DOTTORATO DI RICERCA IN ETOLOGIA ED ECOLOGIA ANIMALE
(XXII CICLO)

Analisi del comportamento e dell'ecologia di *Crematogaster scutellaris* (Hymenoptera, Formicidae): organizzazione spaziale delle colonie, preferenze alimentari ed interazioni competitive

Tesi di

Filippo Frizzi

Coordinatore Prof. Alberto Ugolini
Tutor Prof. Giacomo Santini
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RIASSUNTO

Il lavoro di tesi affronta prevalentemente aspetti ecologici e comportamentali di *Crematogaster scutellaris* (Hymenoptera, Formicidae), una specie ampiamente diffusa in gran parte dei boschi e delle colture del bacino del Mediterraneo. Alcune delle sue principali caratteristiche, quali la costruzione di nidi solitamente ricchissimi di individui, un rapido reclutamento di massa e l'utilizzo di una efficace arma chimica, ne fanno una forte competitrice, permettendole di ricoprire un elevato ruolo nei ranghi di dominanza. Gli aspetti che prevalentemente sono stati analizzati riguardano, schematicamente: i) la selezione alimentare nei confronti di risorse a diverso contenuto di nutrienti, ii) i fattori che influenzano la distribuzione spaziale dei nidi e iii) la competizione, sia intra che interspecifica. In letteratura, le informazioni a riguardo sono scarse, e i risultati della tesi hanno contribuito a fare una maggiore chiarezza su ognuno di questi temi. Si è dimostrato, attraverso analisi di aggressività intraspecifiche prima e genetiche poi, che la struttura coloniale di questa specie può essere polidomica. Inoltre, avendo riscontrato delle difformità nei livelli di parentela all'interno della stessa colonia, è stato possibile formulare un'ipotesi di poliginia per questa specie, ovvero la presenza contemporanea di più regine afferenti alla stessa colonia. Riguardo agli aspetti alimentari, *C. scutellaris* ha mostrato capacità selettive nei confronti di risorse primarie come carboidrati, aminoacidi e acqua in base alla variabilità, dovuta prevalentemente a fattori stagionali, delle proprie esigenze nutritive. Nell'analisi della competizione interspecifica, oltre a *C. scutellaris*, sono state prese in esame altre tre specie: *L. paralienus*, *L. emarginatus* e *L. neglectus*. A differenza dei lavori precedenti, in questa sezione la specie principale esaminata è *L. neglectus*. Questo a causa del suo elevato valore ambientale e conservazionistico, dato dalla notevole invasività e dalla rapidissima diffusione mostrata sul territorio europeo; l'analisi delle dinamiche di competizione con altre specie può quindi essere un elemento utile per uno studio approfondito sui suoi metodi di dispersione. Più in generale, i test di aggressività svolti hanno permesso di valutare alcuni aspetti delle strategie comportamentali negli scenari di conflitto tra specie, evidenziando come gli individui appartenenti a due di queste (*L. paralienus* e *L. emarginatus*) siano in grado di stimare l'entità del proprio gruppo di appartenenza adattando il livello di aggressività alle diverse situazioni in caso di scontro. Altri test effettuati su gruppi di individui, hanno permesso di valutare la possibilità dell'uso di particolari modelli, creati nel 1916 dall'Ing. Lanchester per prevedere gli esiti delle battaglie aeree, in scenari di conflitto tra gruppi di formiche. I modelli, di due tipi detti Linear e Square, si diversificano per il fatto che il primo (Linear), a differenza del secondo (Square), non considera gli effetti dell'entità del gruppo nel prevedere l'esito di una battaglia. Dai dati emerge come questo tipo di approccio analitico possa essere utilizzato in ambiti simili, aprendo possibili scenari futuri di studio che potrebbero chiarire quali siano gli aspetti comportamentali ed ecologici coinvolti.

1. INTRODUZIONE

Le formiche (Hymenoptera: Formicidae) sono insetti sociali presenti sin dal Cretaceo. Il loro successo evolutivo è testimoniato dal considerevole ammontare delle specie, circa 10.000 quelle descritte, distribuite in 296 generi e 16 sottofamiglie (Bolton 1994, 1995). Si stima che almeno altre 5.000 specie restino da descrivere. La straordinaria capacità adattativa alle diverse situazioni ambientali ha fatto sì che questi insetti si diffondessero pressoché in tutte le aree biogeografiche della terra, saturando le diverse nicchie trofiche presenti sia a livello pedologico che vegetazionale (Holldöbler and Wilson 1990). Organismi dall'alta duttilità biologica e adattativa, le formiche possono essere complessivamente classificate come ingegneri dell'ecosistema (Jones et al. 1994), organismi capaci cioè di causare cambiamenti fisici nell'ambiente biotico o abiotico e di controllarne direttamente o indirettamente la disponibilità di risorse, influenzando così in modo drastico le catene trofiche e gli ecosistemi. La loro complessa organizzazione sociale, peculiare rispetto ad altri insetti sociali quali api (Hymenoptera: Apidae) o vespe (Hymenoptera: Vespidae), elemento fondante nel loro successo evolutivo, è oggetto di attento studio e di analisi sia a livello ecologico che etologico. Negli ultimi anni, numerosi lavori hanno cercato di fare chiarezza su alcuni punti controversi in riguardo alle dinamiche degli assetti delle colonie tramite nuove forme di analisi di popolazione, la più diffusa delle quali è certamente l'analisi genetica (Giraud et al 2001, Fournier et al. 2002, Feldhaar et al. 2005, Schlüns et al. 2009, solo per fare alcuni esempi), ma anche semplicemente restringendo i confini del dibattito intorno ad alcuni termini descrittivi, quali 'polidomia', 'policalia' o lo stesso 'nido' (Debout et. al., 2007).

Le formiche sono gli unici insetti sociali che presentano polidomia. Secondo la definizione proposta da una recente review, (Debout et. al., 2007), una colonia è polidomica nel caso in cui siano presenti almeno due nidi separati spazialmente da una distanza superiore alla massima distanza che intercorre tra due celle presenti all'interno dello stesso nido. Per nido, si intende qualsiasi struttura che al suo interno racchiuda operaie e prole (principalmente larve e pupe). La presenza della regina non è fondamentale, in quanto le operaie sono spesso in grado di allevare autonomamente la prole trasportandola direttamente dal nido di origine verso nidi periferici. E' invece basilare la presenza della prole stessa, la quale, oltre ad essere evidentemente indispensabile per il rinnovo generazionale, induce importanti espressioni comportamentali quali la cura parentale e la difesa del nido. La polidomia risulta così essere una più o meno complessa rete di comunicazione tra nidi diversi all'interno della stessa colonia. Il primo a definire il termine 'polidomia' fu Forel (1874), secondo il quale una colonia risulta polidomica semplicemente per la presenza di almeno un nido senza regina e/o prole.

Nonostante la gran parte delle specie di formiche, monodomiche e polidomiche, sia multicoloniale, costituita cioè da popolazioni che consistono di entità funzionali indipendenti l'una dall'altra (Bourke & Franks, 1995), esistono alcune specie le cui popolazioni sono unicoloniali, caratterizzate da frequenti interazioni non aggressive tra tutti i nidi della popolazione e scarsa variabilità genetica. In sostanza, l'intera popolazione funziona come una unica ed estesa colonia polidomica (Passera, 1994; Reuter et al., 2001; Tsutsui & Case, 2001; Giraud et al., 2002; Elias et al., 2005). L'unicolonialità rappresenta una struttura coloniale efficiente (Keller, 1995; Tsutsui et al., 2000) ma instabile, essendo strettamente connessa al grado di adattamento della specie alle dinamiche ambientali (Keller, 1995). La multicolonialità, d'altro canto, supporta una maggiore variabilità, lungo un continuum che spazia dalla monodomia alla polidomia.

La polidomia è talvolta un fenomeno stagionale; la colonia sverna in un unico nido (raramente più di uno) frazionandosi in due o più unità occupando nidi differenti durante la stagione di massima attività, riunendosi poi nuovamente l'inverno successivo (Higashi, 1979; Alloway et al., 1982; MacKay & MacKay, 1984; Rosengren et al., 1985; Herbers & Grieco, 1994). Inoltre, una colonia polidomica può contenere sia una (monoginia) che più regine (poliginia), e ogni nido può indifferentemente contenere femmine fertili o meno, garantendo un maggiore stabilità della colonia su lunghi periodi. La polidomia può infatti essere evolutivamente interpretata come una sorta di strategia 'paracadute': se un nido viene distrutto, altri restano disponibili per la colonia. Questo indipendentemente dal fatto che la colonia sia monoginica o poliginica, anche se risulta evidente una maggiore rilevanza per quest'ultima tipologia. Infatti, nel caso delle colonie monoginiche, è necessario che il nido dove risiede la regina non venga distrutto perché la colonia si mantenga. Nel caso delle colonie poliginiche, a seguito di una completa distruzione di uno o più dei suoi nidi, è sufficiente che sopravviva una delle femmine fertili perché la colonia si conservi.

In passato, particolare enfasi veniva posta sugli effetti della poliginia nell'evoluzione sociale di questi insetti, mentre gli effetti della polidomia venivano spesso sottostimati (e.g. Ross & Carpenter, 1991; Bourke, 1991). Il crescente interesse nei confronti di una analisi più approfondita della complessità strutturale delle colonie, rende necessaria una maggiore chiarezza interpretativa su alcuni punti come appunto polidomia o poliginia, elementi fondamentali per lo studio ecologico e comportamentale di questi organismi.

In alcuni testi, è possibile trovare come sinonimo di 'polidomia' il termine 'policalia'. Policalia è un termine forgiato ancora da Forel (1874) per descrivere una struttura coloniale organizzata in più nidi, ognuno contenente una popolazione più o meno indipendente ma necessariamente costituita da prole, operaie e una o più femmine riproduttive. In Debout et al. (2007), si suggerisce di utilizzare il termine 'policalia' nel caso specifico in cui siano osservati comportamenti non aggressivi tra nidi diversi di una colonia polidomica.

La poliginia probabilmente è la metodologia riproduttiva più diffusa tra gli insetti sociali, in particolare tra le formiche (Keller, 1993). Comparazioni interspecifiche suggeriscono che la polidomia sia un passo evolutivo secondario che accompagna o segue l'evoluzione della poliginia da un antenato monoginico. Sebbene la polidomia sia spesso associata alla poliginia (Holldöbler & Wilson, 1977; Rosengren & Pamilo, 1983; Ross & Fletcher, 1985; Keller, 1991), sono note molte specie sia monoginiche che polidomiche (e.g. Way, 1954; Ichinose, 1987; Cerda et al., 1994). La correlazione tra polidomia e poliginia non è dunque così stretta; l'unico dato che è possibile osservare con certezza è la diffusa polidomia tra specie sia poliginiche che, in misura minore, monoginiche, mentre la monodomia è quasi esclusiva di specie monoginiche, benché esistano rari dei casi di poliginia associata a monodomia (Holldöbler & Wilson, 1977). La frequente associazione tra poliginia e polidomia, è probabile conseguenza di fattori simili che promuovono entrambe. Un elemento determinante nel favorire la poliginia sembra essere il costo di dispersione (Keller, 1995), importante anche per lo sviluppo della polidomia (Debout, 2007).

