impact might be negligible. Given a rough estimate of adult plant crop of about 10000 fruits (Jordano 1989), each colony of *A. senilis* is likely to remove at most 0.16 % of a plant crop per day. Even if one plant of *P. lentiscus* can be visited by two to three colonies of *A. senilis*, ant impact on seed dispersal remains negligible compared to bird effect.

To conclude, we have shown that dispersal of non-myrmecochorous plants by ants in Mediterranean habitats may have been an underestimated phenomenon. Fleshy fruits are an important component of some ants’ diet, but their contribution to seed dispersal may vary between plant species, depending on crop size. This emphasizes the variable degree of asymmetry in the interdependence between ants and plants. More generally, our results suggest that although plant rewards are apparently non-specific and could potentially attract a large variety of consumers, a few partners have a decisive impact on seed fate. These “keystone dispersers” greatly affect plant population dynamics and probably exert strong selection pressures on dispersal-enhancing diaspore traits

CHAPTER 4

Frequent colony relocations do not result in effective dispersal in the Gypsy ant *Aphaenogaster senilis*[3]

ABSTRACT / RESUMEN

Dispersal is an important step in animal’s life cycle, one consequence of which is reducing local mate and resource competition. Dispersal is often achieved during one unique special movement, from the birthplace to a new appropriate area where to settle and reproduce. However, in species in which this special movement is limited by life history trade-offs, we may expect dispersal to be promoted also by routine movements occurring throughout the animal's life and stimulated by other activities like foraging or the search of nesting conditions.

Here, we employ a multidisciplinary approach consisting of computer simulations, mark-recapture and genetic data to better understand the role of colony relocations as dispersal strategy in the ant *Aphaenogaster senilis.* Contrary to expectations, our results show that colony relocations do not result in effective dispersal as evidenced by mark-recapture and genetic data. Furthermore, simulations showed that

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[3] Juan A. Galarza, Roger Jovani, Xim Cerdá, Ciro Rico, Ángel Barroso and Raphaël Boulay. 2012 Oikos 121: 605-613.

successive colony relocations did not follow a constant direction, but occurred either in a randomly changing direction or followed a circular trajectory, indicating limited effective dispersal. We also found a general lack of inbreeding and significant population viscosity between neighbouring colonies suggesting that relocations may act as a balancing strategy between these two processes. We discuss the results in terms of their evolutionary and ecological significance, and highlight future directions of research towards the understanding of dispersal strategies in colonial species.

La dispersión es una fase importante en el ciclo biológico de cualquier especie, una de cuyas consecuencias es la reducción de la densidad local de individuos y de la competencia por los recursos. La dispersión se realiza generalmente mediante un movimiento único y especial, desde el lugar de nacimiento a otra área apropiada donde asentarse y reproducirse. Sin embargo, en especies en las que estos movimientos especiales están limitados por circunstancias de su ciclo vital, podemos esperar que la dispersión sea promovida también por los movimientos rutinarios que se producen a lo largo de su ciclo de vida y que sea estimulada por otras actividades como la búsqueda de alimento o la de condiciones de anidación.

En este caso hemos empleado un enfoque multidisciplinar, que ha consistido en simulaciones informáticas, marcado y recaptura, y análisis genéticos, para comprender mejor el papel de las migraciones coloniales en la estrategia de dispersión de *Aphaenogaster senilis*. Contrariamente a lo esperado, nuestros resultados muestran que las migraciones no dan lugar a una dispersión efectiva, como ponen en evidencia, tanto los datos de marcado y recaptura, como los datos genéticos. Además, las simulaciones muestran que las sucesivas migraciones no siguen una dirección constante, si no que se producen cambios de dirección aleatorios o bien siguen trayectorias circulares, lo que indica un limitado efecto de dispersión. No hemos encontrado endogamia y sí un parentesco genético significativo entre colonias vecinas (viscosidad poblacional), sugiriendo que las migraciones pueden actuar como una estrategia de balanceo o equilibrio entre estos dos procesos. Se discute el significado evolutivo y ecológico de los resultados y se indican orientaciones futuras para la investigación de las estrategias de dispersión en especies coloniales.

4.1 INTRODUCTION

Dispersal is a key process in animals with major implications in ecology, evolution and conservation (Clobert et al. 2001; Wang & Smith 2002). Both theoretical and empirical studies have largely unravel the importance of dispersal processes in inbreeding avoidance (Bollinger et al. 1993; Waser et al. 1986), kin competition (Hamilton & May 1977; Kisdi 2004; Ronce & Promislow 2010) and gene flow among geographically distant populations (Fahrig & Merriam 1985; Hansson 1991; Galarza et al. 2009).

Conceptually, dispersal is often viewed as the spread of individuals from their natal site to settle and reproduce in new areas (Johnson 1969; Greenwood & Harvey 1982; Hawkes 2009). Given its importance, dispersal can derive from a special movement that evolved specifically to allow the displacement of individuals away from their natal site, generally early in their life cycle. Such special movements are well exemplified in species with important dispersal polymorphism. In other species, however, dispersal results from routine movements associated to other activities like foraging or exploration leading to effective dispersal if reproduction takes place in different locations (Van Dyck & Baguette 2005).

Ants are interesting models in population ecology because different species have evolved various dispersal modes in relation with colony foundation (Hölldobler & Wilson 1990; Bourke & Franks 1995). On the one hand, the virgin queens of many species bear long wings activated by a hypertrophied thoracic musculature that allow them flying over relatively long distances (Peeters & Ito 2001). After mating during a nuptial flight, they shed their wings and almost immediately start searching for a nest location to initiate a new colony by themselves. While independent colony foundation exposes queens to important mortality rate due to predation and competition with established colonies (Gordon & Kulig 1988; Wiernasz & Cole 1995, 2003; Adams & Tschinkel 2001; Boulay et al. 2007d), it is though to guarantee enough gene flow to prevent isolation by distance (Helmkampf et al. 2008).

On the other hand, some ant species found new colonies by colony fission. Here, queens either have small non-functional wings or are completely wingless (Molet & Peeters 2006; Molet et al. 2008; Amor et al. 2011). They leave their mother nest accompanied by a group of workers to found a new colony at a walking distance. This strategy increases queen survival during colony foundation but, at the same time, it limits the distance at which the new colony can establish (Pamilo 1991). This strategy is expected to translate into high genetic structuring and limited gene flow, even within short distances (Seppä & Pamilo 1995; Giraud et al. 2000; Clémencet et al. 2005 Berghoff et al. 2008) resulting a high relatedness among neighboring colonies (population viscosity), which in turn, may give rise to local resource competition (Wilson et al. 1992; West et al. 2001).

Although some ant species rarely abandon their nest during decades (e.g. *Pogonomyrmex occidentalis*) (Keeler 1988), others perform frequent colony relocations. Extreme nomadism occurs in army ants of the Dorylinae, Leptanillinae and Ecitoninae subfamilies, in which it is mainly tied to predation. These species do not build a structured nest but only form temporal bivouacs where an enormous mass of workers aggregate around the queen.

Beside these species with a particular lifestyle, colony relocations also occur with a relatively high frequency in several genera, including *Solenopsis* (Fuller et al. 1984; Tschinkel 2006) *Pheidole* (Droual 1984), *Temnothorax*, formerly *Leptothorax* (Dornhaus et al. 2004) and *Aphaenogaster* (Smallwood 1982; McGlynn et al. 2004; Boulay et al. 2010). The causes of colony relocations can be very diverse, however. Some species relocate their colony after an external perturbation such as flooding (Tschinkel 2006) or when microclimatic conditions change (Smallwood 1982; Gibb & Houchuli 2003; Heller & Gordon 2006). For other species, colony relocation may also occur as a response to an attack by competitors (Cerdá & Retana 1998; Brown 1999; Dahbi et al. 2008) predators (LaMon & Topoff 1981; Droual 1984) to reduce the parasitic load (Droual 1984; Gordon 1992; McGlynn et al. 2004) or to be near available food resources (Mabelis 1979; Maschwitz & Hänel 1985).

Even though the proximate causes of colony relocation are relatively well understood in several ant species, the genetic consequences of such movements at the population level remain widely unexplored, particularly for fission-performing species. For example, if on the course of several relocations, each colony tends to keep a relatively constant direction, we may expect the distance between related colonies to increase progressively, and thus reducing population viscosity. By contrast, if colonies tend to repeatedly use the same set of nests, forming a kind of circular movement, relocations may have little consequences on the population genetic structure. Such nest re-utilization after a few relocations (i.e. serial monodomy), has recently been described in the gypsy ant *Aphaenogaster araneoides* (McGlynn 2010).

In the present study, we examine the effects that colony relocations have at the population level in delineating genetic structure and population viscosity in another gipsy ant *A. senilis*, which founds new colonies by fission. Specifically, we determined:

1. If relocations occur randomly within the landscape.
2. If they have an effect in population viscosity.

For this purpose, we monitored colony movements through a mark-recapture approach combined with population genetic analyses and computer simulations. In addition, we document the process of colony fission, which is poorly understood in ants.

4.2 MATERIAL AND METHODS

4.2.1 Study sites

The study was conducted at four sites inside the Reserva Biológica de Doñana (namely *Comedero*, *Beles*, *Jaulón* and *Visita*) and in fifth place in the Algaida (Puntal de Boza) located in Sanlúcar de Barrameda (**Fig. 1.4, 1.6**). The study area is described in the introduction section 1.4.

4.2.2 Colony monitoring by mark-recapture

In order to study the process of colony relocation, 21 focal colonies were excavated between January and March 2006 at *Beles*, *Jaulón* and *La Algaida*. Focal colonies were chosen so they were separated by at least 10 m. Once in the lab, they were cooled down 10-30 min on ice at 0 ºC to mark all the workers and the queen with a dot of paint (Mitsubishi pencil) on the abdomen (**Fig 4.1**). Different colours were used for different colonies originating from the same site. All the marked workers, the queen and the brood were then released in the field at the spot of capture no later than a week after nest excavation.

Figure 4.1 *A. senilis* workers marked with a dot of paint on the abdomen

To ensure that paint marks remained visible over time, we conducted a preliminary pilot study in spring 2005, when 400 workers from an excavated nest were marked with two dots of paint on the abdomen and on the thorax and were then returned to the same place. After a month, we recaptured 86% of the originally marked ants, 98% of them still showing both marks and 2% showing only one mark. The much higher abundance of ants with two marks clearly suggests that those ants captured without marks were mostly new ants not marked in the first place. This indicates that the paint marks have a relatively long life and that the progressive disappearance of marked ants in focal nests was mostly due to high worker mortality in late spring.

Similarly, in a previous study (Boulay et al. 2009b), we have determined the production cycle of both queen and workers through a 6-year follow-up of more that 300 colonies. The results showed a significant reduction in worker abundance during spring followed by a peak abundance in early summer. This suggests a worker turn-over for this species of approximately 11-13 months when the majority of worker force is replaced. Therefore, both mark endurance and worker turn-over are well within the time frame of the present study. To ensure that colony monitoring could follow up during up to ten months that this study lasted, we repeatedly marked unmarked workers collected at the entrance of focal nests.

Figure 4.2 *Aphaenogaster senilis* nest entrance ornamented with vegetal remain

The location of marked colonies was then monitored once a week until they were lost and no later than November 2006. Colony location was determined by carefully scanning an area of 10m around the last location. The area was scrutinized between one or two people depending on the complexity or the vegetation for at least 20 minutes. *A. senilis* nests are often ornamented with flower petals or other vegetal remain, and can be clearly seen (**Fig. 4.2**). When a marked worker was detected, she was hand fed a small piece of biscuit and followed back to her nest. If the colony had relocated, we measured the distance from last location and the distance to the origin (e.g. the first colony location after its release). Colonies lost during the course of the experiment were assumed to have moved at least 10m. Nest half-life (Nhl), which describes the number of days for the colonies to relocate, was calculated according to the formula of (King & Sallee 1956): Nhl = R x ln(2), where R is the average residence time (the average number of days between two relocations).

The area around the last nest location was also checked for possible fission of the focal colonies. When a fission was confirmed by the presence of same-marked ants in two nests, both the mother and daughter nests were excavated. They were brought to the lab to count the workers but they were not released in the field and the monitoring of their trajectory was stopped.

We tested whether the probability of colony relocation was influenced by previous relocations by fitting a generalized linear model (GLM) using the glmer command for R

(The R Core Team 2010) with the binomial error distribution and logit link function. The probability of relocation during the current week (Preloc) was the response variable and the week (Wk), having relocated or not the previous week (RelocPrev) were considered as fixed factors in the full model. The sampling site (Sp) and the Colony (Col) were considered random factors. Wk was also included in the random part of the model to account for temporal variations within colonies. The sign of the RelocPrev estimate indicated whether the probability of relocating increased or decreased after a first relocation.

We also fitted a second generalized linear model with the log transformed relocation distance (Dreloc) as a response variable, Wk and Sp as fixed factors and Col as random factor. Backward model selection was conducted by comparing Akaike Information Criteria (AIC) using Log likelihood ratio tests until all non-significant factors were removed. The scripts for both models are given in the Supplementary material.

Finally we tested the correlation between relocation distance and colony size (number of workers at 1st capture) by mean of simple linear model.

4.2.3 Computer simulations

To identify possible patterns described by colonies after several relocations, we compared real relocations with those expected under a random-walk. First, we tested whether ant colonies showed any directionality over successive relocations. The real data was compared with random-walks simulated with NetLogo© 4.0.4 (code available from authors upon request). For each real colony 1,000 random trajectories were simulated using the observed number of relocations, their distances and order in which the relocations were undergone. Thus, the only aspect in which the simulations differed from the real data was on the turning angles performed by colonies from one relocation event to the next. Then, from each simulation we extracted the two values that we also gathered from real colonies, namely the distance between the final location and the origin and the cumulated distance between each intermediate location and the origin.

The statistical significance of the difference between real and simulated data was estimated as the proportion of simulations with values lower (or larger) than the real value (i.e. $p = 0.5$ means that the real value is equal to the average of 1000 simulations, and p = 0.01 would mean that only 1% of simulated data was larger, or lower, than the real value).

4.2.4 Genetic Structure and Population Viscosity

A total of 261 adult workers were collected in 61 colonies located at *Comedero, Visita,* and *Beles* in April 2008. At each site, we first selected four to seven colonies separated by at least 50m. We then sampled their two or four nearest neighbours. For each colony, a mean of 3.5 workers were genotyped at 6 species-specific polymorphic microsatellite markers (Galarza et al. 2009).

The total number of alleles per locus and colony was obtained using GENETIX v.4.01 (Belkhir et al. 1997). Observed and expected heterozygosities within sites were calculated using the software package Arlequin v. 2.0 (Schneider et al. 2000). Deviation from Hardy-Weinberg expectations (HWE) and linkage disequilibrium within sampling sites were estimated according to the level of significance determined by means of 10,000 Monte Carlo iterations using GENEPOP v.3.4. (Raymond & Rousset 1995). For these tests, a reduced dataset was used which included one randomly selected worker from each colony.

The false discovery rate (FDR) procedure (Benjamini & Hochberg 1995; Verhoeven et al. 2005) was employed to correct for possible type I errors when performing multiple tests. This procedure removes the fraction of false positives among all tests that are declared significant. For example, we set a FDR of 5% meaning that (on average) 5% of the tests declared significant are actually false positives.