La distribuzione attesa dei nidi in una colonia polidomica è di tipo aggregato, sia dal punto di vista spaziale (Herbers, 1985, 1989) che genetico (Herbers & Grieco, 1994). In uno studio su sottopopolazioni poliginiche e polidomiche di *Formica truncorum*, sono state evidenziate significative distanze genetiche tra i diversi nidi, non emerse in sottopopolazioni monoginiche e monodomiche (Sundström, 1993). Altri studi effettuati su *Formica polyctena* (Beye et al., 1997) supportano la tesi di una netta strutturazione genetica delle colonie polidomiche, risultato del processo di 'budding' (fenomeno per quale nuove femmine fertili si spostano in nidi separati accompagnate da alcune operaie) normalmente associato alla polidomia (Elias et al., 2002).

Come la struttura genetica sia relazionata ai pattern di distribuzione dei nidi in ogni popolazione polidomica e come questa influenzi la competizione e la selezione sulla sex ratio a scala locale, sono tutt'ora questioni aperte (Debout et al., 2007).

Dal punto di vista ecologico, la polidomia può fornire numerosi vantaggi. Su tutti, la dispersione dei nidi in un area estesa accorcia i percorsi di foraggiamento, riducendo così i costi del central place foraging (McIver, 1991). Di contro, la polidomia comporta anche maggiori costi, dovuti prevalentemente alla perdita di prole durante il trasporto tra i siti di nidificazione, alla maggiore probabilità di disidratazione delle operaie e alla riduzione della variabilità genetica della popolazione. E' evidente che la bilancia costi/benefici deve pendere dalla parte dei benefici perché la polidomia si imponga dal punto di vista sia comportamentale che evolutivo.

Un bilancio energetico positivo e una disponibilità di cibo che comprenda tutti i nutrienti necessari ai processi metabolici, sono elementi di primaria importanza per la sopravvivenza e la riproduzione di qualsiasi organismo (Raubenheimer & Simpson, 1997), sia a livello individuale che, nel caso di organismi sociali, a livello coloniale. Negli ultimi anni, sta crescendo l'interesse nei confronti dell'analisi del bilancio dei nutrienti nella dieta degli organismi, fattore determinante per numerosi fenomeni sia biologici che comportamentali (Sterner & Hessen, 1994; Simpson & Raubenheimer, 2001; Kay, 2002). Di fronte a diverse opportunità alimentari, un organismo deve essere in grado di compiere una scelta attiva nell'assimilare la risorsa che in quel momento fornisce il maggiore apporto energetico, valutando altresì il contributo dal punto di vista dei nutrienti. La comprensione dei fattori che portano a tale scelta, dei quali le caratteristiche delle risorse scelte possono essere validi indici (Howard, 1987; Völkl et al., 1999), è utile non soltanto per valutare i pattern di utilizzo dell'habitat (Brodbeck et al., 1990; Rank, 1992), ma anche per sondare processi ecologici di livello superiore, come le interazioni competitive (Kronfeld-Schor & Dayan, 1999; Savolainen, 1991) e l'impatto del consumatore sulla popolazione delle prede (Crist & MacMahon, 1992; Hjälten, 1997). Nella dieta degli insetti, il corretto bilanciamento tra carboidrati e proteine è risultato essere fondamentale (Dadd, 1985); numerose specie hanno dimostrato di essere in grado di valutare le proprie esigenze alimentari e di selezionare le risorse in base ai diversi livelli dei due nutrienti (Waldbauer and Friedman, 1991; Kay, 2004).

La disponibilità e la distribuzione delle risorse è un fattore altamente influente anche per la competizione. La gerarchia competitiva delle formiche è basata sia su differenze nella struttura della colonia che sul numero delle foraggiatrici (Savolainen & Vepsäläinen 1988). Specie dominanti sono generalmente in grado di allontanare altre specie, talvolta con fenomeni di predazione soprattutto nel caso in cui la colonia sia in regime di carenza proteica (Driessen et al., 1984). Modificazioni delle caratteristiche ambientali possono variare le gerarchie e promuovere o ridurre la competizione tra specie. Un taglio raso, ad esempio, può ridurre la disponibilità di risorse per specie adattate ad habitat stabili (Rosengren & Pamilo, 1978). In caso di singola specie dominante, i fattori ambientali vanno ad influire ampiamente sulle dinamiche di competizione intraspecifica (Sorvari & Hakkarainen, 2004).

Un aspetto particolarmente interessante dal punto di vista ecologico ed evolutivo è la possibilità, da parte di numerose specie di formicidi, di costituire rapporti mutualistici con altri insetti, in particolare con omotteri. Molte specie di Afidi o Coccidi succhiatori di linfa, producono escreti zuccherini, detti comunemente 'melata', dei quali alcune specie di formiche possono nutrirsi. In alcuni casi, le formiche sono in grado di proteggere gli omotteri dalla predazione, un processo detto anche 'allevamento' per gli aspetti utilitaristici che presenta, incrementandone la popolazione e la produzione di melata (e.g. El-Ziady and Kennedy 1956, Banks 1962, Way 1963, Banks and Macaulay 1967, Addicott 1979, Pierce and Mead 1981, Buckley 1987a, b, Völkl 1992). Anche in questi casi, come avviene per alcune specie dominanti in scontri tra formiche, nel caso in cui nella dieta della colonia venga a ridursi l'apporto proteico il rapporto mutualistico può mutare in comportamento predatorio (Cushmann and Addicott, 1991; Bronstein, 1994).

Scopo di questa tesi è stato di valutare aspetti etologici ed ecologici di *Crematogaster scutellaris* (Hymenoptera: Formicidae)(Fig.1.1), un formicide ampiamente diffuso in tutto il bacino del mediterraneo, dal sud della Francia fino al nord Africa, sia in ambienti naturali che antropizzati. I nidi di *C. scutellaris*, costruiti all'interno di fusti arborei o di tronchi e rami morti, sono di norma molto estesi e altamente ricchi di individui (Bernard 1968; Casevitz-Weulersse 1972). Questa specie, grazie ad alcune caratteristiche sia comportamentali che biologiche, risulta essere un competitore molto efficiente (Cammell et al. 1996; Way et al. 1997; Santini et al. 2007). Una peculiarità del genere *Crematogaster* è la struttura del peziolo, che a differenza di altre specie di Mirmicine presenta una attaccatura marcatamente spostata verso la parte superiore del piano trasversale, sia sul torace che sull'addome. Questo permette loro una elevata mobilità dell'addome, sia lateralmente che frontalmente, fino ad una completa flessione oltre la testa (Fig. 1.2). L'utilità di tale singolarità strutturale risiede nell'uso della efficace arma chimica, importante strumento utilizzato dalla specie per imporre la propria gerarchia di dominanza. Una ghiandola posta sull'addome distalmente rispetto al peziolo, secerne una miscela velenosa ricca di aldeidi tossiche, la quale viene 'posata' sul contendente grazie alla particolare flessione dell'addome (Pasteels et al., 1989; Marlier et al., 2004).

Una delle più importanti chiavi per il successo di questa specie è il sistema di reclutamento, estremamente rapido e massivo al momento del reperimento di nuove risorse da parte degli individui foraggiatori (Bernard, 1968). Oltre a questo, *C. scutellaris* presenta una notevole capacità di difesa della risorsa stessa, riuscendo a dominarla anche nei confronti di specie più opportuniste (Tucci, 2006).

Il giornaliero di attività della specie è molto ampio, fino ad avere, nei periodi di maggiore attività che vanno da metà primavera fino ad inizio autunno, un ciclo di attività pressoché continuo. Le ore con la più alta punta di attività sono quelle nelle quali la temperatura sia compresa circa tra 20°C e 35°C, e sono variabili in base alla stagione (Santini et al., 2007).

La dieta di *C. scutellaris* è estremamente generalista. Sebbene altri lavori abbiano evidenziato una scarsità di predazione (Morris et al., 2002) e una dieta formata da sostanze liquide per l'80% (Soria et al., 1994), comportamenti predatori in questa specie sono stati ripetutamente osservati (e.g. Gonzalez & Campos, 1990; Morris et al., 1998; Schatz & Hossaert-McKey, 2003). Attraverso l'analisi di isotopi stabili, è stato inoltre dimostrato che la predazione e lo scavenging sono la componente principale della dieta di questa specie (Ottonetti et al., 2008). Nonostante questo, *C. scutellaris* è anche in grado di costituire rapporti mutualistici con diverse specie di omotteri (Morris et al.,1998; Schatz and Hossaert-McKey, 2003; Ottonetti et al., 2008). Particolare attenzione viene posta in questa tesi (Cap. 3) nei confronti del rapporto mutualistico che intercorre tra *C. scutellaris* e *Saissetia oleae*, un omottero Coccide parassita degli olivi. La presenza nelle coltivazioni di questo insetto,

Fig. 1.1. Crematogaster scutellaris.

può produrre gravi danni alle piante, dovuti soprattutto alla formazione della cosiddetta ‘fumaggine’[1].

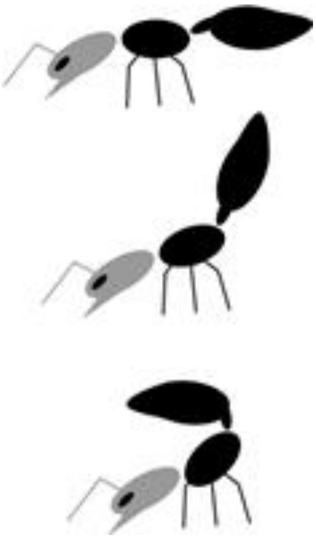

Fig. 1.2. Flessione dell’addome tipica del genere *Crematogaster.*

Anche se sono presenti studi che suggerirebbero per questa specie monoginia (Baroni Urbani & Soulié, 1962) e possibilità di polidomia (Casevitz-Weulersse, 1973), le informazioni sulla sua organizzazione sociale non sono ancora univoche. In questa tesi, si è cercato di fare chiarezza sui metodi di distribuzione e di organizzazione sociale di *C. scutellaris*, cercando di analizzarne il comportamento in habitat sia naturali che antropici (Cap. 2).

L’habitat prevalente nel quale si sono svolti i lavori della tesi è un oliveto situato nei pressi di Calenzano, in provincia di Firenze (Italia). La scelta dell’oliveto fornisce alcune facilitazioni per lo studio della distribuzione spaziale e della competizione intraspecifica. Infatti, essendo gli alberi di olivo organizzati in filari regolari ed essendo gli unici siti disponibili per edificare il nido, la mappatura della presenza/assenza e la distribuzione spaziale dei nidi di *C. scutellaris* è risultata puntuale e correttamente analizzabile. Inoltre, la matrice arida e con carenza di risorse in cui sono immersi gli alberi di olivo, crea delle zone ben distinte di abbondanza e scarsità. Questo sembra avere un effetto di esasperazione sulla competizione intraspecifica, nostro oggetto di

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[1] A causa della presenza di melata prodotta da *S. oleae* e da altri omotteri parassiti, è possibile lo sviluppo di funghi saprofiti, i quali producono fitti intrecci di miceli dalla pigmentazione scura detti appunto ‘fumaggine’ che privano l’albero dell’apporto luminoso. La conseguenza sulla pianta è una netta riduzione della produzione fino, nei casi più gravi di infestazione, alla morte (Daane & Caltagirone, 1989).

studio, oltre a provocare forti modificazioni sulla composizione specifica dell'intera popolazione di formicidi (Retana and Cerdá, 2000; Arnan et al., 2007).

Oltre ai fattori che influenzano la distribuzione dei nidi, la tesi affronta aspetti che riguardano il comportamento alimentare di *C. scutellaris*. (Cap. 3). In particolare, la domanda è stata se la specie sia in grado di operare una scelta attiva nei confronti di diverse possibilità alimentari oltre che per al loro apporto energetico anche per la loro composizione. Gli esperimenti, eseguiti ancora nell'oliveto sopra descritto, prevedevano test sia di scelta individuale della risorsa (acqua, carboidrati e proteine) che di reclutamento, a seguito di una ciclica variazione artificiale nella disponibilità dei nutrienti. L'approccio dell'analisi di campo, in quest'ultimo caso, risulta essere particolarmente importante, dato che la presenza del forte stress idrico al quale sono sottoposte le colonie nel periodo estivo (a causa della rarefazione degli alberi di olivo le zone d'ombra sono scarse) è un fattore che abbiamo supposto essere determinante per le scelte alimentari a livello sia individuale che di colonia. In precedenti simili lavori, la tendenza è quella di focalizzare l'attenzione sulla scelta di nutrienti organici quali zuccheri o proteine, considerando l'apporto idrico come fattore separato e non integrato nel bilancio complessivo dei nutrienti (Kay, 2002, 2004; Abril et al., 2007).

Un ulteriore aspetto dell'ecologia di *C. scutellaris* esaminato nella tesi (Cap. 4) riguarda la competizione intraspecifica, in particolare è stata valutato il comportamento aggressivo nei confronti di conspecifici alieni e come questo si modifichi in funzione della distanza internidiale. L'approccio sperimentale è stato combinare gruppi di individui provenienti da nidi diversi direttamente sul campo, in arene neutrali formati da contenitori chiusi di plastica trasparente. In questo modo, anche i fattori ambientali venivano considerati e immessi nell'analisi. I test si sono svolti sia nell'oliveto che in un bosco degradato adiacente prevalentemente formato da pini marittimi (*Pinus pinaster*) e roverella (*Quercus pubescens*) in un'ottica di confronto tra dinamiche competitive in due habitat che presentano in linea teorica due distribuzioni delle risorse diverse, aggregata (oliveto) e uniforme (bosco).

I primi risultati dei test hanno permesso la definizione nell'oliveto di 'clusters', raggruppamenti, costituiti da nidi reciprocamente non aggressivi e di formulare delle prime ipotesi di struttura coloniale polidomica per la specie. Un elemento interessante emerso dai dati del bosco, è che nel caso in cui si fossero verificati eventi aggressivi tra individui appartenenti a nidi limitrofi (ca. 5 metri di distanza), questi risultavano molto più rapidi e intensi. Questo fenomeno, registrato anche per altre specie di formiche (Gordon, 1989; Knaden & Wener, 2003), si contrappone al cosiddetto 'dear enemy phenomenon', il quale prevede una scarsa tendenza all'aggressività nei confronti di conspecifici che abbiano areale confinante. Entrambi i fenomeni comportano un riconoscimento dell'altro individuo, tramite apprendimento di segnali sia di natura ambientale che chimica.

Il passo successivo è stato quello di sviluppare dei markers genetici (Cap 5) per 9 loci su DNA microsatellitare al fine di stimare l'eventuale correlazione che intercorre tra distanza genetica, distanza spaziale e aggressività attraverso una analisi di parentela degli individui. L'elevato grado di polimorfismo riscontrato per questi loci, incoraggia verso un loro utilizzo per analisi di popolazioni anche a scala estremamente ridotta. I primi esperimenti eseguiti su individui provenienti da alberi dell'oliveto (Cap. 6), tendono a confermare i confini delle colonie disegnati tramite test di aggressività e soprattutto a supportare l'ipotesi precedentemente costruita di polidomia per questa specie. Inoltre, è stata evidenziata una diversità nei rapporti di parentela tra i clusters presi in esame, questo probabilmente a testimonianza del fatto che la specie, oltre ad essere polidomica, presenta diversi livelli di poliginia e/o di poliandria.

Nella sezione che riguarda il lavoro sulla competizione interspecifica, (Cap. 7), verrà posta una particolare attenzione alla biologia e all'ecologia di *L. neglectus*, una specie nota negli ultimi anni in Europa per essere una specie aliena, invasiva, particolarmente rapida nella sua espansione e in certi casi altamente dannosa. E' sufficiente dire che fino al 1990 la specie era inesistente in Europa, e che da poche popolazioni scoperte in quell'anno in Ungheria si è passati ad una copertura quasi totale dell'intero territorio continentale. Soltanto alcune aree a nord ne sono ancora prive, ma l'ampio range di condizioni ambientali nel quale *L. neglectus* può vivere fa pensare ad una sua ulteriore espansione (Ugelvig et al., 2008). Questo lavoro, pur riguardando anche *C. scutellaris*, è primariamente incentrato sulle dinamiche di aggressività di questa specie, avendola ritenuta di alto interesse ambientale e conservazionistico. Sono stati effettuati test di aggressività tra *L. neglectus* e altre tre specie: *C. scutellaris, Lasius emarginatus* e *Lasius paralienus*. Nella prima parte del lavoro, sono stati eseguiti test individuali cercando di valutare un eventuale effetto del 'gruppo' sul livello di aggressività. In sostanza, è stato verificato se la provenienza da gruppi più o meno numerosi influisse sul comportamento aggressivo del singolo. Nella seconda parte, sono stati costruiti degli andamenti temporali della mortalità attraverso scontri effettuati non più tra singoli ma tra gruppi di individui, prendendo in esame le specie di cui sopra a due a due, con *L. neglectus* come specie di riferimento. Gli andamenti sono stati poi comparati attraverso due particolari modelli teorici, detti modelli di Lanchester, sviluppati nel 1916 dall'omonimo ingegnere inglese Frederick Lanchester per l'industria bellica, e che avevano lo scopo primario di prevedere l'esito delle battaglie aeree. Il primo modello, detto 'Linear', prevede che l'esito della battaglia sia dipendente unicamente dalla capacità individuale di combattimento, mentre il secondo, detto 'Square', oltre all'abilità individuale inserisce l'entità del gruppo di appartenenza come parametro per stabilire l'esito dello scontro (Lanchester, 1916). Altri studi confermano la possibilità di questo tipo di approccio in scenari di conflitto tra formiche (McGlynn, 1999, 2000; Powell & Clark, 2004; Tanner, 2006; Schöning, 2007).

Lo studio sia comportamentale che biologico di *Crematogaster scutellaris*, coadiuvato da recenti tecnologie di analisi come i markers genetici, può fornire importanti elementi nella valutazione ecologica di numerose situazioni ambientali, data l’elevata distribuzione anche in habitat antropizzati e degradati della specie.

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2. SPATIAL PATTERNS OF THE ANT *CREMATOGASTER SCUTELLARIS* (HYMENOPTERA, FORMICIDAE) IN A MODEL ECOSYSTEM

2.1. INTRODUCTION

The last few decades have seen an increasing interest in the study of spatial patterns in ecology (e.g. Levin, 1992; Durrett & Levin, 1998; Grimm et al., 1996; Gustafson, 1998; Dale, 1999; Liebhold & Gurevitch, 2002; McPherson & Jetz, 2007, just to mention a few). Detailed knowledge of spatial patterns may reveal the underlying processes and clarify their function (Perry et al., 2002). For example, spatial patterns of both plants and animals could result from various processes and forces, such as intra- and interspecific competition, disturbance, predation or environmental heterogeneity, which may operate at different scales. Analysis of the resulting spatial arrangements can reveal much about the underlying processes, although care is required in inferring causation since many different processes could generate similar spatial patterns (Wiegand & Moloney, 2004). One of the difficulties is to separate the effects of underlying, and often subtle, environmental heterogeneity from intrinsic biological dynamics, even in habitats that appear homogeneous at the organisms' scale (e.g. Vandermeer et al., 2008).

Ants form a large proportion of the arthropod fauna in many terrestrial ecosystems, where they play a key role in determining the structure and function of local communities (Hölldobler and Wilson, 1990). Knowledge of the ecology and behaviour of ants, and the way they interact with other taxa, may help to understand the dynamics of such communities. The analysis of ant spatial patterns, from both a descriptive and theoretical perspective, has long been a prominent field of research. In fact, it still stimulates ecological debate on key topics. For example, Vandermeer et al. (2008) recently examined the spatial distribution of the tropical ant *Azteca instabilis* in a uniform environment. In addition to finding a clustered arrangement of nests, they showed that the cluster distribution behaved according to a power law, a pattern consistent with a so-called 'robust critical state', a hotly debated topic in ecology (Sole et al., 1999; Pascual et al., 2002; Pascual & Guichard, 2005; Sole & Bascompte, 2006; Solow, 2005).

The ant *Crematogaster scutellaris* is a common species in both natural and human-managed ecosystems throughout the Mediterranean basin, where it may form large colonies consisting of up to several thousand specimens (Bernard, 1968; Casevitz-Weulersse, 1970, 1972, 1991). Several studies have shown that this species is one of the most highly ranked competitors in Mediterranean ant communities (Cammell et al., 1996; Way et al., 1997; Schatz and Hossaert-McKey, 2003; Santini et al., 2007). The keys to its success are its ability to mass recruit large numbers of conspecifics onto newly discovered resources (Bernard, 1968), the use of chemical weaponry during interspecific encounters (Pasteels et al., 1989; Marlier et al., 2004) and its wide thermal niche, allowing the species to be active most of the day (Santini et al., 2007). Finally, *C. scutellaris* may play a double role in the function of ecological communities, being able to act both as a predator and as a homopteran tender (Morris et al.,1998; Schatz and Hossaert-McKey, 2003; Ottonetti et al., 2008). One of the main factors limiting the spread of the species stems from its nesting habits. It usually nests inside tree trunks or dead logs (Bernard, 1968; Casevitz-Weulersse, 1970, 1972, 1991), whose availability may severely affect its spatial distribution. In turn, this can have severe cascade effects on the surrounding communities.

In this study, the spatial distribution of *C. scutellaris* nests was analysed in an olive orchard in central Italy (Tuscany). Olive orchards are a peculiar agro-ecosystem since most of the resources are highly packed in well defined and regularly spaced patches (trees) embedded in a poorer habitat matrix (grasses or bare ground) characterised by much harsher abiotic conditions (extreme heat, water scarcity). A similar spatial packing of both resources and shade may exacerbate competitive interactions and have strong effects on the structure of ant assemblages (Retana and Cerda, 2000; Arnan et al., 2007). In this type of ecosystem, the C. scutellaris distribution is highly dependent on the spatial arrangement of trees, which represent the only available nesting sites. Furthermore, although *C. scutellaris* is potentially able to perform long forays from the nest to feeding sites, previous investigations have shown that it relies mainly on resources found on the trees and seldom forages on the ground, at least in the studied agro-ecosystem (Santini et al., 2007; Ottonetti et al., 2008). All these features make the *Crematogaster-Olea* association an ideal model system for the analysis of spatial patterns in ecology.

2.2. MATERIAL AND METHODS

2.2.1. STUDY AREA AND SAMPLING

The study was carried out in an olive orchard near Florence (northern Tuscany, Italy – 43° 53' 8" N, 11° 9' 40" E). The plantation extended over about 40 hectares on the slopes of a hill (maximum elevation 140 m above sea level). The climate is Mediterranean, with hot summers and mild wet winters. The trees were arranged in a regular fashion and the average distance among trees was about 5-6 m. Management practices in the orchard were limited and mainly consisted in mowing (between June and July every year) and pruning (about once every 2-3 years). Neither fertilizers nor irrigation were used. No chemicals were used, except for occasional dimethoate treatments against the olive fruit fly Bactrocera oleae (Gmelin). The last dimethoate treatment was applied three years before the study. Most of the olive trees were of the same age (more than 100 years). However, several trees were damaged during a severe freeze in 1985. Damaged trees were cut back to about 20-40 cm height and new plants were established by allowing 4-6 major resprouts to grow. Hence, the orchard was a mosaic of trees with large trunks and plants consisting of a variable number of resprouts surrounding a central log. In the orchard, olive trees were the only available nesting sites for Crematogaster and no nests were found outside the trees.