The level of genetic structure was assessed by calculating the overall F_{ST} values (Weir & Cockerham 1984) including all colonies within each sampling site. Significance was obtained by 10,000 iterations executed in GENEPOP v.3.4. (Raymond & Rousset 1995) and FDR corrections were applied for multiple tests. Similarly, a two-way hierarchal analysis of molecular variance (AMOVA) was performed in Arlequin v. 2.0 (Schneider et al. 2000) to evaluate whether genetic variation was greater among or within sampling sites.

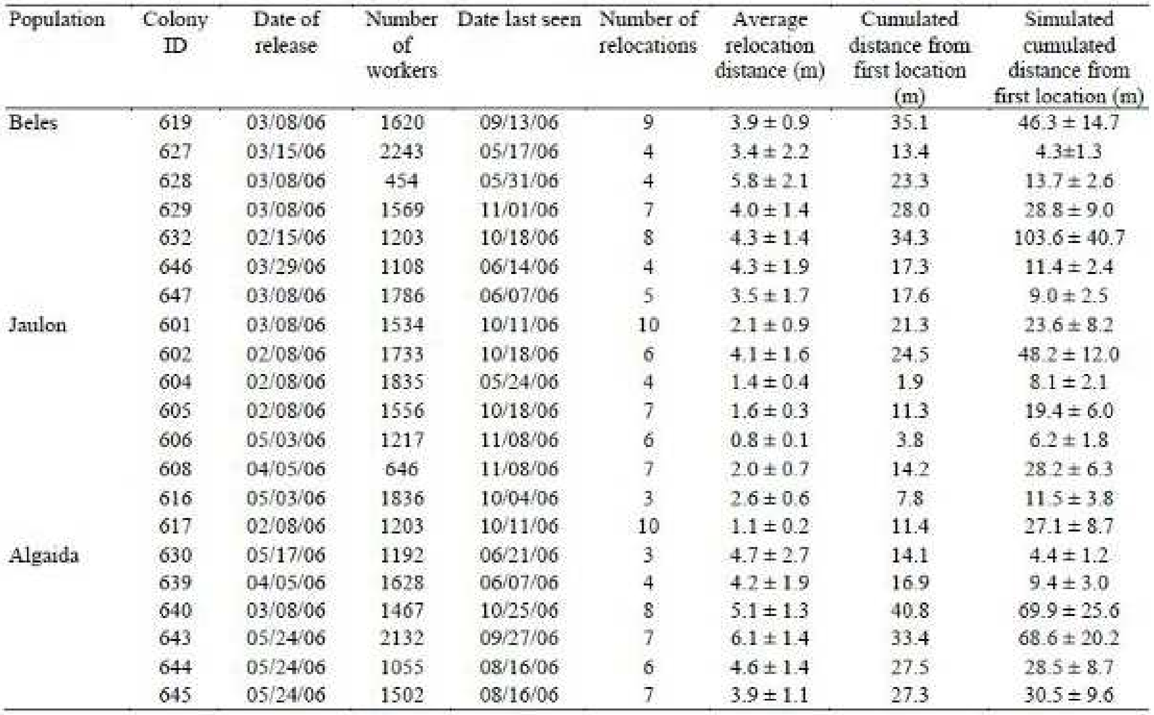
In order to determine the degree of population viscosity, we first calculated the relatedness coefficient (*R*) between colonies (i.e. average pairwise relatedness between individuals from different colonies) using Relatedness 5.0 software (Queller & Goodnight 1989). We then performed a spatial autocorrelation analysis using GenAlEx v. 6 (Peakall & Smouse 2006) to test whether significant relatedness (i.e. higher than random) occurs between pairs of colonies within a given distance class. We set an increment of 5 m for each distance class up to 100 m. Statistical significance for the null hypothesis of no significant relatedness was determined by creating 95% confidence intervals around *R*-values through 999 random permutations. Under the hypothesis of restricted effective dispersal due to colony fission, relatedness values should decrease as a function of distance, and spatial autocorrelation should be observed at short distances only.

4.3 RESULTS

4.3.1 Colony monitoring by mark-recapture

All monitored colonies relocated to some extent during the study period (**Table 4.1**). On average, they changed 6.14 ± 0.46 (hereafter, mean±SE) times of nest between their release in the field (starting late February 2006) and the last time they were observed (at most in November 2006). About 20% of the surveyed colonies each week were found in a new emplacement (**Fig. 4.3**). The average colony residence time was 29.0±3.6 days and the nest half-life was 20.1 days.

Table 4.1 Data from the 21 focal colonies used for mark-recapture and random-walk simulations



Population	Colony ID	Date of release	Number of workers	Date last seen	Number of relocations	Average relocation distance (m)	Cumulated distance from first location (m)	Simulated cumulated distance from first location (m)
Beles	619	03/08/06	1620	09/13/06	9	3.9 ± 0.9	35.1	46.3 ± 14.7
	627	03/15/06	2243	05/17/06	4	3.4 ± 2.2	13.4	4.3±1.3
	628	03/08/06	454	05/31/06	4	5.8 ± 2.1	23.3	13.7 ± 2.6
	629	03/08/06	1569	11/01/06	7	4.0 ± 1.4	28.0	28.8 ± 9.0
	632	02/15/06	1203	10/18/06	8	4.3 ± 1.4	34.3	103.6 ± 40.7
	646	03/29/06	1108	06/14/06	4	4.3 ± 1.9	17.3	11.4 ± 2.4
	647	03/08/06	1786	06/07/06	5	3.5 ± 1.7	17.6	9.0 ± 2.5
Jaulon	601	03/08/06	1534	10/11/06	10	2.1 ± 0.9	21.3	23.6 ± 8.2
	602	02/08/06	1733	10/18/06	6	4.1 ± 1.6	24.5	48.2 ± 12.0
	604	02/08/06	1835	05/24/06	4	1.4 ± 0.4	1.9	8.1 ± 2.1
	605	02/08/06	1556	10/18/06	7	1.6 ± 0.3	11.3	19.4 ± 6.0
	606	05/03/06	1217	11/08/06	6	0.8 ± 0.1	3.8	6.2 ± 1.8
	608	04/05/06	646	11/08/06	7	2.0 ± 0.7	14.2	28.2 ± 6.3
	616	05/03/06	1836	10/04/06	3	2.6 ± 0.6	7.8	11.5 ± 3.8
	617	02/08/06	1203	10/11/06	10	1.1 ± 0.2	11.4	27.1 ± 8.7
Algaida	630	05/17/06	1192	06/21/06	3	4.7 ± 2.7	14.1	4.4 ± 1.2
	639	04/05/06	1628	06/07/06	4	4.2 ± 1.9	16.9	9.4 ± 3.0
	640	03/08/06	1467	10/25/06	8	5.1 ± 1.3	40.8	69.9 ± 25.6
	643	05/24/06	2132	09/27/06	7	6.1 ± 1.4	33.4	68.6 ± 20.2
	644	05/24/06	1055	08/16/06	6	4.6 ± 1.4	27.5	28.5 ± 8.7
	645	05/24/06	1502	08/16/06	7	3.9 ± 1.1	27.3	30.5 ± 9.6

There was no linear trend in the probability of relocation throughout the experiment (**Fig. 4.2**; $F_{1,39}$= 0.87, P = 0.358), suggesting a low effect of our initial colony excavation upon their relocation behaviour. If this had been the case, we would have expected a higher relocation rate just after the manipulation.

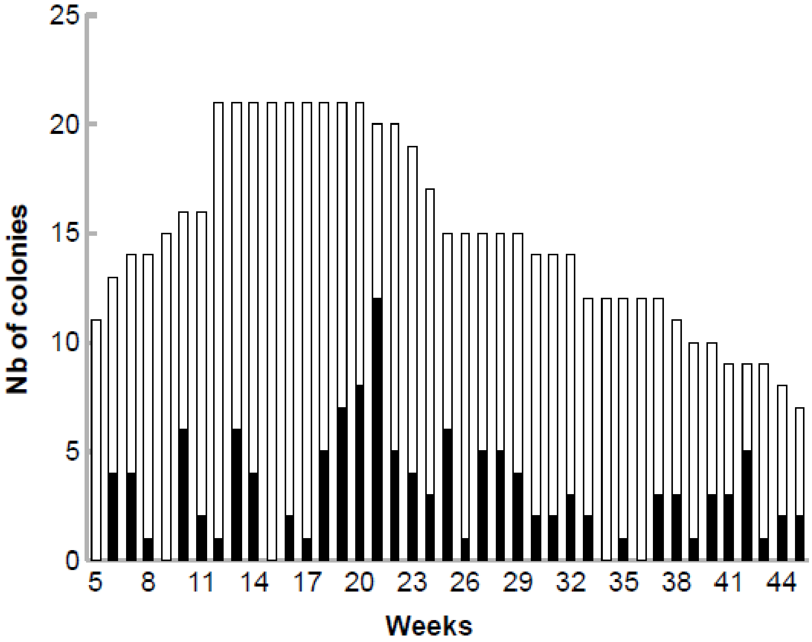


Figure 4.3 Number of *Aphaenogaster senilis* colonies surveyed each week (of the calendar) and number of colonies that relocated (black segments).

Generalized linear model selection based on the AIC did not retain Wk in fixed and random factors (its removal lead to small decrease of the model AIC). This indicated that the probability of relocation did not differ between weeks, even within colonies (see ESM for details of model selection). However, a colony that had relocated on one week had a higher probability to relocate again the following week (estimate: 0.43 ± 0.23; z = 2.13, P = 0.033). This was clearly demonstrated by the highly significant increase of the AIC when the factor RelocPrev was removed from the model (χ^2 =14.34, Df=1, P < 0.001). The variance explained by differences between colonies was very small (variance < 10^{-11}) compared to the varience explained by differences between sampling sites (0.217).

Relocation distances showed a long-tailed frequency distribution (**Fig. 4.4**) with relocations shorter than 2m and longer than 8m representing 50% and 19% of all relocation events, respectively. Relocation distances did not differ significantly between weeks as indicated by the non significant variation of the AIC when this factor was removed (χ^2 = 3.29, Df=1, P = 0.069. As for the probability of relocation, relocation distances mostly varied between sampling sites (variance = 0.73) rather than between colonies within sampling sites rather than between colonies (variance $<10^{-3}$). Relocation distance was not significantly correlated with colony size (linear model: $F_{1,19} = 0.01$, P =0.88).

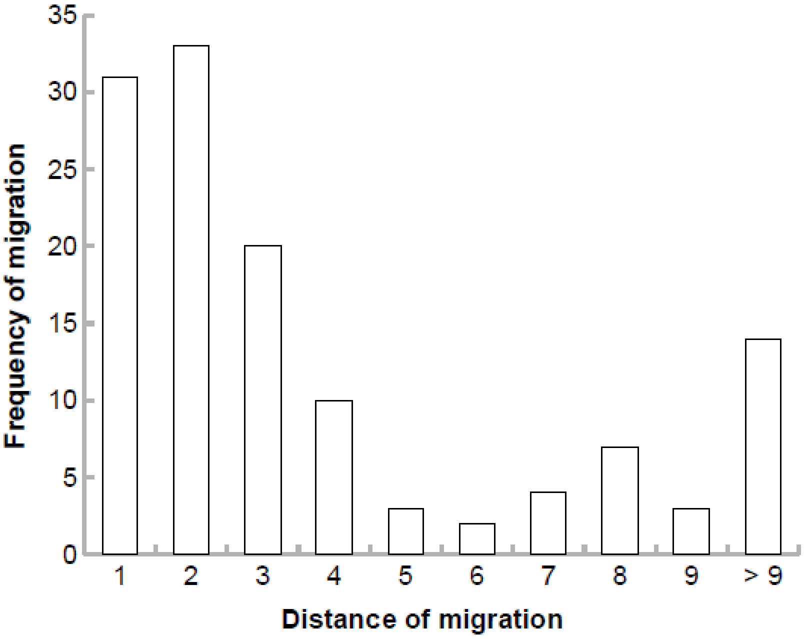


Figure 4.4 Frequency distribution of relocation distances of the 21 focal *Aphaenogaster senilis* colonies. X-axis: Distance of migration, unit in meter (m)

Only two out of the 21 focal colonies fissioned during the course of the survey. Both fissions occurred in August 2006 at *La Algaida*. In both cases the mother (marked) queen was found in a new nest located 2.3 or 2.7 m away from the old nest, which contained a single unmarked queen. These queens were surrounded by marked and unmarked workers, which confirmed the fission.

4.3.2 Computer simulations

Random-walk simulations indicated that successive relocations did not follow a constant direction. On the contrary, the general movement described after several relocations was either random or, in a few cases, tended to be circular, that is, colonies re-occupied several times the same nest location. Colonies moved away from their initial location as much or less than predicted by a random walk.

An example of such simulations for colony n° 619 is given on **Fig. 4.5**. This colony realized eight relocations of, on average 3.1±1.2m which led it at the end of the study to occupy a nest located more than 10m away from its origin (black arrow). A total of 1,000 simulations preserving the same relocation distances in the same order but allowing the colony to choose a random turning angle between consecutive relocations indicated that the distance between initial and final locations could range from 0.6 (the colony almost return to the origin, left end of the distribution) to 20.7m (directional relocations, right end of the distribution) with an average of 8.5m (grey arrow). Thus, colony nº 619 moved at least 17.6% longer than what would be predicted by random walk, but this slight difference was not statistically significant (P = 0.349).

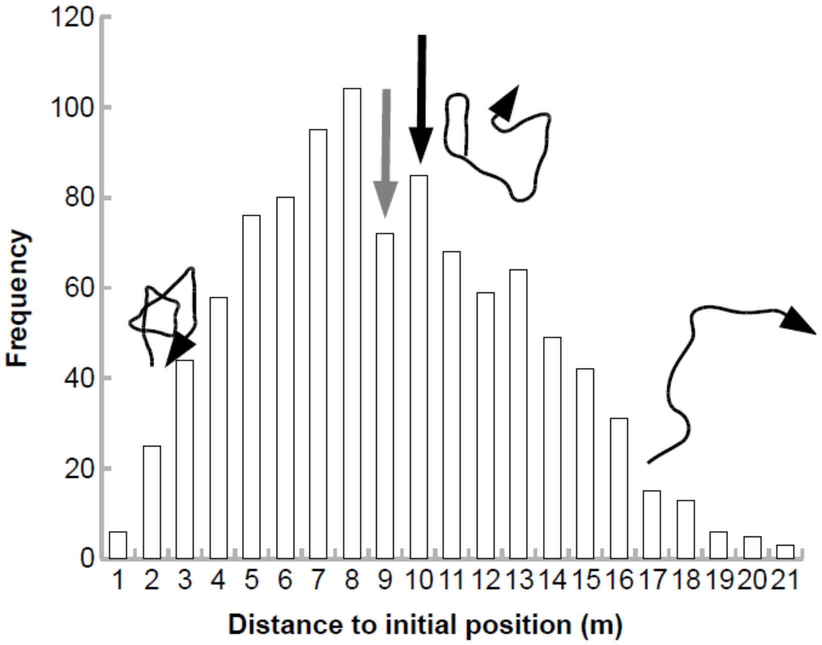


Figure 4.5 Frequency distribution of 1,000 random-walk simulated relocation distances between the initial and the last locations for colony nº 619.