In total, 531 trees in an area of about 2.5 hectares were surveyed in June 2006 and their position was recorded on a map. Trees were classified in three categories (SHAPE) based on trunk shape and circumference at breast height (CBH). The three groups were:

> a) NT, trunk absent. Trees formed by a central log (max height 40 cm from the ground) surrounded by a variable number (4-6) of major resprouts (max resprout circumference <50 cm).
> b) MT, medium trunk. Trees with a single trunk and CBH<=100 cm (minimum trunk circumference= 50 cm).
> c) GT, large trunk. Trees with a single trunk and CBH>100 cm.

Because Crematogaster scutellaris nests directly inside tree trunks, this classification is intended to reflect the suitability of a tree to host a colony. In olive trees, as size increases the trunk usually becomes progressively empty and irregular in form, providing more opportunities to establish a nest. Despite differences in trunk size due to cultural practices, the tree crowns had a fairly regular form (roughly corresponding to a cylinder) and size. Differences in crown volume for trees of different sizes were estimated from a random subsample of 90 trees. For each tree, the maximal crown diameter (cd) and crown height (ch) were measured and an approximate volume index was obtained as the volume of the cylinder: π (cd/2)2*ch.

Trees were surveyed for Crematogaster nests during the summer (June/July) and early fall (October) 2006 and 2007. Surveys were carried out on sunny days from 9.00 to 12.00 and from 17.00 to 19.00. During all the surveys, the average temperature under the tree crowns during the day was 22-30°C. The presence of Crematogaster on each tree was assessed by hammering on the trunk. A trunk was scored as hosting a nest only if the hammering elicited defensive swarming of ants from nest holes. The occasional presence of isolated ants scouting on trees was not considered. It was impossible to determine the presence of brood or queens, except on a few occasions. The space between two adjacent trees hosting colonies was carefully and repeatedly examined during the 2006 and 2007 samplings to reveal interconnecting ant trails. Nests on nearby trees, not separated by an empty tree in between, were considered to form a cluster. Cluster size was computed as the number of nests forming the cluster.

2.2.2. AGGRESSION TESTS

Confrontation tests in an arena were used to assess aggressive responses between pairs of nests. In particular we wish to test if nests found on adjacent trees (i.e. trees not separated by one or more empty tree in between) could belong to a same colony. Ants from a nest were induced to forage on a tuna bait placed in an open transparent plastic container (diameter 10 cm, height 10 cm) pinned over a tree trunk. Each container was closed after 20±2 ants were recruited on the bait and was then used for a confrontation test within the next 15 minutes. Each group of ants and container was used only once. The test consisted in joining two containers to allow the mixing of ants. The ant behaviour was followed for four minutes and the type of interaction was noted. Interactions were scored as: a) no aggression, when the transferred ants mixed with resident ants with no evident reaction or this was limited to antennation and b) aggression, when attacks involving biting, charging and repeated raising of the abdomen were observed (see Marlier et al., 2004 for a thorough description of intraspecific aggressive behaviour in Crematogaster scutellaris). It was impossible to test all the possible pairs of nets due to the high number of combinations. When clusters of at least four nests were detected, at least two tests (involving four different nests placed at the extremes of the cluster) were performed. For smaller clusters one or two nests were tested. Furthermore, at least two nests per each cluster were confronted against surrounding nests not belonging to the cluster and located within a radius of 25 meters from the centre of the cluster. Finally, ten control tests were also carried out, confronting groups of ants from a same tree, to control for any possible effect on ant behaviour due to manipulative stress. A total of 138 tests was thus carried out during summer 2007.

2.2.3. DATA ANALYSIS

The stability of ant nests between any pair of surveys was evaluated by two indexes, as used by Cerdá et al. (2002):

i) Renewal index, RN, corresponding to the percentage of new nests that appeared at time t2 with respect to time t1: RN = (N2/Total2) * 100, where N2 is the number of new nests at time t2 and not present at time t1, and Total2 is the total number of nests found at time t2;

ii) Stability index (ST), corresponding to the percentage of nests present at both t1 and t2: ST = (S/Total1) * 100, where S is the number of nests present at both t1 and t2, and Total1 is the total number of nests found at time t1.

The spatial arrangement of nests was analysed with standard methods of point-pattern analysis (Diggle, 1983; Ripley, 1988; Cressie, 1991). In particular, kernel analysis was preliminarily used to explore large-scale variations in the spatial arrangement of nests and trees in the study area and Ripley's K-functions were used to describe the small-scale spatial correlation structure. K values, which are computed from the expected number of points (trees, nests) in a circle of radius r centred at an arbitrary point were estimated for values of r in the range 5-20 metres. Because the estimation of K(r) values is affected by edge effects arising from the unobservability of points outside the study area, Ripley's isotropic correction was used to reduce estimate bias (Ripley, 1988). Furthermore, because K(r) values at a smaller scale may also be affected by large-scale clustering, the analysis was also conducted on separate subplots of the study area, as suggested by Wiegand and Moloney (2004). To stabilize the variances, K(r) functions were transformed into the corresponding L(r)-functions as $L(r) = \sqrt{K(r)/\pi} - r$, according to Besag (1977) and Ripley (1988). Statistical significance of the observed L(r) values was assessed by comparing the observed data with Monte Carlo envelopes from the analysis of multiple (1000) simulations of a null model (Bailey and Gatrell, 1995; Wiegand and Moloney, 2004). Because nests are restricted to trees and the position of trees is fixed, each simulation consisted in a random relabelling of trees (Wiegand and Moloney, 2004). L(r) values exceeding the 95% confidence envelope at the considered r distances were considered as significantly different from the null model.

To model the probability (pi) that an i-th tree hosts a *Crematogaster* nest, the vector of the occupied/unoccupied status of each tree (yi) was fitted to the following set of candidate models:

$$\log(pi/1\text{-}pi) = \alpha \qquad \text{(model\_1)}$$

$$\log(pi/1\text{-}pi) = \alpha + \beta 1\ \text{SHAPEi} \qquad \text{(model\_2)}$$

$$\log(pi/1\text{-}pi) = \alpha + \beta 2\ \text{NEIGHi} \qquad \text{(model\_3)}$$

$$\log(pi/1\text{-}pi) = \alpha + \beta 1\ \text{SHAPEi} + \beta 2\ \text{NEIGHi} \qquad \text{(model\_4)}$$

$$\log(pi/1\text{-}pi) = \alpha + \beta 1\ \text{SHAPEi} + \beta 2\ \text{NEIGHi} + \beta 3\ \text{SHAPEi*NEIGHi} \qquad \text{(model\_5)}$$

where α is the intercept term, SHAPE is trunk shape of the i-th tree (as described above) and NEIGH is an index expressing the presence of occupied nests on nearby trees. For any given tree, NEIGH was computed as the number of trees hosting a *Crematogaster* nest in an 8-m radius. Hence, the NEIGH variable corresponded to the autocovariance of an autologistic model with equal weighting for all the trees inside the 8-m radius (Wintle and Bardos, 2006). Due to the quasi-regular spatial distribution of trees, with average inter-tree distance between 5 and 6 m, this 8-m threshold value was chosen to capture the effects of neighbouring colonies on nest presence. Trees at the border of the study area, for which NEIGH could not be estimated with confidence, were not used in this analysis. Thus, the total number used for the analysis was reduced to 430. Models were fitted following a Bayesian approach based on MCMC sampling (Wintle and Bardos, 2006; McCarthy, 2007). This approach was preferred to classical logistic regression based on maximum likelihood because previous information (Pianiri, 2007) were available, suggesting guess values for the effect of tree size on nest presence (β2 coefficients, see below). These values could be used as ‘priors’to models, increasing the precision of estimates (McCarthy & Masters 2005). In general, unless explicitly stated, for all model parameters, non-informative priors were implemented using Gaussian distributions with mean =0 and precision τ = 10-6, where τ =1/σ2 (Wintle and Bardos, 2006; McCarthy, 2007). For β2 coefficients, informative priors were also used and the results were compared with those obtained using non-informative priors. In particular, priors for β2,MT and β2,GT, i.e. the coefficients expressing the effect of MT and GT-type trees with respect to NT trees, were generated from Gaussian distributions with means=1.1246 and 1.8488 and precisions τ = 3.847693 and 7.035862, respectively (see Pianiri 2007 for details).

Separate models were fitted for all the four sampling times, to avoid having complex models with repeated measures on the same units. Models_1-5 were ranked on the basis of the Deviance Information Criterion (Spiegelhalter et al. 2002). DIC is a measure of the fit of the model to the data that is penalized for the model’s complexity; it is intended as a generalisation of Akaike's Information Criterion (AIC, Burnham and Anderson 2002). Models with smaller DIC values represent more parsimonious descriptions of the data than models with larger DIC. The rules of thumb suggested by Burnham and Anderson (2002) for comparing models with AIC also apply to DIC (Spiegelhalter et al. 2002). For each model, two separate chains were run with 100000 samples. Parameter estimates and DIC values were obtained from 50000 samples, after the previous 50000 were excluded as a burn-in. The Gelman and Rubin (1992) test and partial autocorrelation plots were used to check for achieved convergence, after the first 50000 iterations. The WinBUGS 1.4.1 package (Spiegelhalter et al., 2003) was used to fit the models.

The form of the relationship between cluster size and its frequency was examined to test if this distribution behaved according to a power law: $n(s) = a+s^{-\gamma}$, with n(s) being the number of clusters of size s and a and γ are constants. Data were fitted according to Edwards (2008) and γ estimated using maximum likelihood method. The goodness of fit was estimated using the Kolmogorov-Smirnov test, according to Newman (2005).

2.3. RESULTS

More than half (302) of the 531 trees surveyed were classified as shape category NT (trunk absent), 81 were medium size (ME) and 148 had a large trunk (GT). No significant difference was observed in crown volumes among the three trunk shape categories (Type III ANOVA: F2,96 = 1.94, P>0.1).

In total, 129 *Crematogaster* nests were recognised during June 2006, while there was a significant reduction of active nests in October 2006, when the total number of occupied trees decreased to 83. During the next summer (2007) 108 active nests were found which decreased to 60 during the fall.

Trails of ants connecting different trees were observed in limited number of cases. During summer 2006 a total of 17 trails was observed. The majority of these (13) was between trees in pairs, while the other four connected multiple trees (up to four). During 2007 a total of 10 trails was observed and only in two cases more than two (four) trees were connected. Only one of the trails observed during 2006 was also observed during next year.

The stability of occupied nests also changed between sampling periods. The renewal (RN) and stability (ST) index values between the different periods are reported in Table 2.1. In general, lower values of both indexes were observed during the summer/fall transitions. Finally, only ~65% of nests remained stable from one summer to the next and only ~49% from one fall to the next.