Four other colonies moved slightly further away than predicted randomly, although in no case was the difference significant. However, sixteen colonies moved less than expected randomly and for five of them the difference was significant at $P < 0.05$ (**Fig. 4.6**). Ten of these colonies were seen reoccupying a nest they had left a few weeks before. Similarly, the cumulated length of the eight relocations realized by colony nº 619 was 35.1m but the cumulated distance between each intermediate location and the origin was only 20m. Overall, the cumulated distance between each intermediate location and the origin was significantly shorter than expected randomly for six colonies (Fig. 4B). This also refutes the hypothesis of successive relocations following a constant (linear) direction.

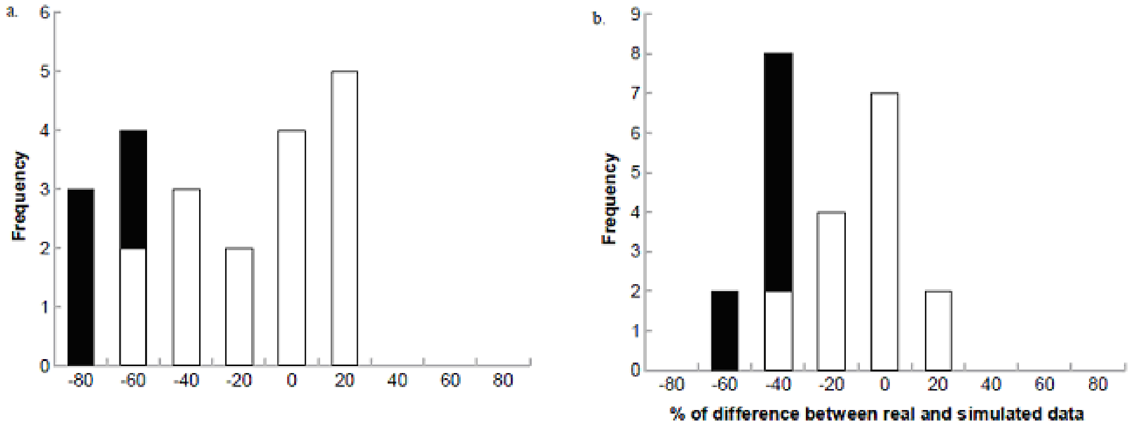


Figure 4.6 Comparison between real and simulated relocations. In simulations, colonies migrate the same distances than in the real data but turn in random directions before each migration. X-axis show the % of difference (e.g. +50% means that the colony was found 3 m away from first location, but that the mean value from the simulations was 2m, that is, ((3–2)/2)*100=50%). A value of zero means that the colony relocated as the average of the simulated random walks. Black and white bars show values departing and not departing significantly ($P<0.05$) from simulated data, that is, from a random walk. A) Distance of the colony from the first location of the colony at the end of the study period. B) Cumulated distances of the colony from the initial colony location. Cumulated distances are calculated by the sum of all partial distances to the initial colony location each time the colony relocates.

4.3.3 Genetic Structure and population viscosity

The total number of alleles per locus within colonies ranged from two to three as expected for haplo-diploid monogynous-monoandrous species. No evidence of linkage disequilibrium was observed between any locus pair. Similarly, none of the probability tests for Hardy-Weinberg expectations remained significant after FDR correction for multiple tests. This suggests that no inbreeding occurs within sampling sites and the loci can be considered independent. Overall F_{ST} values within sampling sites were relatively high ranging from 0.039 in *Beles*, to 0.040 and 0.043 at *Visita* and *Comedero* respectively. This denotes a high degree of genetic structuring within the sampling sites. Similarly, the hierarchical AMOVA indicated that the majority of genetic variation occurred among colonies within sampling sites (**Table 4.2**).

Table 4.2 Two-way hierarchal analyses of molecular variance (AMOVA). Significance of fixation index values obtained after 1023 permutations are given in parenthesis.

Source of Variation	d.f.	Percentage of Variation	Fixation Index
Among Sampling Sites	2	1.79	F_{CT} =0.01787 (0.00293)
Among colonies within sampling sites	58	39.51	F_{SC} = 0.40231 (0.0000)

Significant population viscosity was revealed by the spatial autocorrelation analysis. The results showed significant autocorrelation between relatedness values at the 0 to 5m and at 25 to 30m distance classes (**Fig. 4.7**). This indicates that although relatedness among neighbouring colonies within these intervals is relatively low, it is still significantly higher than expected by chance.

It is important to notice, however, that a single correlogram may not reflect accurately the true non-random spatial genetic pattern. The autocorrelation largely depends on the extent of the genetic structure, the size of the distance class chosen and the associated number of samples per distance class (Peakall et al. 2003; Peakall & Smouse 2006). Thus, each sampling site could have a distinct spatial autocorrelation pattern. To account for this possible site effect, we performed independent autocorrelation analyses within each site using the same parameters as above. The results were consistent across the three sites indicating significant autocorrelation at the 0-5 and 25 to 30 distance classes. Nevertheless, the result for 25-30 m distance class should be taken cautiously as no pairs of samples were available in *Visita* for this interval. Therefore, the result of the autocorrelation analysis suggests a genetic-patch-size (sometimes referred to as genetic neighbourhood) of < 5 m for colonies of *A. senilis*.

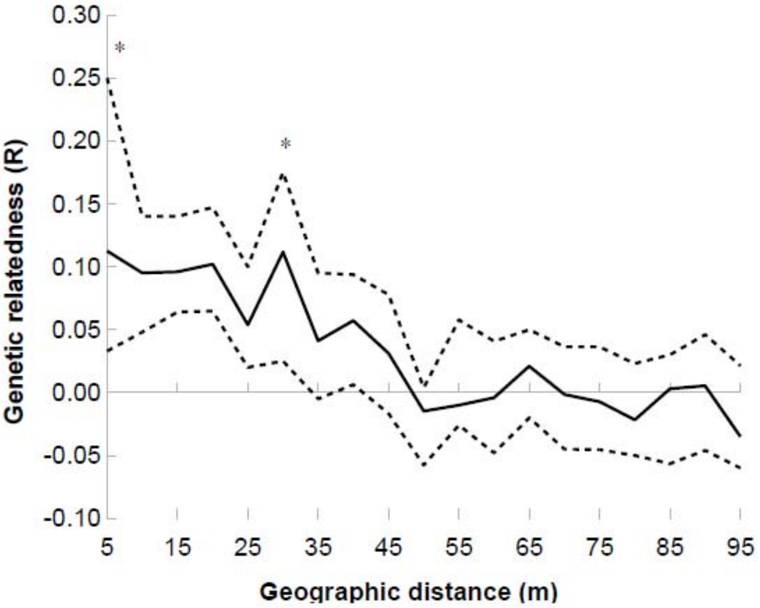


Figure 4.7 Pairwise relatedness values (R) of *A. senilis* colonies across increasing geographic distance classes. Dotted lines indicate upper and lower 95% confidence intervals of *R* after 999 permutations. Significant autocorrelations are denoted with an asterisk

4.4 DISCUSSION

The present study shows that 1) Successive colony relocations in *A. senilis* do not follow a constant (linear) direction but describe a random or circular movement; 2) They do not prevent population viscosity at a local scale; 3) A high genetic structuring exists within the sampled area. Thus, frequent colony relocations are not a mean to increase effective dispersal in this fission performing gypsy ant.

4.4.1 Colony relocations

Over the six-month survey, nest occupancy had a half-life of 20.1 days which is very similar to what was found by Smallwood (1982) for *Aphaenogaster rudis* (20.6 days) in West Virginia. All colonies relocated several times during the study, most often over a few meters, though the relocation distances varied between sampling sites. This difference of relocation distance might be due to intrinsic properties of each locality including the availability of nest sites or colony density. Future studies could test these hypotheses.

Two fission events were detected during the summer 2006. In both cases, the old queen moved to another nest with 69% and 66% of the workers while the new queen inherited the old nest and the remaining workers. This pattern is similar to what is known in other species, including *Cataglyphis cursor* (Lenoir et al. 1988; Chéron et al. 2011) and the honeybee *Apis mellifera* (Seeley 1997). It differs, however, from what happens in *Cataglyphis floricola* in which the queen remains in the old nest (Amor et al. 2011). In both observed cases of fission, the mother colony (which contained the old queen) settled at a very short distance from the daughter colony (2.3 and 2.7 m).

The result of our simulations indicated that colonies either moved randomly or described a circular trajectory by using several times the same nest, which suggests an opportunistic behaviour of the ants during relocation. What stimulates relocations in *A. senilis* has not been investigated in great detail yet. The lack of a clear temporal pattern (non significant difference in the probability of relocation between successive weeks) suggests that relocations might be driven by a series of factors. In a recent study, no increase in migrations was found following an experimental reduction of neighbour colony density indicating that intra-specific competition may not determine the probability of relocation in this species (Boulay et al. 2010). In *A. senilis* like in other congeneric species, sun exposure and attacks by predators and parasites were shown to stimulate nest relocation (Smallwood 1982; McGlynn et al. 2002; McGlynn et al. 2004). Other nest disturbances (including flooding, perturbations induced by large mammals and human activities) or food shortage could also trigger emigration, as in other Myrmicines (Hölldobler & Wilson 1990; Wilson et al. 1992).

Insight in the decision-making rules during emigration in other species indicates that after a sudden perturbation, scouts start to explore the environment in all directions until the most suitable nest site has been discovered (Franks et al. 2003). Scouts may also use latent knowledge about potential nest locations around their current nest (Franks et al. 2007). Then, recruitment and social transports take place allowing moving the entire colony to the new site (Avargues-Weber & Monnin 2009), (**Fig. 4.8**). In *A. senilis*, the fact that the probability of colony relocation was higher when it had already occurred the week before suggests that the ants may successively try several nest locations until the best site is adopted for the next three to five weeks.

Figure 4.8 Nest relocation in *A. senilis* colony. a) Social transport. b,c) Transport of larvae and egg. d) Queen relocation (photo:F. Amor)

After the ants have abandoned their nest, the whole structure of galleries and chambers most likely remains intact for a while which allows their further reutilization by the same or other ants. Hence, the first suitable area discovered by a colony may, just by chance, be the one they had abandoned a few months earlier. In some occasions, a colony may also use a nest that was previously occupied by one of its neighbours (Boulay pers. obs.). By doing so, the ants reduce the cost of excavating a new nest each time they have to relocate. This could explain the random pattern of colony relocation as well as the circular trajectories shown by our simulations.

4.4.2 Genetic Structure and Population Viscosity

Our results are inline with a recently published microsatellite-based study (Chéron et al. 2009) which provided evidence that the socio-genetic organization of *A. senilis* colonies conforms to that of a monogynous-monoandrous system.

Likewise, a strong genetic structuring within and among sampling sites was observed in our study. Considering the high mutation rate and bi-paternal inheritance mode of microsatellites, such a genetic structure has probably been stable for at least a few hundred generations. This suggests that, although gene flow between distant colonies may occasionally occur (most likely by flying males), such events are rare and not of sufficient magnitude to homogenize gene pools between interbreeding colonies. Such a pronounced genetic structure has also been observed in other fissioning ant species like *Diacamma cyaneiventre*, *Nothomyrmecia macrops* and *Cataglyphis cursor*, where divergence values (F_{ST} or analogous) between populations range from 3% to 15% (Doums et al. 2002; Sanetra & Crozier 2003; Clémencet et al. 2005). Interestingly, in these previous studies as well as in ours, there was a general lack of inbreeding and a relatively high genetic variability. In the case of *A. senilis*, this could be due to male-biased dispersal and/or some, perhaps yet unobserved kin-recognition mechanism that prevents inbreeding among nearby colonies.

On a very small scale (< 5 m), the spatial autocorrelation analysis showed relatedness values higher than expected by chance between any two random colonies within that distance (**Fig. 4.7**). This suggests that nearby colonies most likely share a common ancestor and so derived from relatively recent fission event(s). In contrast to species with independent colony foundation, colony fission drastically limits the distance at which effective dispersal is achieved.

Available data in other ant species have found contrasting results when estimating population viscosity across a range of geographic distances depending on the genetic marker used (Seppä & Pamilo 1995; Chapuisat & Crozier 2001; Tsutsui & Case 2001; Berghoff et al. 2008). Some of these previous studies have evaluated the relationship between genetic similarities and geographic distance using both mitochondrial and nuclear markers. All results agree in a stronger genetic differentiation (orders of magnitude) at the mitochondrial level relative to nuclear loci, even within a few meters distance. This can be explained partly because the effective population size of the mitochondrial genome is only one quarter that of nuclear, and because dispersal is male-biased in most fissioning ants.

In the present study we measured female dispersal directly (mark-recapture) and infer male dispersal indirectly by microsatellite markers. Our results indicate that males are mainly the dispersing sex and that they disperse over greater distances than fission events and colony relocations (female dispersal). Nonetheless, neither male nor female dispersal seem to prevent population viscosity within the short-distance range. This appears to be a general trend already well recognized in polygynous species that reproduce by colony fission (Chapuisat et al. 1997; Giraud et al. 2000; Tsutsui & Case 2001). However, recent genetic evidence suggests that population viscosity at micro-geographical scale can also be a common phenomenon in monogynous fissioning species (Doums et al. 2002; Sanetra & Crozier 2003). Thus, other traits such as male mating success and queen philopatric behaviour warrant further investigation.

4.5 CONCLUSION

Our results highlight the conceptual distinction between animal movement and effective dispersal (or gene flow). In species with dispersal polymorphism, effective dispersal is usually achieved by a fraction of the population with specialized phenotypes (including behaviour) moving through a landscape until settlement habitat is encountered and colonization (i.e. reproduction) takes place. In other species like fission-performing ants, dispersal occurs at the colony level and is limited by specific constrains such as the participation of apterous workers in fission. In this case, routine movements could acquire a particular importance to promote gene flow. However, our results suggest that the consequence of routine movements on population genetic structure will greatly depend on the orientation of successive displacements.

Hence, in *A. senilis*, the lack of a constant directionality of successive relocations does not seem to outweigh the effects of limited dispersal as evidenced by high population viscosity within short distances. Occasional gene flow via male dispersal between nearby colonies appears to prevent inbreeding, but it is not of sufficient magnitude to completely cancel out population viscosity. The possibility of other cryptic inbreeding-avoidance mechanisms cannot be ruled out. Overall, fission creates a neighbourhood area of genetically related colonies, which is maintained in spite of frequent relocations. A consequence of this could be the relatively low level of aggressiveness among neighbour colonies observed in this species, minimizing the effects of local-resource competition (Ichinose et al. 2009). Further studies on a variety of organisms, including other fission-performing ants, are necessary to address a possible evolutionary link between limited special-movement dispersal and enhanced routine movements as a regulatory process between population viscosity, inbreeding and local resource competition.

CHAPTER 5

Is the gypsy ant forced to go? Colony relocation searching for optimal temperatures and constrained by intra-specific competition[4]

ABSTRACT/ RESUMEN

Animals employ movement for a variety of purposes, including the use of resources (food, shelter and mates). Social insects live in nests that protect them against environmental changes and enemies (competitors, parasites and predators). Nest relocation is a common strategy to search the best compromise between good resources and enemy avoidance. For workers to relocate their nest, the risks associated with relocation (i.e. predation of the brood and the queen during and just after relocation) should be highly compensated by the new site conditions. We followed two populations of a monogynous species, *Aphaenogaster senilis* during five years, in order to know whether abiotic and/or biotic factors affected the process of relocation. Under the Mediterranean conditions of our study sites, we predicted that nests would move searching for favorable abiotic conditions (temperatures) to colony growth while

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[4] Ángel Barroso, Elena Angulo, Raphaël Bolulay, Fernando Amor, Alain Lenoir, Xim Cerdá. In prep.

constrained by intra-specific competition. We showed that in spring, when brood biomass increases enormously, spatial arrangement of colonies is overdispersed suggesting colony mutual exclusion. High competition for space in spring is not accompanied by intensified aggressive interactions or higher foraging distances. In spring and fall, colonies move to bare soil where they can benefit from heat necessary for brood pupation. However, in summer colonies avoid the elevated ground temperatures by moving the nest under the scrubs. This is confirmed experimentally: in summer shaded nests remained longer without moving than control ones; and observationally: lower temperatures were recorded at different depths in the soil between nests under scrubs than nests in bare soil (high and risky temperatures). We conclude that *A. senilis* has a seasonal pattern for nest relocation motivated mainly by high temperatures of the Mediterranean summer, while intra-specific competition is higher in spring when the colony invest in its growth.

Los animales se desplazan con muy diversos fines, entre los que se incluye la explotación de los recursos (sean éstos alimento, refugio o pareja). Los insectos sociales viven en nidos que los protegen frente a los cambios ambientales, los enemigos o los competidores. La emigración del nido (traslado) es una estrategia frecuente para alcanzar en cada momento el mejor compromiso entre la obtención de buenos recursos y el evitar a depredadores y competidores. Los riesgos del traslado deberían estar ampliamente compensados por las ganancias de las nuevas condiciones, puesto que el traslado de nido es arriesgado, especialmente en especies monogínicas donde la reina se expone durante la migración tanto a los enemigos como a las condiciones abióticas. En este capítulo se analiza cómo afectan los factores abióticos y bióticos al proceso de la migración. Para ello se siguieron durante cinco años dos poblaciones de *Aphaenogaster senilis*. Dadas las condiciones mediterráneas de nuestras zonas de estudio, predijimos que las colonias se moverían buscando unas condiciones abióticas (temperatura) que favoreciesen su crecimiento, mientras que los traslados estarían limitados por la competencia intraespecífica. Hemos observado que en primavera, cuando la biomasa de larvas es muy grande, el patrón de la distribución espacial de los nidos es regular, lo que sugiere que hay una exclusión mutua entre colonias. Esta competencia por el espacio en primavera no supone una alta frecuencia de interacciones agresivas en los cebos. Las interacciones agresivas son más frecuentes en verano y otoño, cuando las colonias están distribuidas al azar. Tampoco las obreras van más lejos en primavera, sino que las distancias de forrajeo son más altas en otoño. En primavera y otoño las colonias nidifican en zonas abiertas y

soleadas, con suelo desnudo, para que el nido se caliente. En cambio, en verano las colonias evitan las elevadas temperaturas del suelo desnudo, emigrando y situándose bajo los matorrales. Esto fue confirmado con un experimento: en verano los nidos sombreados experimentalmente, se mantenían durante más tiempo sin emigrar que los controles. Asimismo, la medida de la temperatura del suelo a diferentes profundidades confirmó que la temperatura era menor en los nidos bajo matorral que en el suelo desnudo (donde se alcanzaban temperaturas de riesgo para las hormigas). Concluimos que *A senilis* tiene un patrón estacional para la emigración de nidos, provocado por las elevadas temperaturas del verano mediterráneo. En primavera, cuando la colonia está invirtiendo mucho en su crecimiento, hay mayor competencia intraespecífica.

5.1 INTRODUCTION

Ant colonies have long been seen to share several life history traits with plants (Bourke and Franks, 1995). Like seeds that disperse and germinate to give rise to a tree, ant queens typically flight from their mother nest to found a new colony independently that may remain in the same nest location for more than a decade. Sterile workers may then be compared to the roots and leaves that extend around a tree to obtain resources necessary for growth. Like in plants, some ant species have evolved a different mode of colony founding through "budding", or colony founding. However, the ant-plant comparison has important limitations. For example, unlike trees, ant colonies are not completely sessile entities but are able to relocate their nest to search for better environmental conditions (McGlynn 2012). The nest protects ant societies against environmental changes (rain, temperatures) and enemies (competitors, predators), providing optimal conditions for brood production and development. Thus the choice of nest location will depend on the equilibrium between exploiting good resources and avoiding predators and competitors.

Nest characteristics vary considerably between species. Leaf-cutter ants, for example, excavate enormous and architectonically complex nests to lodge tens of thousands of workers; this contrasts with tiny *Temnothorax* societies that often occupy small galls, acorns or twigs. For most ant species, the nest represents an important resource and its construction and maintenance require a great deal of energy. However, accidental nest disturbances, demographic changes and/or attack by all sorts of enemies may force a colony to move to another site. Nest occupancy duration and relocation efficiency vary greatly between species. Colonies of *Leptogenys diminuta* contain 500 workers that relocate their nests every 1-6 days in less than 90 min (Kumar and Veeresh 1990); *Pogonomyrmex barbatus* very rarely relocate and may occupy the same nest years after year, but when forced to relocate, the colony with tens of thousand of workers moves during 20-25 days (van Pelt 1976). A relatively large body of literature describes the process of nest relocation in numerous species (see e.g. McGlynn 2012). Nevertheless, as McGlynn (2012) pointed out, studies analyzing their causes are still very scarce (but see Gordon 1992; Cerdá & Retana 1998; McGlynn et al. 2004; Dahbi et al. 2008).

The gypsy ants, belonging to *Aphaenogaster* genus, are known to relocate their nests very frequently (Talbot 1951; Mizutani and Imamura 1980; Smallwood 1982;

Weseloh 1994; McGlynn et al. 2004; Richards 2009; Galarza et al. 2012; Lubertazzi 2012). In some species, colonies move seasonally in spring in search of environmental conditions (temperatures) more favorable to brood growth (Talbot 1951; Mizutani and Imamura 1980; Smallwood 1982; Lubertazzi 2012). Although the outcome of relocations can be viewed as a population process, it is useful to focus first on the behavior of individuals (colonies in the case of ants), as this underlies the collective aspects (Dingle and Drake 2007). Microhabitat requirements for *Aphaenogaster* colonies during winter, spring or summer are likely to be very different, and constitute one the cause of seasonal relocations.

Previous works have examined how intraspecific competition regulates population growth in *Aphaenogaster senilis* (Boulay et al. 2010) as well as the genetic effects of frequent colony relocations at the population level (Galarza et al. 2012). The results of both studies indicate that *A. senilis* relocations are mostly hazardous in their direction, that neighbor colonies are often genetically related and that the density and foraging area at the study site seems to be at carrying capacity. However, what clues determine where colonies move is not understood. Here we present the results of a population survey conducted in two plots located in South-western Spain, from July 2002 to October 2006. Our aim was to elucidate if nest relocations are motivated by the search for optimal nest environment that favor larval growth and reduce intraspecific competition. For this purpose, we measured the spatial pattern distribution of colonies during five years, their foraging areas, and the microhabitat parameters of nests in each period (cover of nest entrance and ground temperature). Given the Mediterranean conditions of our study sites, we predicted that nests move from low covered areas in the spring (searching for sunny places and warmer temperatures for overwintering) to high covered areas during summer (searching for optimal not extreme temperatures for colony growth). We tested experimentally if the rise of temperature in spring motivates colony movement to the shade. Moreover, abiotic requirements might be constrained by intraspecific competition, and for this reason we explored the spatial patterns of nests, foraging and interactions between nests.

5.2 MATERIALS AND METHODS

5.2.1 Field observations: nest spatial patterns, foraging and microhabitat use

The study was conducted at la Algaida (ALG) and las Beles (BEL). A 50x50m plot was delimited at both sites to survey *A. senilis* nests. Colonies located inside the plots and in a 6m outer band surrounding the plots were exhaustively mapped on several occasions in spring, summer and fall between July 2002 and October 2006 (a total of eight and thirteen mappings were carried out at ALG and BEL, respectively; see **Table 5.1** for more details). Mappings were conducted on sunny days with intensive ant activity. A grid of 61 food baits composed of small pieces of biscuits (11 rows with 6 or 5 baits; distance between baits in the same row 10 m; distance between rows 5 m) was installed early in the morning before *A. senilis* started foraging. The presence of *A. senilis* workers on the baits was monitored during 3-4 days from 9AM to 5PM (the normal daily range activity of the species) by 3-4 observers. Foragers were tracked back from the baits to the nest.

Nest spatial patterns

Nest locations were mapped manually by triangulation using the plot corners as fixed references. Maps of nest locations were digitalized to calculate the distance between each active nest and its nearest neighbor using Hawth’s Analysis Tools for ArcGis 9.3. Nests spatial distribution patterns were analyzed using the Clark and Evans (1954) R statistics that compares the observed mean nearest neighbor distance (NND) to the random distribution. The Sinclair correction (Sinclair 1985) was applied to account for edge effect. Moreover, all colonies located in the outer band and foraging on the baits grid were used to calculate the rA of the colonies located inside the plot. Differences of NND between spring, summer and fall were analyzed using a general lineal model (STATISTICA 8.0, StatSoft Inc 2007) with season and sites as fixed factors.

Foraging distances

The foraging distance was estimated from the distance between the baits and the nests. Foraging distances were compared between seasons (spring, summer and fall) and sites (ALG and BEL) by fitting a generalized linear model with the Poisson distribution and log link function (hereafter GLM_P). Across mappings, nests were considered different units. We also tested whether the maximum foraging distance (distance to the more distant bait) varied according to the number of baits visited by a colony. We explored whether these nests having a higher foraging activity also have larger foraging areas. We used the number of baits attained by each nest as a measure of foraging activity, and the distance to the nearest neighbor nest as a measure for nest foraging area. We performed a GLM_P on the distance to the nearest neighbor with the number of baits attained by the nest, followed by post-hoc analyses when corresponding.

Interactions at baits

Interactions between workers from different nests on the baits were classified as aggressive (biting) or tolerant (workers detected each other without showing aggressive behavior). Distances between nests with co-occurring workers at baits were calculated from the maps using ArcGis 9.3. Thus, we tested whether this distance varied between seasons and if the distance had an effect on worker behavior, i.e. if aggression (instead of pacific coexistence) was related with distance between neighbor nests or with the distance between the nest and the bait. We tested whether aggression of ants from a given nest at bait was affected by the season, the number of baits visited (more baits - larger colonies), the distance to the bait, the absolute difference of the distances to the bait of co-occurring nests and the distance between co-occurring nests. We performed a GLM_B modeling the aggression as a function of the season (spring, summer or fall), the number of baits visited and the three types of distances.

Microhabitat use

We compared whether the number of nests during the period of study differed between sites and seasons with a factorial ANOVA. The proportion of active nests located on bare ground vs under shrub cover was compared between sites (ALG or BEL) and seasons (spring, summer and fall) with a generalized linear model using the Genmod procedure for SAS9.1 (SAS Institute 2004). The model was fitted with the binomial distribution and logit link function (hereafter, GLM_B). Both variables and their interaction were introduced as fixed factors. Contrast analysis was employed to test differences between levels of significant factors.

5.2.2 Effect of experimental shade on nest relocations

Shading experiment

At the end of May 2005, in each study site, 30 *A. senilis* colonies nesting in bare soil were selected and individually marked with metal rods. Half the colonies were experimentally shaded (shaded). Shading was provided by a 0.8 x 0.8 m piece of black standard shade cloth maintained horizontally 20-30 cm above the nests (**Fig. 5.1**). The other half of the colonies remained untouched (control). During seven weeks, we surveyed every week if the colonies were still present at the initial location or if they had relocated their nest. During the whole experiment, we also measured ground surface temperature every 15 min at six shaded and eight control nest entrances at the BEL site using two HOBO 4-channel Data-logger (two channels placed at two shaded nests were destroyed by mammals at the beginning of the experiment making them useless).

Figure 5.1 Experimental shade (black standard shade cloth) installed above an *Aphaenogaster senilis* nest in BEL site (May 2005).

The time to colony relocation was compared between experimentally shaded and control nests and between sites using the Cox's regression with Weibull distribution (survival analysis). Differences of ground temperatures between treatments (shaded vs. control) along the seven weeks of the experiment were tested with a repeated-measures ANOVA (STATISTICA 8.0, StatSoft Inc 2007).

Ground temperatures of nests located within or outside vegetation cover

To estimate the effect of vegetation on nest temperature, ground temperature was recorded during four consecutive summer days (13-16 July 2004) at BEL site, in four nests located on bare soil and in four nests located under scrub cover. Temperature was recorded simultaneously every 15 min with three HOBO 4-channel Data-logger at the nest entrance, at and at 5 and 25 cm deep in the ground. In BEL site, a more detailed study on above-nest plant traits was also performed (**see Appendix 2**).

5.3 RESULTS

5.3.1 Nest spatial patterns

Spatial patterns of nests obtained from the mean NND analyses showed a regular or a random distribution, depending on the sampling (**Table 5.1**). From the 21 samplings, the general trend was randomness for summer (7 out of 8 samplings) and fall (4 out of 6 samplings), but significantly overdispersed (or regular) for spring (6 out of 7 samplings). That is, in spring nests were located significantly more distant from each other than what is expected randomly. Exceptions to this general pattern occurred at ALG in summer and fall 2002, with a regular pattern but in the limit of significance (P = 0.032 and 0.047, respectively), BEL in fall 2003 (also a regular significant pattern), and BEL in spring 2006 (a random pattern).

NND ranged between 1.1 and 16.6 m and varied between seasons and (General Lineal Model, $F_{1,718} = 13.54$, $P < 0.0001$) and sites ($F_{1,718} = 35.11$, $P < 0.0001$). The interaction between site and season was not significant ($F_{1,718} = 2.02$, $P = 0.133$). Hence, NND was significantly smaller in summer than in spring and fall and at ALG than at BEL (**Fig. 5.1a**).

Table 5.1 - Statistics of mean nearest neighbor distances (mNND, in m) calculated for each sampling period in two Doñana sites (ALG and BEL). n is the number of distances measured, density is the nest density estimated in each plot (nests / ha), R is the coefficient of Clark and Evans (1954) with Sinclair (1985) correction. R values no significantly different to one indicate a random pattern, values significantly below one indicate clumping (aggregation), while values significantly above one indicate regularity (overdispersion).