Transition	**Stability Index (ST)**	**Renewal Index (RN)**
Summer - Fall 2006	63.57	1.20
Fall 2006 – Summer 2007	83.13	36.00
Summer – Fall 2007	50.93	8.33
Summer 2006 – 2007	65.11	22.22
Fall 2006 – 2007	49.11	31.67

Table 2.1. Values of stability and renewal indexes between the different sampling periods.

In all the sampling periods, 373 trees never hosted a *Crematogaster* nest, while 158 trees were occupied at least once. The frequency distribution of the number of times an occupied tree hosted a nest (hereafter referred to as 'occupancy') is shown in Figure 2.1. Nest occupancy was not independent of tree shape (Likelihood ratio $\chi 2$=-16.30, P<0.001). More than 60% of trees that were steadily occupied during all the sampling periods (occupancy = 4) belong to shape class GT, and only a few to class NT (small trees). On the contrary, nests occupied only once were more equally divided among the three shape classes, with a slightl prevalence of small (NT) trees.

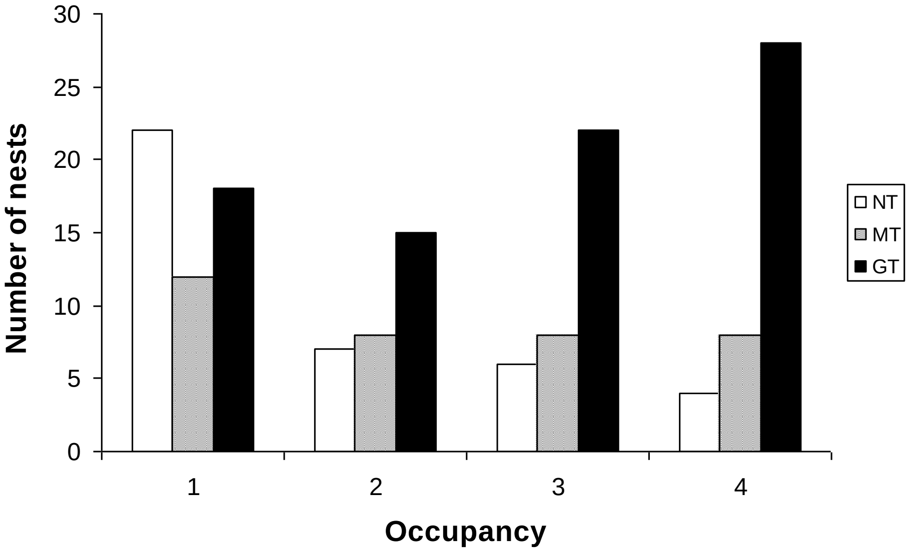

Figure 2.1. Frequency distribution of the number of times each tree hosted a nest (occupancy index), subdivided by tree shape (SHAPE). Only trees occupied at least once were considered. Trees without trunk (NT), white bars; medium trunk (MT), dashed bars; large trunk (GT), black bars.

Although the trees were evenly spaced, the nests were not. On a large spatial scale, nests were neither randomly nor uniformly scattered throughout the study area; in fact, kernel analysis showed areas with higher or lower nest density (Figure 2.2A). A similar pattern was observed in the spatial arrangement of large plus medium trees (GT and MT types, Figure 2.2B) and there was a broad correspondence between areas of high GT/MT tree density and high ant nest density. Similarly, L function values computed from nests and GT/MT trees were significantly higher than expected according to the null model for all the considered r values, indicating an aggregate pattern of both nests and large trees. However, a difference emerged when the analysis was repeated at smaller spatial scales. The analysis for both nests and GT/MT trees was repeated separately for two sub-areas (Figure 2.2C) where the tree density was higher. Estimated L (Figure 2.3A) functions for ant nests were still significantly higher than expected according to the null model for all the sampling periods, showing an aggregated pattern at all the considered r distances. The only exceptions to this pattern were the distributions of the summer and fall 2007 nests in sub-area 2, when the pattern was indistinguishable from a random one. In contrast, no significant difference from a random pattern was evident in the distribution of GT trees in both sub-areas (Figure 2.3B).

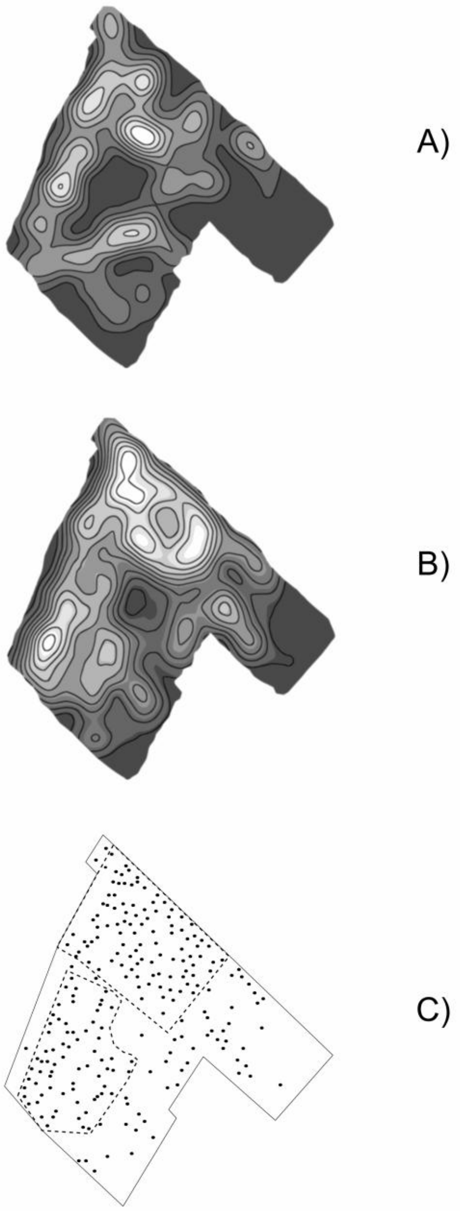

Figure 2.2. Examples of kernel maps (A-B). Crematogaster nests observed during Summer 2006 (A) and large plus medium trees (B). Lighter values indicate higher density. Maps were obtained using a bivariate kernel smoothing using a quadratic function with bandwidth=15 m. C) Locations of large and medium trees. Dashed lines show the subareas 1 (upper) and 2 (lower).

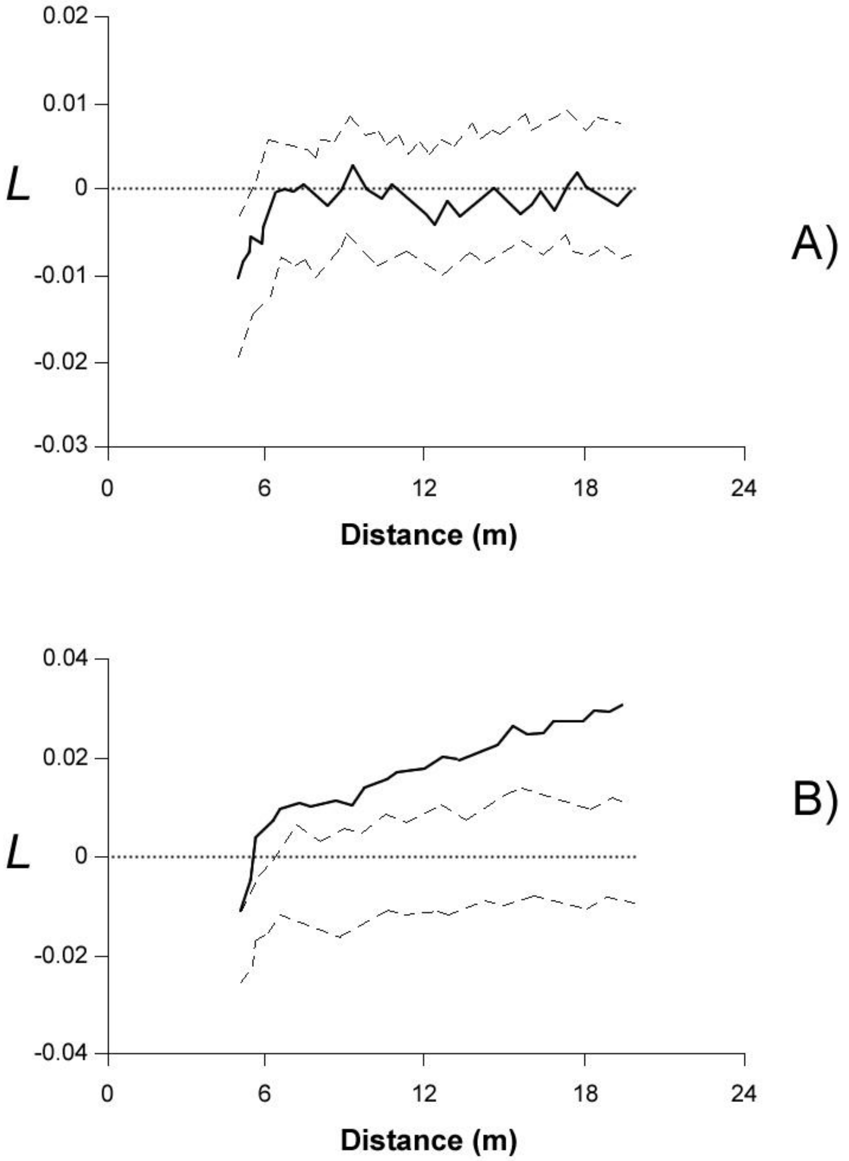

Figure 2.3. Examples of L functions for large and medium trees (A) and ants nests (B) during the summer 2006 in sub-area 1. Estimated L function, continuous line. Simulated confidence intervals, dashed lines. Expected value for random pattern, dotted line.

Model	Model formula	Priors	2006		2007	
			Summer	Fall	Summer	Fall
1	α	N	480.0	380.8	418.1	311.1
2	$\alpha + \beta_1$ $SHAPE_i$	N	407.6	317.8	369.2	290.5
2	$\alpha + \beta_1$ $SHAPE_i$	I	406.7	317.7	368.5	289.0
3	$\alpha + \beta_2$ $NEIGH_i$	N	427.3	351.4	386.5	300.9
4	$\alpha + \beta_1$ $SHAPE_i + \beta_2$ $NEIGH_i$	N	372.7	299.6	345.5	283.3
4	$\alpha + \beta_1$ $SHAPE_i + \beta_2$ $NEIGH_i$	I	**371.5**	**298.9**	**344.6**	**281.8**
5	$\alpha + \beta_1$ $SHAPE_i + \beta_2$ $NEIGH_i + \beta_3$ $SHAPE_i*NEIGH_i$	N	381.9	301.7	356.8	287.1
5	$\alpha + \beta_1$ $SHAPE_i + \beta_2$ $NEIGH_i + \beta_3$ $SHAPE_i*NEIGH_i$	I	381.2	299.5	355.3	286.3

Table 2.2. DIC values from the different models. Priors indicate if informative (I) or non informative (N) priors were used. Lowest DIC values observed during each study period are shown in bold.

Results of the MCMC analysis are reported in Table 2.2. The best model explaining the presence/absence of *C. scutellaris* during summer/fall samples was always model_4 with informative priors, which included both tree shape (SHAPE) and the neighbourhood index (NEIGH) as explanatory variables. The more complex model (model_5), which also included the SHAPE*NEIGH interaction, had much less support. Simpler models (models_1-3) showed poorer performances, the worst being model_1 (intercept only model). Models using informative priors always had slightly lower DIC than models with non-informative priors. Figure 2.4 shows the estimated values for β2 coefficients (corresponding to the SHAPE variables) with their 95% Bayesian credible intervals obtained from the best models. In most cases, parameter values are very close, with a wide overlap of Bayesian credible intervals, suggesting no difference among sampling periods. When β2 values inside each period are examined, the values of estimated coefficient β2,MT and β2,GT appear almost the same, with a lower overlap during the 2006 samples. However, none of their lower Bayesian credible intervals encompassed the 0 value, confirming the significantly higher chance of a GT or MT tree being occupied by a nest with respect to a NT tree.