Site	Year	Period	mNND	n	Density	R	*P*	Spatial pattern
ALG	2003	spring	5.3	37	148	1.27	**0.004**	**regular**
	2004	spring	4.4	50	200	1.21	**0.005**	**regular**
	2004	spring	4.5	52	208	1.26	**0.001**	**regular**
BEL	2003	spring	5.5	39	156	1.34	**0.0001**	**regular**
	2004	spring	5.6	37	148	1.34	**0.0001**	**regular**
	2005	spring	6.5	27	108	1.31	**0.004**	**regular**
	2006	spring	7.0	17	68	1.13	0.201	random
ALG	2002	summer	4.7	40	160	1.18	**0.032**	**regular**
	2003	summer	4.0	49	196	1.09	0.145	random
	2004	summer	3.9	48	192	1.07	0.221	random
BEL	2002	summer	3.9	42	168	0.98	0.425	random
	2003	summer	4.7	26	104	0.94	0.295	random
	2004	summer	4.4	35	140	1.03	0.394	random
	2005	summer	5.7	21	84	1.03	0.425	random
	2006	summer	6.3	18	72	1.04	0.401	random
ALG	2002	fall	5.0	36	144	1.17	**0.047**	**regular**
	2003	fall	4.6	31	124	1.01	0.468	random
BEL	2002	fall	5.2	32	128	1.15	0.078	random
	2003	fall	5.3	35	140	1.24	**0.009**	**regular**
	2005	fall	7.5	17	68	1.20	0.090	random
	2006	fall	6.1	25	100	1.19	0.066	random

5.3.2 Foraging distances

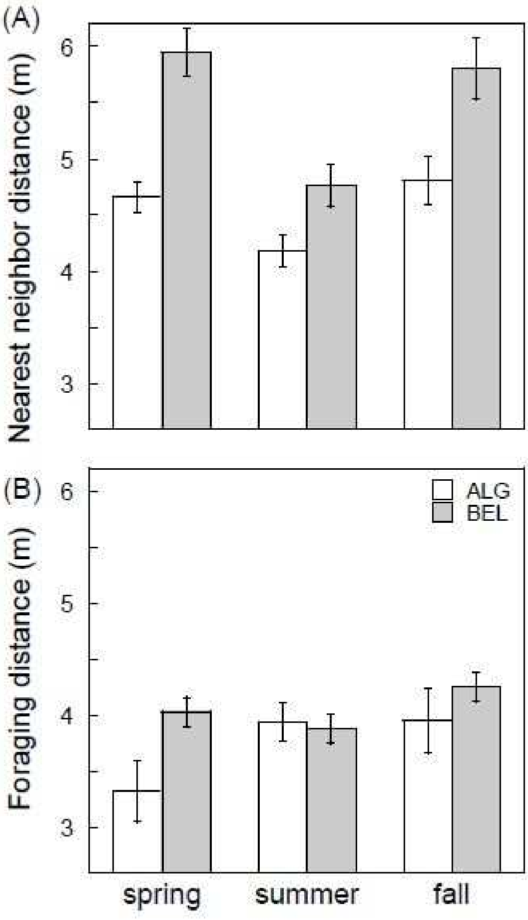


Figure 5.1 Mean nearest neighbour distance (A) and mean foraging distance (B) of *A. senilis* colonies during the different seasons in the two study sites (ALG, white bars; BEL, grey bars). Error bars are SE values

We measured trajects of workers between nests and baits in a total of 614 different colonies. Foraging distances from nest to bait were significantly different between seasons and sites, and the interaction was also significant (GLM$_P$, χ^2 = 143.51, P < 0,0001, χ^2 = 333.7, P < 0,0001, χ^2 = 226.97, P < 0,0001, **Fig. 5.1b**). Foraging distances ranged from 0.05 to 12.1 m and they were longer in BEL than in ALG and in fall than in summer or spring (**Fig. 5.1b**). Maximum foraging distance was also significantly higher between nests foraging on increasing number of baits (GLM$_P$, χ^2 = 18812, P < 0.0001; mean values ± SD: 3.4 ± 1.8, 5.4 ± 1.3, and 6.9 ± 1.4 m, for one, two, or three or more baits respectively). For each sampling period and site, mean maximum foraging distance was independent of nest density (Pearson's R= 0.009, P > 0.05).

The relationship between the maximum foraging distance and the NND was not statistically significant (Pearson's R= 0.019, P > 0.05). NND were higher between nests that occupied a higher number of baits (GLM$_P$ χ^2 = 1344.7, p < 0.001). Distances to the nearest neighbor were significantly lower for nests attaining only one bait (5.0 ± 0.1m, N = 307) than for nests that occupied two or more baits (5.8 ± 0.2 m, N = 118 for two baits, and 5.8 ± 0.3 m, N = 47 for three or more baits).

5.3.3 Interactions at baits: effects of season and nest distance

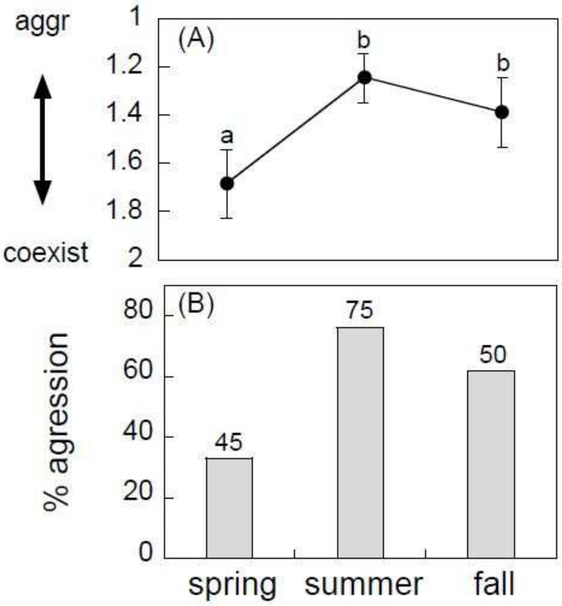


Figure 5.2 - Results of the observed intraspecific interactions of *A. senilis* at baits during each season. (A) Weighted marginal means values from GLM_B model. Error bars are SE values (different letters indicate significant statistical differences from post-hoc contrast analyses). (B) Percentage of aggressive interactions (numbers on the top of each bar are the total number of observed interactions).

Aggressive interactions at baits between workers from different nests were significantly different between seasons (χ^2 = 18.71, *P* < 0.0001, Fig. 2b) being less frequently observed in spring than in summer or fall (**Fig. 5.2a**). However, nests that occupied more baits were not significantly more aggressive (GLM_B, χ^2 = 0.50, *P* = 0.477). Foragers were not more aggressive when baits were close to the entrance of their nest: aggression was independent of the distance between the nest and the bait (χ^2 = 2.13, *P* = 0.145). Their behavior at baits when entering in contact with alien conspecific was also independent of the distance between both nests and independent of the relative distances of both nests to the bait (χ^2 = 0.10, *P* = 0.755 for the distance between co-occurring nests and χ^2 = 0.25, *P* = 0.620 for the absolute difference of the distances to the bait of co-occurring nests).

5.3.4 Microhabitat use

The total number of nests varied between sampling and plots from 17 to 52 (densities between 68 and 208 nests/ha, **Table 5.1**, **Fig. Appendix 1.1**). The number of nests was not different between seasons (ANOVA, $F_{15,2}$ = 1.42, *P* = 0.273) but it was different between sites ($F_{15,1}$ = 11.68, *P* = 0.004) with a higher number of nest in ALG (28.5 ± 2.4) compared to BEL (41.8 ± 3). The interaction was not significant ($F_{15,2}$ = 0.75, *P* = 0.488).

The percentage of nests located under plant cover varied between seasons (**Fig. 5.3**; GLM_B, χ^2 = 181.3, *P* < 0.001) but not between sites (χ^2 = 1.87, *P* = 0.171). Hence, in summer a higher proportion of active nests were located under plant cover than in fall and spring. The interaction between site and season was significant (χ^2 = 48.72, *P* < 0.001).

Differences between seasons at BEL site were higher than in ALG site. Furthermore, in spring a majority of nests (70%) were located on bare ground at BEL while this value it was lower at ALG (47%) (**Fig. 5.3**).

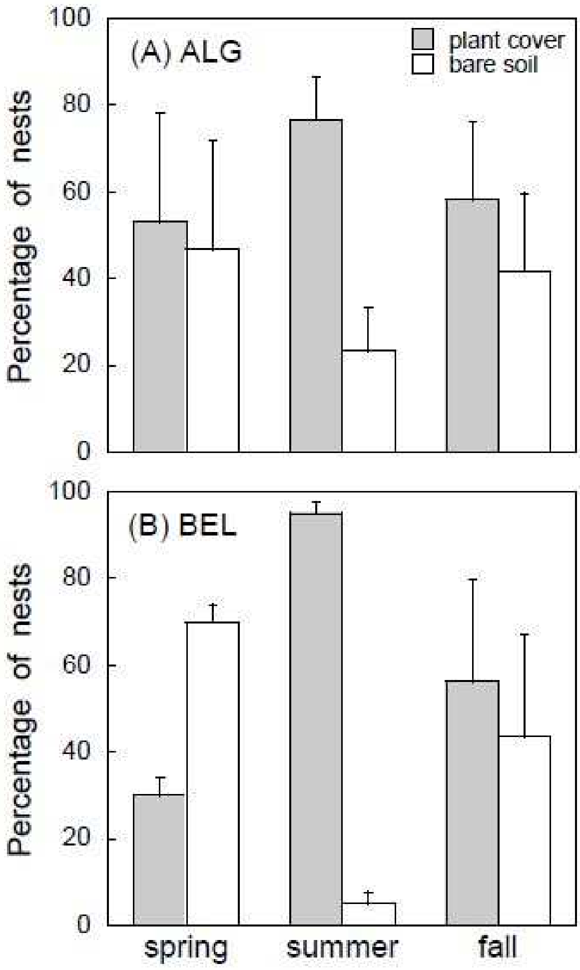


Figure 5.3 - Percentage of *A. senilis* nests occupying different microhabitats (white bars, in bare soil; grey bars, under scrub cover) in the different seasons in (A) ALG site and (B) BEL site. Error bars are SD values.

5.3.5 Effect of experimental shade on nest relocations.

Ground temperatures under the experimental shades were significantly lower than those in the ground of control nests (**Figure 5.4a**). Temperature varied significantly between treatment (Repeated measures ANOVA, $F_{1, 11} = 225.9$, $P < 0.0001$) and weeks ($F_{6, 66} = 114.5$, $P < 0.0001$). The hypothesis that in summer the nests moved to avoid the high temperatures was experimentally confirmed. Shaded nests remained longer at the initial location than the controls that were naturally exposed to the sun (control vs shaded, Cox Regression, deviance = -10.83, df = 58, $P = 0.0009$) (**Figure 5.4b**). There were significant differences between sites (ALG vs BEL, deviance = -6.59, df = 58, $P =$

0.0102), in BEL site nests moved faster than in ALG, but the interaction between the treatment and the site was not significant (deviance = -0.70, df = 57, *P* = 0.4002).

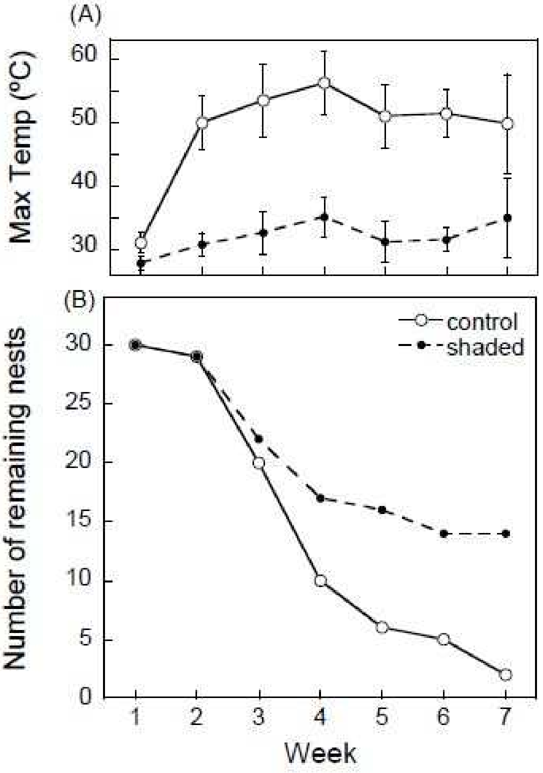


Figure 5.4 - Mean maximum ground temperature (A) and number of *A. senilis* nests that remained without moving (B) during the shading experiment. Total number of experimentally shaded nests (exp) and naturally sunny nests (control) were 30 per treatment. Temperature values (in ºC) are the week average of daily maximum temperatures; error bars are SD values.

Pattern of temperature under experimental shades were similar to those observed in wild. During the summer, the daily pattern of ground temperature differed greatly between nests located under scrubs and on bare ground (**Fig. 5.5**). Temperature on bare ground reached 54 º C at the ground level and 46 º C at 5 cm deep (where brood chambers are placed). By contrast, under plant cover, surface and 5-cm deep temperatures did not exceed 36 ºC and 33 ºC, respectively. Temperature at 25 cm deep were very similar in both microhabitats (31 and 29 ºC on bare soil and under scrub, respectively).

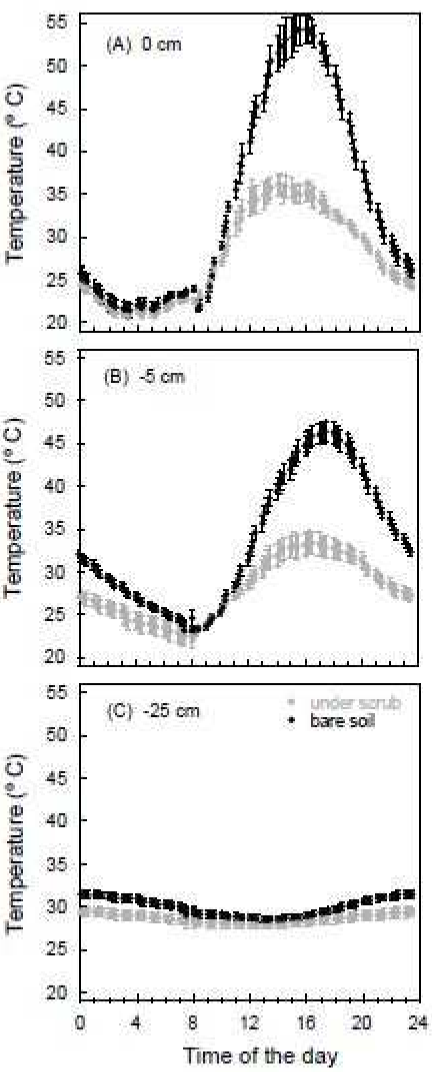


Figure 5.5 - Mean daily curves of temperature in two different microhabitats (black dots, in bare soil; grey dots, under scrubs) at different soil level: (A) at the ground surface; (B) at 5 cm deep; and (C) at 25 cm deep. Error bars are SE values.

5.4 DISCUSSION

The results of this study show that colony relocations in *A. senilis* have a seasonal pattern. During the summer, most colonies are inside or below the vegetation, whereas in spring and fall, the colonies are in the bare ground. These frequent colony relocations lead to changes in the spatial distribution patterns of the colonies. Colonies are closer to the nearest neighbor and frequently randomly distributed in summer, whereas distances between colonies are higher in spring and fall. Aggressive interactions at baits are more frequent in summer, suggesting that during this season intraspecific competition may be stronger.

In the Costa Rican gypsy ant *A. araneoides*, colonies relocate very frequently (McGlynn et al. 2003, 2004), and they are serially monodomous: each colony maintains multiple nests for its exclusive use, but only occupy one nest at time (McGlynn 2007). In the Spanish gypsy ant, empty nests are less frequently reoccupied: only in ten occasions (out of 129 relocations) the same colony reoccupied a nest that they had left few weeks before (Galarza et al. 2012). In some polydomous species colony relocations occur seasonally, colonies overwinter as a unit then break up into fractions over the active season and coalesce again in autumn (Mackay and Mackay 1983; Herbers 1986; Traniello and Levings 1986; Tsuji 1988; Banschbach et al. 1997; Backus et al. 2006; Heller and Gordon 2006). However, *A. senilis* is strictly monodomous: each colony occupies only one nest, and seasonal nest relocations are linked to a change of nesting microhabitat preferences.

5.4.1 Spatial competition in *A. senilis*

A regular pattern of nest distribution has long been thought to evidence strong competition between colonies (Bernstein and Gobbel 1979, Levings and Traniello 1981, Levings and Franks 1982). However, spatial arrangement of colonies cannot always be used directly as a test of competitive effects, since other spatial arrangements can also result from strong competition between colonies (Ryti and Case 1986, 1992). In some species, as *Veromessor pergandei*, mature colonies tend to reduce the survival of young ant colonies (Ryti and Case 1988) and overdispersion of colonies results from resource competition and perhaps founding queen predation by established colonies (Ryti and Case 1986). In the harvester ant *Pogonomyrmex barbatus*, competition with neighbors rarely causes the death of established colonies (Gordon and Kulig 1998).

Our observed *A. senilis* nest densities were lower (ranging from 68 to 208 nests/ha) and NND were higher (averages ranging from 3.9 to 7.5 m) than those observed in two Catalonian populations (Gómez and Espadaler 1996: 438 and 440 nests/ha and 3.3 m for NND in both populations). Gómez and Espadaler (1996) consider that these Catalonian populations are likely close to the habitat carrying capacity. In their experiment about colony density reduction, Boulay et al. (2010) observed that 6 months after density reduction, colony density did not differ between experimental and control plots. At the beginning of the experiment, colony density was 173 and 179 nests/ha, in control and experimental plots, respectively. Six months later densities were 148 and 200 nests/ha, and twelve months later, 148 and 167 nests/ha. Our observed density values are similar to those of the beginning and end of Boulay et al. (2010) experiment, indicating that our populations may be also close to the carrying capacity. During the last years of sampling (2005 and 2006) we observed in BEL site a decrease in colony density (from

148-140 nests/ha in 2004 to 68-100 nests/ha in 2006; **(Fig. Appendix 1.1)** for total number of nests) probably due to the combination of a very dry period (Serrano et al. 2008) and the emergence of a plant disease attacking *Halimium halimifolium*, the plant species that *A. senilis* preferred to nest below in summer (**Fig. Appendix 2.1**).

After experimental reduction in *A. senilis* colony density, Boulay et al. (2010) observed that the maximum foraging distance of the colonies increased significantly in the experimental plots, from 4.2 m to 6.3 m (control plot distances before and after the experiment were 3.9 and 4.5 m respectively). The maximum foraging distances observed in our study (averages ranging between 3.1 and 4.7 m) were similar to those of their "normal" density plots (control and experimental plots before experiment, control plots after experiment), and also similar to those of the Catalonian populations (3.9 and 4.9 m) (Gómez and Espadaler 1996). Unfortunately, we could not directly measure the size of the colonies without destroy them, the only indirect measurement about colony size provides from the number of baits that each colony exploited: the bigger colonies, with greater worker force, are expected to exploit more food resources at baits (McGlynn et al. (2002) found in *Aphaenogaster araneoides* a strong correlation between home range and colony size). Bigger nests (those foraging to more than one bait) had a significantly higher maximum foraging distance, and were further from their neighbors than smaller nests. Probably, colony size affects the spatial nest distribution.

To invoke a competitive ghost arising from the ant colony spatial patterns could be misleading. Nevertheless, spatial intraspecific competition between colonies has been experimentally demonstrated in *A. senilis*: a reduction in colony density stimulated that remaining colonies founded new nests by colony fission (Boulay et al. 2010). Fission creates a neighborhood area of genetically related colonies, which is maintained in spite of frequent relocations (Galarza et al. 2012). In this situation, with a colony-fission founding species, queen and young colonies survival is guaranteed, but during the first steps of founding, these daughter colonies will be close to their mother colonies. To avoid high competition between mother-daughter colonies, successive nest relocations will be done during next weeks after fission.

5.4.2 Seasonal changes in microhabitat selection

Brood development has a relatively narrow temperature requirement. Nest temperature may affect not only the rate at which it develops but also its survival rate and the orientation of diploid larvae into either the queen or worker caste (Brian 1963, 1973; Kipyatkov and Lopatina 1990; Tinaut et al. 1999; Kipyatkov et al. 2004, 2005; Sanada-Morimura et al. 2006; Boulay et al 2009). In *A. senilis* temperature affect pupae production, that is much faster at 30 ºC than at 24 ºC (Boulay et al. 2009). Spring and fall are the seasons of *A. senilis* pupae production (Boulay et al. 2009). However, pupation may be limited by temperature so that the ants have to move away from the vegetation in spring and fall to benefit from longer time at optimal temperature. In summer, extreme surface temperature (in Doñana, temperature in the most superficial chambers may attain 53 ºC, R. Boulay and F. Amor, unpublished data) may force *A. senilis* colonies to return to the vegetation (as suggested by the result of the experiment and temperature measurements inside and outside vegetation). In other species, workers can determine the brood rearing temperature by moving the larvae at different places within the nest (Tinaut et al. 1999; Porter and Tschinkel 1993; Penick and Tschinkel 2008). However, when the summer nest is inside the vegetation, *A. senilis* workers can forage by climbing directly to plants and walking far from the ground surface. In this case, a more efficient strategy could be to move the entire colony to a cooler place than to stop completely the foraging activity. Colonies of *A. senilis* have not a big worker population, and the species has a well coordinated emigration behavior, allowing a fast nest relocation (Avargues-Weber and Monnin 2009).

In early spring, at the beginning of the activity period, the majority of *A. senilis* are in the bare ground, in warmer places. This may allow an optimal temperature for brood development. At the end of spring or beginning of summer, when temperatures become higher, *A. senilis* colonies move from the open ground to the vegetation. Colonies seem to prefer dense and tall individuals of two plant species, *Halimium halimifolium* and *Stauracanthus genistoides* (**Fig. Appendix 2.1 and Appendix 2.2).** Chew (1987) observed that *Myrmecocystus mexicanus*, in Arizona desert grassland, never nested under shrub canopies of creosote bushes. In a similar way, colonies of *Veromessor pergandei*, rarely have their nest entrance under perennials, and the species did not show special requirements for a particular plant species or density of plants (Ryti and Case 1986). However, in Doñana, dense scrubs provide a shelter for *A. senilis* nests against extreme temperatures. In summer, during the daytime, soil temperature at 5 cm deep (where superficial chambers are located) is around 30 ºC when nests are under the vegetation, which is the optimal temperature for brood development (Boulay et al. 2009). However, if the colonies should remain during summer in the same spring nest, these

5cm deep chambers should attain 45 ºC, a lethal temperature for brood and very close to the critical thermal limit of workers (46 ºC according with Cerdá et al. 1998a). Evidently the ants can move the brood deeper, but the temperature in the surroundings of the entrance will be critical for the foraging workers, limiting foraging activity. The vegetation offers not only low temperatures for the whole nest but also a good environment for ants to forage within the scrub. Thus, thermal advantages of *A. senilis* nest moving are evident. In spring, temperatures out of scrub are more adequate than under the scrub, while nesting under a scrub during summer shield the colony and provide an optimal thermal habitat for brood. Colony chambers are frequently around plant roots probably to maintain better the soil moisture, because scrubs have a vertical root distribution typical from desert habitats: 60 % of root biomass occur in the first 25 cm (Martínez García and Rodríguez 1988). The observed differences between sites in the frequency of by plant covered nests (in ALG site there are less nests below plants than in BEL site) may be caused by differences in vegetal physyognomy (see Supplementary Material, plate 1). ALG vegetation is mainly composed by tall and large shrubs (that provide big shadow), and in ALG site the water table is less deep than in BEL site (and colonies may need less to nest around the roots to maintain moisture).

Shading experiments have been frequently used to analyse microhabitat preferences in ants. In the Australian golden spiny ant, *Polyrhachis ammon*, Gibb and Hochuli (2003) observed that shading treatment reduced maximum nest temperature in spring, but there were not significant differences between treatments in the number of nests remaining actives. In the Costa Rican ant *Ectatomma ruidum*, McGlynn et al. (2010) found that colonies preferred to nest in experimentally shaded plots. In the case of the meat ant *Iridomyrmex purpureus*, Greenslade (1975) reported that shaded nests were more likely to be abandoned than well-insolated nests. In the Florida harvester ant, *Pogonomyrmex badius*, shading of the mound surface of colonies caused an increase in the number of migrations (Carlson and Gentry 1973). A similar trend was observed in *Aphaenogaster rudis*: the emigration rate of shaded colonies was higher (Smallwood 1982). *A. rudis* lives in the forest floor, in this habitat, colonies take advantage of the sunny patches in order to warm the nest and accelerate brood development when forest temperature is relatively low (Smallwood 1982). On the contrary, in Doñana, summer temperature is too high, and *A. senilis* must take advantage of shaded patches, as it has been confirmed in our shading experiment: nest relocations are mainly due to thermal requirements of the species.

5.4.3 Inability to assess a good nest site? Trial and error

In the harvester ant *Pogonomyrmex californicus*, nest relocations significantly increased NND and exhibited a strong tendency to be in a direction away from the neighbor colony (De Vita 1979). In a similar way, in *Messor andrei*, nest relocation substantially reduced overlap between the foraging areas of neighbouring colonies (Brown 1999, Brown and Gordon 2000, Behav Ecol Sociobiol). On the contrary, in *A. senilis*, the general movement after several relocations is either random or circular (Galarza et al. 2012). Moreover, a colony that had relocated on one week had a higher probability to relocate again the following week, suggesting that ants may successively try several nest locations until the best site is adopted (Galarza et al. 2012).

Temnothorax ants assess an array of nest site attributes when choose a nest site (Mallon et al. 2001; Pratt et al. 2002; Franks et al. 2006; Pratt 2008), even the quality of the habitat surrounding the site: they prefer to move to nests located close to a previously explored food-rich area (Cao and Dornhaus 2012). In the case of *A. senilis*, because it is an omnivorous and scavenger ant (Cerdá et al. 1988, 1998b; Barroso et al. in press), food distribution is both spatially and temporally unpredictable, therefore, there are not best food areas and according with the frequent relocations, probably they cannot assess the site quality before nest moving. A similar pattern has been described in *Proformica longiseta*, a high-mountain ant nesting under rocks (Tinaut et al. 1999). When a colony nested under a rock with optimal dimensions for heating and accelerate brood development, it remained longer and produced sexuals. However, *P. longiseta* is not able to recognize the optimal rocks, and no site selection is performed a priori: if they moved to a "bad" rock, they abandoned it early, until find a "good" rock. Probably this "trial and error" process is also performed by *A. senilis* colonies, they try several successive nest locations until find the best site, from both abiotic (environmental) and biotic (competitive) point of view, to remain longer (Galarza et al. 2012).

5.4.4 Seasonal changes in strength of competition?

If we consider that competition can be (cautiously) detected from spatial arrangement of colonies and overdispersion suggest colony mutual exclusion, competition between *A. senilis* colonies should be higher in spring, when colonies are overdispersed. In spring *A. senilis* has the highest foraging activity level in Doñana because is the period of highest brood presence (Barroso et al. in press). It could be possible that only during this period, colonies suffer strong competition. However, in spring there are relatively few encounters at baits, indicating a reduced overlap between neighbor colonies' foraging areas. On the contrary, in summer and fall, when colonies are randomly distributed, encounters between workers from different colonies are more frequent and more aggressive (**Fig. 5.2**). Aggressions only aim to defend the food source and to expel the intruders belonging to another colony by pulling and biting, but only in few occasions the intruders are injured (**Fig. 5.6**). Yamaguchi (1995) described ritualized combats during food-robbing in *Messor aciculatus*, where the winner ejected, but did not injure the loser. Gordon and Kulig (1996, Ecology) observed a similar behavior in *Pogonomyrmex barbatus*, and they suggested that exploitative competition might have more important effects than interference competition on founding colony survival and thus on the spatial distribution of nests.

Figure 5.6 Pictures of intraspecific *Aphaenogaster senilis* aggressive interactions at baits. Aggressions aim to defend the food source and to expel the intruders belonging to another colony by pulling and biting, but only in few occasions the intruders are injured. (photo:F. Amor & X. Cerdá)

5.5 CONCLUSION

Nest relocation is a risky event for the colony, in polygynous species this risk is reduced (Gibb & Hochuli 2003), however, in monogynous species emigration is a risky process. During *A. senilis* emigration, the queen walks to the new nest, when the flow of ants is maximal (Avargues-Weber and Monnin 2009). This exposition of the only queen to different stressors (e.g. predation risk, heat shock, loss, etc) may be assumed by the colony only if the gains of relocation compensate. Gypsy ants, of the *Aphaenogaster* genus, are well adapted for frequent relocations. In Doñana, where the abiotic conditions of habitat environment change greatly from one to another season, *A. senilis* colonies move searching for the optimal environment. Search for the optimal nest site may be a "trial and error" process, where the colony, after the arrival to the new site, assesses not only abiotic environment but also biotic competitive environment.

APPENDIX

Appendix 1 - Figure A1.1

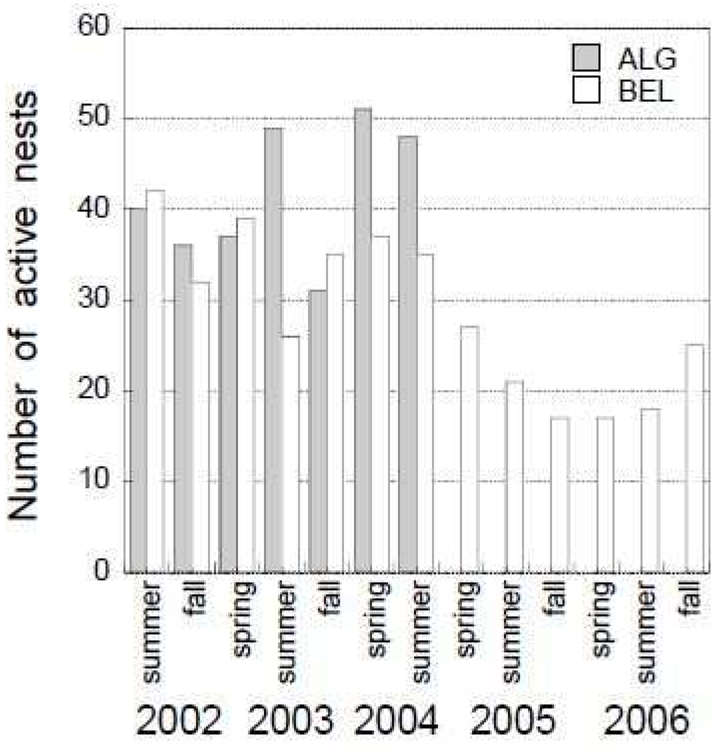


Figure A1.1 - Total number of active nests in each study site (ALG and BEL) during each sampling period. Values are nests inside the 50 x 50 m plots.

Appendix 2 - Details on microhabitat selection (plant choice) by *A. senilis* colonies

A2.1 Methods

A2.1.1 Nest selection of scrub species

We compared whether nests were placed in different scrub plant species regarding their availability or whether they selected specific scrubs in summer. In BEL site, we selected nests within scrub plants in summer 2002 and 2005 for which the plant species was recorded and we calculated the number of nests under each scrub species. Besides, we estimated the proportion of available scrub species for the same site and periods as follows: for each plot of 50 x 50 m we carried out 11 linear transects of 50 m separated 5 m. We counted the length that each plant species covered within each transect with five categories ("jaguarzo" *Halimium halimifolium, H. commutatum*, "aulaga" *Stauracanthus* *genistoides*, *Lavandula stoechas*, and other: *Urginea maritima*, *Asparagus aphyllus*, *Carthamus lanatus* and death *Halimium* sp.). For each plot we then calculated the percentage of length covered by each scrub species excluding the length that was not occupied by plants (bare soil). To test whether nests were placed randomly in scrubs or some plant species were more selected that other, we performed a log-linear analysis comparing the percentage of scrub availability (percentage of length) with the percentage of nest located within each species (log linear analysis, STATISTICA 8.0, StatSoft Inc 2007). The year was included in the analysis, because from 2002 to 2006 there was a regional drought and a disease in scrubs that affected the study area. We searched for the best model with a forward stepwise method. The fit of a model is good when the observed frequencies are non significantly different from the frequencies expected by the model (using χ^2 statistics). The best model is the one that included the least number of interactions necessary to fit the observed frequencies.