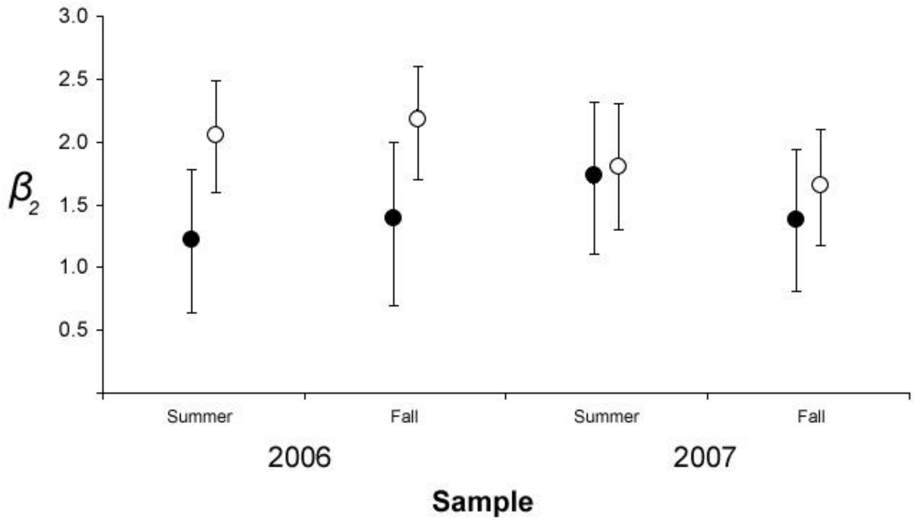

Figure 2.4. Estimated values of β2 coefficients. These coefficient express the effect of MT (solid circles) and GT-type trees (open circles) in determining the occupancy of a tree by a nest, with respect to NT trees (value 0). Vertical bars represents 95% Bayesian credible intervals.

Finally, in all four sampling times, most of the spatial clusters were formed by a few trees, with only a small number of large clusters. The relationship between cluster abundance and cluster size fell off according to a power law throughout the study period (Table 2.3). Estimated values of the γ exponent were close, ranging from a minimum of 1.728 (Summer 2006) to a maximum of 2.279 (Fall 2007).

Sample		**γ**	**K**	**n**	**P**
2006	Summer	1.728 (0.018)	0.150	32	>0.1
	Fall	1.978 (0.031)	0.086	35	>0.4
2007	Summer	1.796 (0.019)	0.213	35	>0.05
	Fall	2.279 (0.056)	0.064	35	>0.2

Table 2.3. Fitting of power law relationships γ. is the exponent of the power law (±SE) K = Kolmogorv-Smirnov test; n = sample size; P probability value.

The results of the aggression tests are summarised in Figure 2.5. No aggression was recorded in control tests (ants from the same nest). In general, the results agreed with those of spatial analysis, with a limited number of aggressions among nests belonging to the same spatial cluster, and greater aggressiveness among those belonging to different clusters. However, in some cases groups of nests identified through aggression tests did not exactly coincided with those identified only on the basis of a proximity criterion. As for example two or more spatially separated clusters of nests may merge into a single bigger group (see eg. group # 4 in Figure 2.5) or nests originally classified as belonging to the same cluster ended up into two separate groups (see eg. the border between cluser#2 and #3).

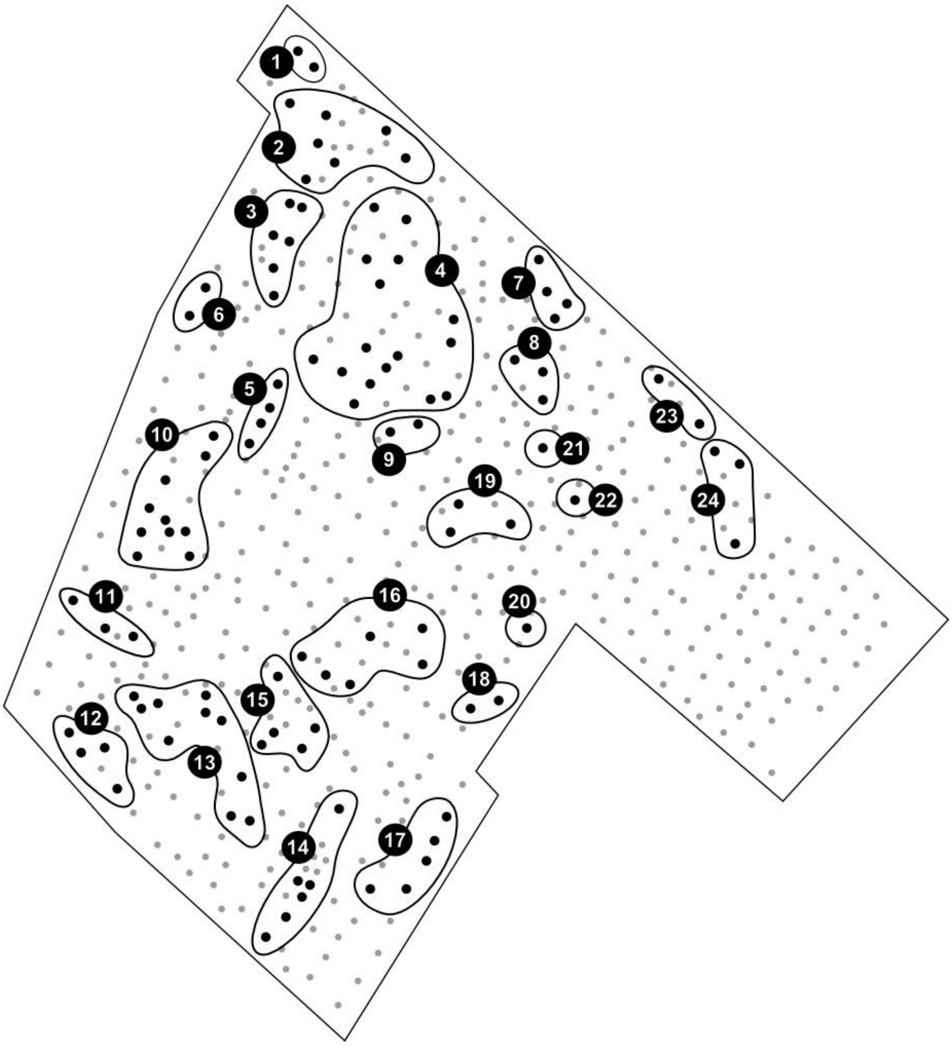

Figure 2.5. Results of aggression tests. Lines encircle groups of nests (black dots) among which no aggressive behaviour was observed. Grey dots are trees not hosting a nest.

2.4. DISCUSSION

The results of this study showed that spatial distribution of *C. scutellaris* nests followed an aggregate pattern. A primary determinant of the observed spatial arrangement of nests is to be seen in the availability of large trees, which indeed were preferred to smaller ones (Casevitz-Weulrersse, 1972, 1973). However, the spatial arrangement of nest was not fully explained by corresponding clumping of more suitable trees. On a large spatial scale, denser nest areas broadly corresponded to areas where large trees were more abundant. At a smaller scale, however, nests tended to be significantly more aggregated in space than large trees. Indeed, statistical modelling of factors affecting nest presence confirmed that both the tree's shape and the presence of neighbouring nests contributed to explain occupation of a tree. A likely candidate to explain this sort of contagious pattern could be seen in a polydomous arrangement of nests, where single nests are modules of a larger colony and are distributed so as to expand the area over which the colony collects food resources. This arrangement is in fact usually associated to an aggregated nest distribution (e.g. Traniello and Levings, 1986; Cerdá et al., 2002; Debout et al., 2007). In addition, other evidences provided by this study, further support the existence of polydomy in C.scutellaris, which indeed it has been suggested before (e.g. Schatz and Hossaert McKey, 2003). Direct evidences come in fact from the observation of continuous exchanges of ants among nearby trees via connecting trails on the ground, confirming that those nests were part of the same colony. Further support stems from aggression tests which showed that aggressiveness was reduced among nests belonging to a same cluster but generally increased among nests of spatially separated clusters. However, although aggression tests are routinely used as a primary tool to investigate colony closure (e.g. Cerdá et al., 2002; Debout et al., 2003), caution is required in interpreting this data because aggression levels among workers from different nests may vary in space and time, irrespective of their relatedness (Debout et al., 2007 and references therein). In particular, the “dear-enemy” phenomenon, which leads to reduced aggression toward closer albeit unrelated colonies (e.g. Heinze et al., 1996; Langen et al., 2000), may grossly bias the estimated degree of relatedness.

A second important feature is represented by the dynamic nature of nests, characterised by a pronounced temporal variability. In general a within-year fluctuation in the number and location of active nests was observed, with an increase during the favourable season (spring-summer) and a decrease during the fall prior of the winter pause. Further changes were oserved when comparing nests arrangement from one year to the next. Although the death of existing colonies and the founding of new ones are possible events, their incidence is probably low on the temporal scale observed here. The dynamic behaviour of the system, coupled with the clustered distribution of nests, suggests that some trees in a cluster may be opportunistically occupied or abandoned, e.g. in response to the presence or absence of suitable resources, despite the fact that excavating a nest is a costly and time-demanding activity, particularly in a tree trunk (Holldobler & Wilson, 1990; Mikheyev and Tschinkel, 2004). Furthermore, the observation that large trees were more steadily occupied than smaller ones suggests that the former may host the "core" of a colony and the latter the "satellites", for which the trunk shape and size are less important. This is supported by the observation that, in two different cases during summer 2006, ants were observed moving between a large tree and a smaller one, where workers actively tended a conspicuous homopteran (*Saissetia oleae*) infestation, whereas during the 2007 sampling the Saissetia clumps had disappeared and no presence of ants on the smaller trees was observed. This fact is probably exacerbated by the physical characteristics of this ecosystem, where most of the resources are packed over the trees and scarce over the ground, especially from June onward. As such, although being able to perform long foraging forays, in this system *C. scutellaris* relies mainly on resources found on the trees and seldom forages on the ground (Santini et al., 2007; Ottonetti et al., 2008).

The frequency distribution of cluster size decreased according to a power law. This results is qualitatively similar to the finding by Vandermeer et al. (2008) for the tropical ant Azteca instabilis, and suggests that self-organizing patchiness may be involved in the *Crematogaster* system studied here stimulating questions on how much such effects are widespread among ants. Caveats are needed, however, since the mechanism underlying this pattern in our study is not yet clear. Density-dependent control by natural enemies, which seems to be the main limiting agent to cluster expansion in the *Atzeca* system described above, may occurs, although it is probably less relevant than in Vandermeer et al. (2008) study. No parasite attack has been observed in several years of research on this species and, to our knowledge, no phorid fly is known to parasitize *C. scutellaris*. Attacks by avian predators, such as woodpeckers, are more likely to occur. Both the green woodpecker (*Picus viridis*) and the wryneck (*Jynx torquilla*) which are known to feed upon ants (e.g. Rolstad et al. 2000; Mermod et al. 2009) are common in the study area and signs of attack of the former were observed on 17 of the studied trees (three of which still hosted a nest during the study period). No detailed data are however available to quantify the actual impact of this predator on the viability of ant colonies. Another potentially important limiting factor is yet to be seen in the strong seasonality of the environment, which mediate water and resource availability to ants and it has been proven to interfere with spatial self-organization in semi-arid ecosystems (Guttal et al. 2007). Due to the important ecological implications of these phenomena, which extend well beyond the simple description of an aggregated pattern (Sole et al., 1999; Pascual et al., 2002; Pascual and Guichard, 2005; Sole and Bascompte, 2006) this point requires further investigation and will be a major goal of future research.