A2.1.2 Nest micro-location within scrubs

When nests were below scrubs, the minimum distance to the border of the scrub was recorded in BEL site. Thus, nests could be located in the scrub border (distance = 0 cm) or at different distances to the center of the scrub (the maximum distance recorded was 102 cm). This measure is an indication of how exposed to the sun are the nests; they are very exposed when they are located in the border of the scrub and they are less exposed (more shadow) as distance from the scrub border increases. We also recorded the maximum height of the scrub. We analysed whether the height of the scrub or the distance of nests from the scrub border varied between seasons (spring, summer and fall). We carried out two generalized linear model with Poisson distribution and log link (hereafter, GLM_P) to relate the distance or the height with the season, followed by post-hoc tests when significant differences were found.

A2.2 Results

A2.2.1 Nest selection of scrub species

The best model was the one that contained the interactions between scrub species and year and scrub species and scrub availability ($\chi^2 = 4.19$, p = 0.522). Nests were located more than expected in jaguarzo (*H. halimifolium*) and aulaga (*S. genistoides*) than in *H. commutatum* or *Lavandula* that are shorter and smaller. The two *Halimium* species decreased (availability and use) between 2002 and 2005 (**Fig. A2.1**) mainly due to a disease coming into the study area. This increased the proportion of other species, such as *Lavandula* or *S. genistoides*. In 2005, 13.8 % of available plant cover were *Halimium* spp. dry plants because disease, but only one *A. senilis* nest was under a dry plant.

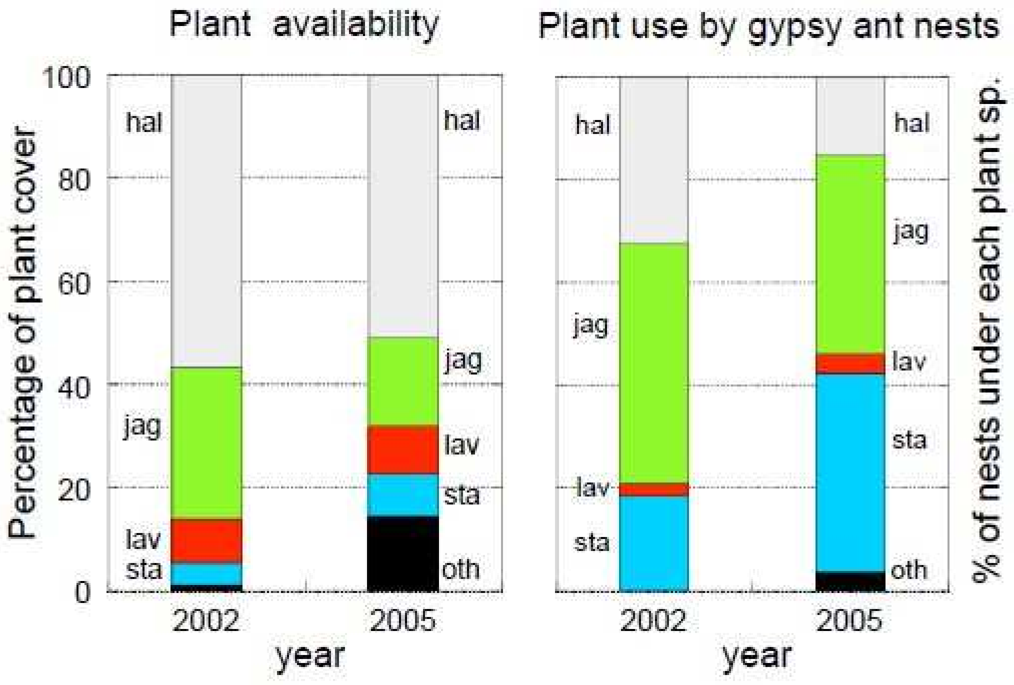
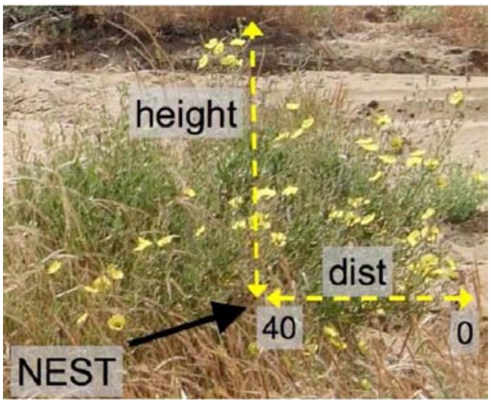


Figure A2.1 Relative plant availability (from vegetation transects) (left) and relative plant use by *A. senilis* colonies that nest under them (right) in summer 2002 and summer 2005 in BEL site. Abbreviations: hal - *Halimium commutatum*, jag - "jaguarzo" *Halimium halimifolium*, lav - *Lavandula stoechas*, sta - *Stauracanthus genistoides*, oth - other (*Urginea maritima*, *Asparagus aphyllus*, *Carthamus lanatus* and death *Halimium* sp.).

A2.2.2 Seasonal nest location within scrubs

Distance of nests within the scrub differed between seasons (GLM_P, $\chi^2 = 1740.0$, $p < 0.001$, **Fig. A2.2 (d)**). Nests were located significantly deeper within the scrub in summer than in fall and spring (mean ± SE distance from the nest entrance to the scrub border in cm: 30.9 ± 0.9, 12.32 ± 1.1 and 7.34 ± 0.7, in summer, fall and spring respectively). Nests were located in scrub plants of significantly different height in the different seasons (GLM_P, $\chi^2 = 616.6$, $p < 0.001$, **Fig. A2.2 (h)**): nests were located below higher plants in summer (52.7 cm, N =77), below medium plants in spring (34.6, N = 43), and below small plants in fall (16.2 cm, N = 22).



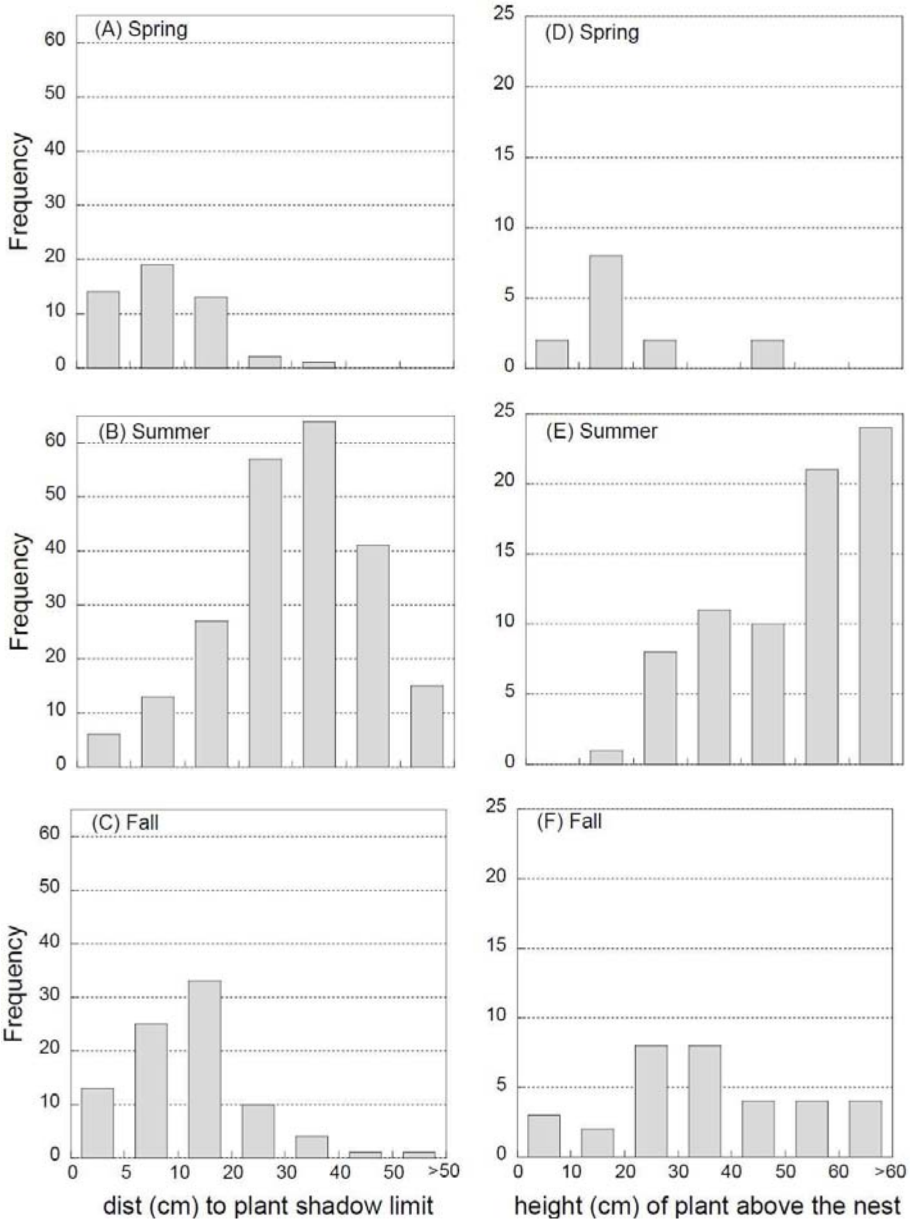


Figure A2.2 Frequency of *A. senilis* colonies nesting in the different seasons under plant with different size categories. Left: categories of distance to the scrub shadow border (d). Right: categories of the maximum height of the plant (h). Data are only from BEL site.

A2.3 Conclusion

Aphaenogaster senilis colonies show to have evident preferences to nest under some plants when environment is harsh (summer). To avoid the high temperatures, they have the nests in the middle of big, dense and tall scrubs, preferably of the species *Halimium halimifolium* and *Stauracanthus genistoides*.

CHAPTER 6

DISCUSIÓN GENERAL

Esta tesis aporta datos que pueden ayudar a entender mejor la ecología trófica, las interacciones con plantas y el uso del espacio en la especie *Aphaenogaster senilis*. Si bien adolece de la limitación de que el estudio se haya realizado en unos pocos lugares cercanos entre sí y ecológicamente semejantes (arenales costeros y "vera" de Doñana), los muestreos han sido intensivos y prolongados en el tiempo dando consistencia a los datos. Metodológicamente se combinan aspectos descriptivos y experimentales y se integran diferentes técnicas, en parte novedosas: uso de isótopos estables y dieta clásica, apertura de caja negra de la colonia, interacciones hormiga-planta desde ambos puntos de vista, modelos espaciales de dispersión, técnicas de marcado y recaptura, análisis genéticos de microsatélites, experimentos de campo y de laboratorio, sistemas de información geográfica y técnicas clásicas de observación de actividad de recolección.

A continuación pasamos a discutir de manera resumida algunos aspectos que consideramos relevantes de su biología y ecología a la luz de las aportaciones de esta tesis y en un contexto más general.

6.1 UNA ESPECIE IMPORTANTE EN LAS REDES TRÓFICAS Y MUTUALISTAS

Hasta ahora se consideraba a *Aphaenogaster senilis* una especie omnívora básicamente zoonecrófaga (*scavenger*). Nuestros resultados confirman su omnivorismo pero, en cambio, sugieren que es, tanto zoonecrófaga, como depredadora de insectos (especialmente áfidos) y de algunos otros invertebrados herbívoros; característica ya observada en varias *Aphaenogaster* americanas (Carroll 1975). En el NE de España, las principales presas de *A. senilis* son también los pulgones (Cros, Cerdá & Retana, datos no publicados). Ecológicamente es muy diferente consumir cadáveres que consumir animales vivos. La depredación supone nuevos bucles de interacción y, una presión de selección directa sobre las especies consumidas e indirecta sobre otras especies, por ejemplo, sobre las plantas de las cuales se alimentan sus presas.

La dieta y el aprovisionamiento de alimento en *A. senilis* varían notablemente a lo largo del año. Hemos demostrado que se trata de una especie oportunista, es decir, que su dieta se adapta a la disponibilidad de alimento animal. También que la actividad de recolección es bastante más intensa en primavera, época en la que ocupa un nivel trófico más elevado alimentándose principalmente de presas de origen animal, mientras en verano-otoño aumenta relativamente su consumo de alimentos vegetales. Por otro lado, *A. senilis* parece ser una especie clave en las redes mutualistas de dispersión de semillas, dispersando tanto a plantas mirmecócoras como a no mirmecócoras con diferente eficiencia. Los estudios realizados durante la última década acerca de las redes mutualistas animal-planta (de polinización y dispersión de semillas) indican que éstas presentan características bastante constantes: 1) son heterogéneas (la mayoría de las especies interaccionan con otras pocas especies, pero unas pocas están mucho más conectadas de lo se esperaría al azar) (Jordano et al. 2003); 2) presentan un patrón encajado (las especies especialistas interaccionan solo con especies generalistas, pero éstas interaccionan también con otras generalistas) (Bascompte et al. 2003); y 3) están construidas mediante dependencias débiles y asimétricas (Bascompte & Jordano 2007). Una de las características que otorgan estabilidad a las redes mutualistas es la presencia de unas pocas especies hiperconectadas que interaccionan de forma asimétrica y encajada con las especialistas. Nuestros resultados y la bibliografía existente sugieren que *A. senilis* es una de estas especies. Sin embargo, en el caso bien estudiado de *A. rudis* (ver Ness et al. 2009) las interacciones son mayoritariamente fuertes, ya que de su servicio de dispersión dependen, casi de forma exclusiva, un amplio conjunto de especies herbáceas. En uno de los estudios de mayor extensión geográfica sobre dispersión de semillas por hormigas en el Mediterráneo (Manzaneda et al. 2007), no se encontró tanta especificidad, existiendo varias especies de hormigas (sobre todo de los géneros *Camponotus, Formica* y *Aphaenogaster*) que actuaban como dispersores legítimos. Hay tres características ecológicas que predicen el número de interacciones mantenidas por una especie en las redes mutualistas: amplitud geográfica, abundancia local y amplitud de la fenología (Bascompte & Jordano 2007). *A. senilis* presenta una distribución geográfica relativamente restringida, sin embargo, es muy abundante en diversos ecosistemas y permanece activa casi todo el año. Todo lo anterior sugiere que *A. senilis* puede ser una especie clave en el mantenimiento de las redes ecológicas donde aparece. Estudios futuros a una escala geográfica más amplia podrían comprobar el grado y la intensidad de interacciones de *A. senilis* con diferentes conjuntos de especies.

6.2 EL CICLO COLONIAL, UNA CLAVE PARA EXPLICAR LA ECOLOGÍA DE *A. senilis*

Como comentamos en el primer párrafo de la introducción, las hormigas son interesantes por la gran cantidad de interacciones ecológicas y por su estatus de superorganismos. La unidad de la población es la colonia. Los factores internos o endógenos a la colonia han sido menos considerados en los estudios ecológicos, que los factores externos abióticos o bióticos. En el Mediterráneo se ha demostrado el papel importante de la temperatura y la competencia interespecífica (Cros et al 1997; Cerdá 1998; Retana & Cerdá 2000). Sin embargo, menos atención se ha dedicado al estudio de la influencia de los factores internos sobre la actividad de forrajeo o sobre la actividad general de la colonia.

Las hormigas son insectos holometábolos con diferentes requerimientos en los estadios de larvas y adultos (Blüthen & Feldhar 2010). La mayor parte de la comida es destinada a la alimentación de las larvas cuyas necesidades son sobre todo proteicas (Dussutour & Simpson 2009) y cualitativamente de micronutrientes. El resto se destina al mantenimiento de los adultos que tienen necesidades mayoritariamente de tipo energético. Por tanto las obreras recolectoras necesitan ajustar su estrategia de forrajeo a las necesidades de la colonia (Dussutour & Simpson 2009). Esto se ha estudiado hasta el momento solo en unas pocas especies (*Solenopsis invicta*, Cassill & Tscinkel 1999; Cook et al. 2011; *Pheidole ceres*, Judd 2005; *Linepithema humile*, Abril et al. 2007; *Rhytidoponera sp.*, Dussutour & Simpson 2009).

¿Por qué aumenta tanto la actividad recolectora en primavera? Está claro que está relacionado con el aumento de la producción de larvas y que es una adaptación a las condiciones ambientales de disponibilidad de proteínas animales. El aumento en la producción de larvas precede al de la actividad de forrajeo, y el pico de máxima producción de larvas se alcanza un mes antes que el pico máximo de forrajeo. En el NE de España (Canet de Mar, Barcelona) se observa algo similar con aproximadamente un mes de retraso con respecto a Doñana, tanto del periodo de mayor producción de larvas como del máximo de actividad de forrajeo (Cerdá et al. 1992; Cros et al. 1997). En años húmedos, en Doñana el otoño puede ser casi una segunda primavera, pero nuestras observaciones -tanto en años húmedos como secos- muestran que no hay un repunte de larvas y que la actividad de forrajeo, en condiciones de temperatura muy similares a las de primavera, es bastante menor. Ello sugiere que factores ambientales como la temperatura podrían modular la actividad de forrajeo, pero que es probable que los factores internos (estimulación de larvas, edad de las obreras, presencia de sexuados) desempeñen un papel igualmente importante.

Las variaciones en la proporción de alimento animal/vegetal podrían estar relacionadas también con las necesidades internas de la colonia: mayores necesidades de proteínas durante el periodo de producción de larvas y mayor necesidad de glúcidos y lípidos durante el resto del ciclo. Durante el verano-otoño, la proporción de alimento vegetal aumenta, y ello supone una mayor cantidad de glúcidos y lípidos para la colonia. Por ejemplo, en el inicio del verano las obreras muestran una gran apetencia por los frutos de *Arum*. Llegan a subirse a los espádices más maduros y arrancar los frutillos o transportan colectivamente los frutos más grandes. En ese periodo la colonia se prepara para la fisión. Hemos demostrado en condiciones de laboratorio que el consumo de los frutos de *Arum italicum* en las colonias sin reina produce el aumento de peso en las larvas de princesas y de obreras, y el aumento del número de machos procedentes de las obreras. Aunque se trata de condiciones artificiales, éstas pueden ser semejantes a las que se dan en las colonias grandes en este momento del año. Entonces, la dilución del efecto de la feromona real puede provocar, tanto la aparición de princesas como la puesta de huevos haploides por las obreras. Es posible que la apetencia por los frutos de *Arum* esté causada por algún estímulo interno estacional relacionado con la reproducción de la colonia. Entre septiembre y noviembre recolectan bastantes frutos de Olivilla (*Phillyrea angustifolia*) y lentisco (*Pistacea lentiscus*) que hemos encontrado almacenados en el interior de los nidos. Estos frutos son ricos respectivamente, en azúcares y en lípidos, y pueden suponer una reserva de recursos para los meses de diciembre y enero en los que la actividad recolectora es muy pequeña, pero la colonia sigue manteniendo una cierta cantidad de huevos y larvas pequeñas. El invierno es probable que sea el periodo de mayor mortalidad de las colonias.

¿Por qué migran las colonias hacia lugares más expuestos al sol en primavera? Es un mecanismo de termorregulación para aumentar la temperatura del hormiguero. Sin embargo la migración se produce entre marzo y abril ¿Por qué no se produce antes, en febrero, por ejemplo? La respuesta podría estar nuevamente en el ciclo. Entre marzo y mayo se alcanza la máxima biomasa de larvas que crecen rápidamente aprovechando tanto el pico primaveral de recursos, como el calentamiento del nido. En esa época las obreras trasladan a las larvas a las cámaras superiores durante las horas centrales del día y a las cámaras más profundas cuando el sol va bajando. Si hay lugares disponibles en sombra, las colonias migran hacia ellas en verano y en otoño tienden a regresar a zonas más despejadas. Sin embargo, la distribución de otoño no es tan clara como la de primavera, bastantes nidos permanecen en zonas menos expuestas al sol. Esta diferente respuesta es más probable que esté relacionada con el ciclo ya que en otoño las colonias tienen pocas larvas.

En resumen, consideramos que solo se puede entender el comportamiento, tanto de las obreras recolectoras como el de la colonia entera, a la luz del ciclo anual.

6.3 HORMIGAS Y DISPERSIÓN DE SEMILLAS EN EL MEDITERRÁNEO

Como ya se ha dicho, en el Mediterráneo se conocen pocas especies de plantas mirmecócoras y la dispersión de semillas por hormigas se asocia más con las hormigas granívoras, muy abundantes en nuestro clima, y que tienen efectos antagónicos depredadores/ dispersores cuyo balance depende de diversos factores ya mencionados. La dispersión de frutos carnosos por hormigas es un fenómeno extendido en América tropical. Se da en plantas leñosas primariamente dispersadas por aves. La realizan en gran parte hormigas de la familia *Ponerinae* de comportamiento subordinado y que no realizan trofalaxia. Existe un paralelismo notable con las plantas del matorral mediterráneo del tipo esclerófilo que tienen un origen tropical anterior al surgimiento del clima mediterráneo, hace unos tres millones de años (Herrera 1984, 1992). Estas plantas son igualmente dispersadas primariamente por aves y por algunas hormigas como *A. senilis*, ecológicamente similares a las *Ponerinae* tropicales. No obstante, mientras en diferentes especies de árboles y matorrales americanos se ha demostrado la importancia de la dispersión por hormigas, no es éste el caso en el Mediterráneo. En nuestro caso, la baja proporción de semillas de las plantas leñosas (*Phillyera angustifolia* y *Pistacea lentiscus*) dispersadas por *A. senilis,* en relación al tamaño de la cosecha, parece indicar una baja efectividad para la planta en las condiciones actuales. Una situación muy diferente se da en el caso de las plantas herbáceas *Arum italicum* y *Ornithogalum ortophyllum,* en la que *A. senilis* dispersa la práctica totalidad de la cosecha de semillas. Es una interacción muy especializada y en apariencia asimétrica. En el caso de *Arum italicum,* los mamíferos no la dispersan (Herrera 1989) ya que sus frutos les resultan venenosos, sin embargo son intensamente consumidos por *A. senilis.* Esta planta se defiende activamente de los vertebrados facilitando el recurso en exclusividad a las hormigas, que obtienen beneficios de su consumo. *Arum italicum* está ampliamente distribuida por la Península Ibérica en un área más amplia que la de *A. senilis.* Sobre ello se plantean varias preguntas: ¿Qué otros dispersores tiene la planta? ¿Se mantiene la misma relación entre las dos especies en toda el área de distribución? ¿Existen rasgos sometidos a coevolución por parejas o en mosaico geográfico? Futuros estudios podrían responder a estas preguntas.

6.4 USO DEL ESPACIO, CAUSAS Y EFECTOS DE LAS MIGRACIONES

¿Por qué migran las colonias?

Ecológicamente hemos demostrado que una de las causas de la migración es la regulación de insolación del nido, probablemente en relación con el máximo primaveral de larvas y con las temperaturas extremas del suelo en verano. Igualmente, parecen tener un cierto papel en el cambio hacia un patrón espacial regular en primavera. Sin embargo, posiblemente existan varias causas más. En una zona próxima al área de estudio de la Algaida, de suelo arcilloso, que se encharca en invierno y en la que hay abundantes matas de juncos, *A. senilis* anida en lugares despejados en verano aprovechando las grietas del terreno y migra hacia la parte superior de los juncos cuando el suelo se encharca. Es posible que también haya una relación con los parásitos ya que casi la mitad de las colonias excavadas tienen ácaros (Lenoir et al. 2012).

Independientemente de las causas ecológicas de las migraciones, su significado evolutivo a largo plazo debe estar relacionado con el tipo de reproducción por fisión que limita la dispersión y tiende a aumentar tanto el parentesco genético como la competencia intraespecífica entre las colonias próximas.

La competencia intraespecífica puede ser un factor importante ya que la reproducción por fisión tiende a aumentar la densidad de las colonias y consecuentemente intensificar la competencia. En el género *Cataglyphis*, las especies que se reproducen por fisión ejercen una mayor explotación de los recursos que las que tienen un fundación independiente (Knaden & Wehner 2006). En nuestras zonas de estudio las colonias están próximas entre sí y, durante la primavera, mantienen una distribución regular. No es raro ver peleas entre obreras de diferentes colonias.

6.5 DISTRIBUCIÓN GEOGRÁFICA DE A. *senilis*

¿Qué limita la distribución de *A. senilis*?

A pesar de su gran plasticidad ecológica, *A. senilis* no está presente por ejemplo en gran parte del Este de la Península Ibérica. En esta zona es sustituida por *A. iberica* una especie muy cercana filogenéticamente. *A. iberica* tiene una menor eficacia en el reclutamiento (Lenoir et al. 2011) y el tamaño de la colonia es aproximadamente la mitad que el de *A. senilis* (media±SE: 524 ± 69 obreras, N=12 colonias, Boulay datos no publicados, frente a: 1260 ±69, Boulay et al. 2007c). Esto sugiere que la distribución de *A. senilis* puede estar limitada por la aridez del terreno que actuaría reduciendo los recursos alimenticios disponibles y haciendo menos eficaz el sistema de reclutamiento. Un ejemplo de la importancia de la humedad para *A. senilis* es que durante el verano, al amanecer, la mayor parte de la colonia suele salir del nido a beber el rocío que se condensa en las hojas de los matorrales. Como ya se ha dicho antes, en La Algaida *A. senilis* está ausente en el matorral de jaguarzo de zonas que han sufrido incendios. En estos lugares la única especie de hormigas es *Tapinoma nigerrimun*. Es un hábitat con muy baja productividad y con suelo arenoso poco estable.

6.6 APLICACIONES: *A. senilis*, ¿un posible insecto auxiliar en agricultura?

La propensión a comer pulgones, orugas o caracoles podría hacer de *A. senilis* un insecto auxiliar en agricultura. Es muy fácil de criar, se adapta a una gran variedad de hábitats y realiza reclutamiento sobre presas pequeñas cuando su densidad es alta (Cerdá et al. 2009). No obstante, posiblemente el mantenimiento de agrosistemas con diversidad alta que incluyan la presencia de *A. senilis* pueda ser una medida más sencilla y eficaz que su introducción artificial. Futuros estudios podrían evaluar esta posible aplicación.

CONCLUSIONES

Ecología trófica:

1.- En Doñana las colonias de la hormiga *Aphaenogaster senilis* permanecen activas durante casi todo el año, pero la recolección de alimento y la producción de larvas se concentra en la primavera. Ambas actividades descienden acusadamente en verano y otoño.

2.- El uso combinado de observaciones directas e isótopos estables demuestra que la dieta de *A. senilis* es omnívora incluyendo una amplia variedad de recursos: insectos (vivos y muertos) y plantas (entre las que aparecen los frutos y semillas de más de once especies). Su nivel trófico es más elevado en primavera y otoño, y desciende entre junio y septiembre. La abundancia en la dieta de los principales grupos de animales se correlaciona con la disponibilidad de los mismos.

Dispersión de semillas:

3.- Los frutos carnosos de *Arum italicum, Phillyrea angustifolia* y *Pistacea lentiscus* representan más de un cuarto de los items recolectados por *A. senilis* entre junio y noviembre. Sin embargo, son generalmente ignoradas (o a veces depredados *in situ*) por otras hormigas.

4.- En el laboratorio, la adición de frutos de *Arum italicum* a una dieta base de insectos incrementa la producción de machos y el tamaño de las pupas de obreras y reinas.

5.-Confirmamos el papel de *A. senilis* como “keystone disperser” (especie dispersora clave). Las semillas de las plantas dispersadas son transportadas hasta más de 8 m desde las plantas madres y depositadas en hábitats favorables, permitiendo su germinación en una tasa relativamente alta. No obstante, el beneficio para la planta puede variar mucho dependiendo del tamaño de la cosecha de semillas de cada especie vegetal y la existencia de dispersores alternativos.

Uso del hábitat: movimientos de las colonias

6.- Las migraciones coloniales sucesivas no siguen una dirección constante sino que sus trayectorias son aleatorias o circulares, lo que, combinado con la reproducción por fisión, produce un efecto de dispersión limitado.

7.- Este limitado efecto de dispersión ocasiona un parentesco genético entre las colonias vecinas (aquéllas situadas a menos de 5 m) mayor del que se esperaría por azar (viscosidad poblacional). Sin embargo, no hay endogamia entre las colonias cercanas, probablemente debido al ocasional flujo genético vía machos. Una posible consecuencia de la formación de áreas de vecindad, sería el bajo nivel de agresividad entre colonias cercanas que se observa en esta especie, reduciendo los efectos de la competencia por los recursos.

8.- Se han documentado por primera vez dos fisiones coloniales en la naturaleza. Tuvieron en común las siguientes características: Se produjeron en colonias de tamaño grande, por encima de la media habitual, con más de 1500 obreras; la formación del nuevo nido fue a corta distancia, poco más de 2 m. y la fisión resultó asimétrica. En ambos casos, la vieja reina se trasladó al nuevo nido con el 69 % y 66 % de las obreras, mientras la nueva reina heredó el viejo nido y las restantes obreras

9.- Las migraciones presentan un patrón estacional: Durante el verano la mayor parte de las colonias tienden a emigrar a lugares sombreados con mayor cobertura vegetal para evitar las temperaturas elevadas que alcanza el suelo. En primavera y otoño tienden a situarse en lugares más expuestos al sol condicionadas por necesidades térmicas del desarrollo de las larvas. La búsqueda de un lugar óptimo de anidación parece seguir un proceso de ensayo y error en el que las colonias, una vez que han llegado a un nuevo nido, evalúan las características ambientales, tanto bióticas como abióticas, del nuevo emplazamiento y pueden migrar nuevamente si las mismas son poco adecuadas.

10.- En primavera el patrón de distribución de las colonias es regular. En verano y otoño es un patrón principalmente aleatorio. La distancia media de forrajeo es significativamente menor que la distancia entre nidos vecinos. Todo apunta a que la competencia intraespecífica es más fuerte en primavera, cuando los requerimientos de las colonias son mayores

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