Finally, whichever the mechanism leading to the spatial arrangement of nests, the observed spatial pattern may have strong influences on the whole ant assemblage and on biotic interactions at the landscape level. Since the presence of *C. scutellaris* may affect the performance and behaviour of more submissive species (Cammell et al., 1996; Way et al., 1997; Schatz and Hossaert-McKey, 2003; Santini et al., 2007), spatial clumping of nests may lead to the formation of a mosaic, with patches dominated by *C. scutellaris* alternating with patches where competition is relaxed and other species may even predominate (e.g. Sanders et al., 2007 and references therein). Furthermore, trees hosting a *C.scutellaris* nest are known to suffer greater predation rates than other trees (Ottonetti 2006) and hence the spatial arrangement of other arthropods is affected as well. A reverse effect is on the contrary expected on homopterans. A survey of trees infestation by the two major homopteran parasites of olive trees, the scales *Saissetia oleae* and *Lichtensia viburni*, in the study area (Ottonetti, 2006) showed a clear association between these and ants. About 90% of infested trees, in fact, also hosted a conspicuous ant nest and 80% of these belonged to *C. scutellaris*.

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3. FEEDING PREFERENCES OF *CREMATOGASTER SCUTELLARIS* (HYMENOPTERA: FORMICIDAE) IN THE FIELD AND THEIR RELATIONSHIP WITH ANT-HOMOPTERAN INTERACTIONS

3.1. INTRODUCTION

All organisms need nutrients such as carbohydrates, proteins or fats for their survival and reproduction, and the availability of these resources strongly affects all the aspects of their ecology and behaviour. To maximize their fitness foragers primarily select resources on the basis of their energy content and abundance (Stephens and Krebs 1986). In many instances, however, foragers have to solve more complex tasks than merely maximize their energy intake. This is the case, as for example, when there is a trade-off between energy gain and the simultaneous intake (or avoidance) of specific nutrients/elements or when foraging activity has to be weighed against the need of avoiding predators, competitors or other stress factors (Clark & Mangel, 2000; Houston & McNamara, 1999).

Many common ants have generalist feeding habits and use multiple sources of protein and carbohydrates at the same time (Hölldobler and Wilson, 1990). While predation and scavenging account for most of their protein intake, an important source of carbohydrates is represented by secretions of plant parasites, such as homopterans (e.g. aphids and scales). These N-poor secretions are rich in carbohydrates (which amount up to 98% of their dry weight) and are composed by a mixture of mono-, di- and tri-saccharides (Mittler 1958; Maurizio 1985; Douglas 1993; van Helden et al. 1994). Further carbohydrate supply may be obtained directly from plants, in the form of floral and extrafloral nectaries (Pascal & Belindepoux, 1991; Wetterer et al., 2001; Goitia & Jaffe, 2009).

The relative value of each resource to ants may vary in time, depending on several intrinsic and extrinsic factors. First, colony needs are known to differ according to development stage, with proteins being usually more requested by larvae and carbohydrates by active workers (e.g. Judd 2005). Furthermore, temporal changes in the availability of resources (e.g. due to preys phenology, homopteran life cycle or plants development stage) may further complicate this picture, making specific resources more or less available, and hence more or less valuable, at different times of the year (Kay 2004). In arid and semi-arid habitats, where water shortage is an important stress factor (Edney 1977), water is to be considered as a resource on its own. In these environments, the decision to take a given food sources has therefore to be traded-off also according to its contribution to water balance.

As changing availability of resources affect ants behaviour, but in turn ants choices may feedback on other species with which they interact (preys, mutualists, plants), generating complex dynamics. Particularly interesting from both an ecological and an evolutionary point of view, is the mutualistic interaction with homopterans (and other insects as well), which provide ants with energy-rich exudates and are in turn are actively protected from their predators and parasitoids (e.g. El-Ziady & Kennedy 1956, Banks 1962, Way 1963, Banks & Macaulay 1967, Addicott 1979, Pierce & Mead 1981, Buckley 1987a, b, Völkl 1992). It is known that this interaction depends on the availability of alternative energy sources, hence the name 'conditional mutualism' used for this kind of interaction (Cushmann and Addicott, 1991; Bronstein, 1994). Eventually, when proteins are scarce and alternative carbohydrates sources available, this interaction may switch to predation (Pontin, 1958; Edinger, 1985; Cherix, 1987; Rosengren & Sundström, 1991; Sakata, 1994, 1996; Offenberg, 2001).

The ant *Crematogaster scutellaris* is a common species in both natural and man-managed ecosystems throughout the Mediterranean basin, where it may form large colonies, amounting up to several thousand specimens (Bernard 1968; Casevitz-Weulersse 1972). Nests are usually excavated directly into tree trunks and dead logs. Previous studies showed that this species is an highly ranked competitor in Mediterranean ant assemblages (Cammell et al. 1996; Way et al. 1997; Santini et al. 2007) and have generalist feeding habits since both predatory and tending behaviour have been described under different ecological scenarios (Morris et al. 2002; Villagràn et al. 1992; Gonzalez & Campos 1990; Morris et al. 1998; Schatz & Hossaert-McKey, 2003; Ottonetti et al. 2008).

In this study, the preferences of *C. scutellaris* for carbohydrates and amino acids were investigated in the field in a typical Mediterranean agroecosystem, the olive orchard.

Preferences for the two types of resources, in a liquid form, were assessed from observation of ants behaviour towards solutions with increasing concentration of carbohydrates and amino acids. These was analysed both at individual (single ant responses) and colony level (recruitment over the resources). Responses were assessed during two different seasons (spring vs summer), corresponding to different stages of colony life cycle (early onset after hibernation and maximal colony activity, respectively) and availability of water and other resources (preys, homopterans). To investigate the effect of local resource and water availability on preferences, the behaviour of ants from colonies living on trees where leaf biomass, and hence resources, were reduced by pruning was compared to that of colonies from intact trees. Furthermore, the behaviour of ants from colonies where resource and water availability was experimentally manipulated through supplementation, was investigated. Finally, the effect of resources and water availability on the tending behaviour of *Crematogaster scutellaris* towards the scale *Saissetia oleae*, was investigated, under the expectation that tending behaviour should decrease when ants were supplemented with carbohydrates but not amino acids or water.

3.2. MATERIALS AND METHODS

3.2.1. STUDY AREA AND SPECIES

The study was carried out in an olive orchard near Florence (northern Tuscany, Italy – 43° 53' 8" N, 11° 9' 40" E). The plantation extended over about 40 hectares on the slopes of a hill (maximum elevation 140 meters above sea level). The climate is Mediterranean, with hot, dry summers and mild wet winters. The trees were arranged in a regular fashion and the average distance among trees was about 5-6 meters. Neither fertilizers nor irrigation were used and management practices consisted only in mowing (between June and July every year) and pruning. Only a fraction of trees are pruned every year and each tree is pruned about once every 5 years. Pruning is however severe, and reduce much (more than 80%) of leaf volume. This reduce the amount of resources available to *C. scutellaris* whose foraging activity is mainly centered on the trees, particularly during the summer months (Santini et al. 2007; Ottonetti et al. 2008) and exposes the nests to direct sunlight, increasing heat and desiccation stress. Temperature recordings of the trunk surface of pruned and non pruned trees (taken at midday using a Fluke 52 II temperature meter) showed that surface trunk temperature of the former were up to 10 °C greater than the latter. More details on the study area can be found in Santini et al. (2007) and Ottonetti et al. (2008). In the orchard, olive trees were the only available nesting sites for *Crematogaster* and no nests were found outside the trees.

In the study area, this species is usually active from April to October. Activity peaks during June/July and sexuates appear during late-August/September (personal observation). Resource availability also changes through time. During the spring, there is abundance of both water and insect preys, while during the summer water and preys are scarcer (personal observation). This effect is exacerbated by the concurrent action of mowing (July) and desiccation due to high temperatures during the summer. However, during mid-summer there is developmental peak of the scales *Saissetia oleae* and *Lichtensia viburni* (Bagnoli 2003), which represent the principal source of carbohydrates to *C. scutellaris* in this system. As a consequence preferences for different type of resources are expected to change through time and availability of resources to a given colony.

3.2.2. PREFERENCE EXPERIMENTS

Ants choice of different nutrients were tested using know-concentration solutions of sucrose and aminoacids. Preferences were assessed both as individual responses and as the degree of recruitment by a colony on a given source, as detailed below. Experiments were carried out during the spring (April) when there is plenty of water and prey and during mid-summer (July-August) when, on the contrary, water and preys are limiting. Furthermore, to investigate the effect of local water and resource availability to different colonies during the summer, responses of ants from pruned and non-pruned trees were compared. All the trees were searched for the presence of homopteran parasites. The majority of intact trees hosted variable numbers of scales but those with clear and extensive infestations were discarded and used only for the 'homopterans experiments' (see below).

AA solutions were made from a Sigma-Aldrich. Dry powder was homogenised for 10 minutes and known amounts of powder diluted in distilled water. The following concentrations (% wt/vol) were used for sucrose: 0, 1, 2, 4 and 16%. The same concentrations were also used for aminoacids, with the exception of the highest (16%), as amino acids amounts greater than 0.4 g/10 ml did not dissolved completely. Solutions were stored at 4 °C and used within a maximum of two days after preparation. After this time new fresh solutions were prepared.

3.2.2.1. INDIVIDUAL PREFERENCES

To assess individual preferences, 63 colonies were selected from the study area during the spring and a further 126 (63 from pruned and 63 from non pruned areas) during the summer. Intact trees were selected from areas where pruning had occurred at least three years before. Each colony was randomly assigned a test solution and used only once. A total of 7 colonies per solution were thus used. Each test consisted in leaving a drop of solution in front of a walking ant on the trunk of a tree, following Kay (2002). A forager which touched a drop with the mandibles was scored as accepting the solution if contact lasted for more than 10 seconds, or rejecting if she moved away. Furthermore, acceptance of a solution was easily recognizable because ants drinking from a drop began to swell the gaster after a few seconds. Ants that never touched a drop were not considered. Remaining of drops were removed from the tree after finishing a test and care was taken to use an ant only once. Ten replicate ants were tested from each colony.

3.2.2.2. RECRUITMENT

As above, 63 trees, each hosting a *Crematogaster* nest, were selected during the spring and 126 during the summer (63 on pruned and 63 on non-pruned trees). A plastic container (diameter 4.5 cm, height 2 cm) was pinned to the trunk of each tree, at approx 30 cm from the main nest entrance and filled with 1.5 ml of one of the above solutions (7 replicate colonies/solution). Care was taken to ensure that at least one ant discovered the cup. Experiments started in the morning by 9.30 am. Trees were regularly surveyed at 30 min intervals and the number of ants feeding in the container counted, for up to 3 hours. In three cases the container were monopolised by *Pheidole pallidula*. These data were discarded from the analysis to avoid confounding due to competitive interactions, and the treatment repeated on different trees. Because several tens of ants may recruit on a cup and their number is impossible to be assessed, cups with more than 15 *Crematogaster* in, were arbitrarily scored as 'recruited'. However, using different threshold in the range 10-40 did not affects the results.

3.2.2.3. SUPPLEMENTATION EXPERIMENTS

To assess the influence of previous resource availability on food choice, 20 trees hosting a *Crematogaster* colony, were selected from the study area during the summer. Each tree was randomly assigned to one of the following group: a) controls, b) water supplemented, c) sucrose supplemented and d) amino acids supplemented. Each group was thus composed by 5 different trees. Three plastic container (diameter 4.5 cm, height 2 cm) were pinned to the trunk of each tree at about breast height. The cups were then filled with the corresponding solution. For both sucrose and aminoacids, suprasature solutions were used. Cups on the control trees were left empty. The solutions were left on the trees for three consecutive days and continuously refilled, when necessary, to compensate for evaporation or consumption by ants. After three days individual preferences were assessed as described above, using a lower (0.031%) and an higher (4%) amino acidic and sucrose solutions. On all the trees, 10 ants for each solution were tested, taking care to use each ant only once. Order of subministration of tests solutions was randomised.

3.2.2.4. HOMOPTERANS EXPERIMENTS

To asses the effect of varying resources availability on the degree of homopteran tending by ants the following experiment was carried out during the summer, when *Saissetia oleae* is at its maximum development (Bagnoli 2003). Twenty *Crematogaster colonies*, each tending a large *Saissetia oleae* clump (>100 specimens), were selected in the study area. Three plastic container (diameter 4.5 cm, height 2 cm) were pinned to the trunk of each tree at about breast height. The cups were then filled with water (n=5), a suprasature sucrose solution (n=5), a suprasature aminoacidic solution (n=5), or left empty as controls. The solutions were left on the trees for three consecutive days and continuously refilled, when necessary, to compensate for evaporation or consumption by ants. Attendance of homopterans was assessed daily from 9 to 12 am. Observations were carried out at the beginning of the experiment (t_0, immediately before filling the cups) and then for three consecutive days (t_1-t_3). To obtain an index of the number of ants tending the homopterans all the ants moving across a marked section of the proximal part of branch hosting the clump were counted. Each day five replicate 3-minutes counts were carried out, at 30 minute intervals. Daily counts for each tree were pooled and an index of change with respect to the pre-supplementation counts computed as:

$$AR = \ln\left(\frac{N_t}{N_0}\right)$$

where Nt and N0 were the total number of specimens moving to homopterans, recorded during day t and day 0, respectively.

3.2.3. DATA ANALYSIS

Results of individual and collective (recruitment) choices responses were analysed using Generalized Linear Models (GLMs). For individual choice experiments the percentage of ants of a colony accepting a solution was used as dependent variable after arcsin transformation (Sokal & Rohlf, 1995) using a Gaussian link. For the recruitment experiments a binomial link was employed, using as dependent variable the recruited/empty status of the solution. Two explanatory variables were used: a) Concentration of solutions (CONC, log2-transformed), available for both spring and summer tests and b) pruned/non-pruned status of the tree hosting the colony (PRUN), available only for summer tests. Five different models of increasing complexity could be formulated. These models were: 1) null model (intercept only); 2) a model with only the CONC variable; 3) a model with only the PRUN variable, 4) a model with both CONC and PRUN; 5) a model with both CONC, PRUN and their interaction. According to our experimental design (see above) only models 1 and 2 were possible for the spring samples, whereas for the summer samples all the models could be used. Models were fitted using maximum likelihood methods (McCullagh & Nelder 1989) and ranked on the basis of their AICc (Akaike's Information Criterion corrected for small sample size) values. The ‘best’ model was chosen as the one having the lowest AICc value (Burnham & Anderson 2002). In short, a difference in AICc values of two models (ΔAICc) less than 2 indicates that the two models are indistinguishable, while ΔAICc =4–7 indicates that the poorer model has considerably less support than the better one and ΔAICc>10 shows that the poorer model has essentially no support (Burnham & Anderson 2002).

Results of supplementation experiments, were analysed using Multivariate Analysis of Variance (MANOVA). Prior of analysis, the data were arcsin transformed and normality, homogeneity of variances and presence of outliers for each response variable (test solution) were checked using exploratory data analysis techniques, as recommended by Quinn & Keough (2002). The Pillai's trace was used as a test statistic, since this statistic is know to be the most robust to deviations from MANOVA assumptions (Johnson and Field, 1993). Differences among treatments were assessed using multivariate contrasts based on the Roy intersection method (Morrison, 1976). Finally, to assess univariate differences among controls and supplemented colonies Dunnett test was also applied for each test solution (Zar, 1999). Results of homopterans experiments were analysed using ANOVA and differences with controls assessed with the Dunnett test.

3.3. RESULTS

3.3.1. INDIVIDUAL PREFERENCES

The results of preference experiments, with models ranked on the basis of AICc values for both sucrose and amino acid solutions are shown in Table 3.1. As for sucrose, during the spring tests, the degree of acceptance increased with increasing concentration (Figure 3.1A) and reached a maximum for the richer solution, which was accepted on average by about 8 out of 10 ants in each tested colony. The model including the concentration of sucrose as explanatory variable had an AICc value considerably lower than the null model (ΔAICc >10) and the coefficient of the CONC variable was highly significant (t_{33}= 4.3, P<0.001). There was however considerable variability in individual responses and the model explained about 40% of total variance.

Model	Model Formula	Individual				Recruitment			
		Sucrose		Amino acids		Sucrose		Amino acids	
		Sp	**Su**	**Sp**	**Su**	**Sp**	**Su**	**Sp**	**Su**
1	Null model	21.9	**34.4**	20.8	45.6	39.8	**93.3**	NA	79.1
2	CONC	**8.8**	35.2	**17.3**	34.9	**29.5**	94.3	NA	81.1
3	PRUN		36.4		42.9		94.9		**72.2**
4	CONC + PRUN		37.3		31.1		95.9		74.3
5	CONC + PRUN + CONC*PRUN		38.2		**24.2**		96.1		76.6

Table 3.1. Results of preference experiments for both individual and recruitment tests. The AICc values of all the models are shown. Lower AICc values for each set of observations are in bold. Sp = spring, Su = summer. NA = not available.

On the contrary, during the summer no clear effect of sucrose concentration nor pruning was observed (Figure 3.1A) and the null model had the lowest AICc value. The second model, in order of AICc, was model 2, which also included the effect of concentration. The difference in AICc with respect to the best model (ΔAICc) was very low (< 1) substantially indicating no difference among the two. In this case the coefficient of the CONC variable was not significant (t_{68}= -1.16, P>0.2). All the other models were comprised in range of ΔAICc lower than 4. This result reflects an high level of acceptance for any solution, without any difference clearly attributable to concentration or pruning.

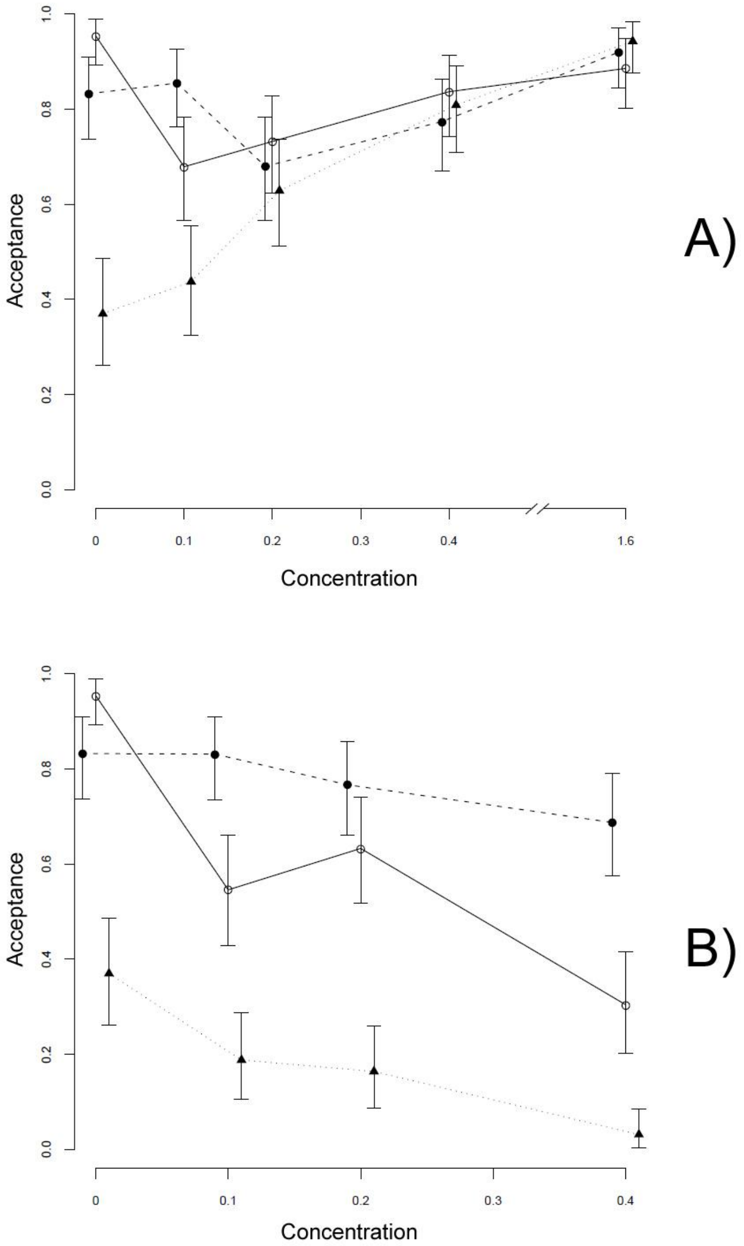

Figure 3.1. Results of preference experiments for individual tests on carbohydrates (A) and aminoacids (B). Spring =solid triangles, summer pruned = open circles, summer intact = solid circles.

A different pattern emerged when considering responses to amino acids (Figure 3.1B). During the spring, amino acid acceptance was very low for all the test solutions (on average 2-4 ants for each tested colony). The model including the CONC variable performed slightly better than the null model, However, although the coefficient of the CONC variable was significant (t_{26}= -2.136, $P<0.05$), with a negative sign, the percentage of total variance explained was very low (0.07%). During the summer, acceptance of amino acids was higher and the response was different for ants from pruned and non pruned trees. The model with the lowest AICc value was in fact model 5, which included the explanatory variables CONC, PRUN and their interaction, CONC * PRUN. The coefficient for the interaction term was significantly different from zero (t_{52}=-3.136, $P<0.01$), supporting the idea that ants responded differently to amino acid concentrations on pruned and non pruned trees (Figure 3.1B). In particular, while on non pruned trees, the degree of acceptance did not significantly changed with increasing concentration, on pruned ones there was an higher acceptance of water, followed by a lower acceptance for all the amino acids solutions.

3.3.2. RECRUITMENT TESTS

All the test solutions were discovered by ants and recruitment, when present, occurred quickly during the first hour after discovery. Results of recruitment experiments broadly paralleled those of individual responses (Table 3.1), although in general lower values were observed. During the spring, recruitment on sucrose was still concentration-dependent (Table 3.1): it occurred only on the two richer solutions (on 4 out of 7 trees, for both) and no event was recorded on lower concentrations and water. Model 2, greatly outperformed the null model (ΔAICc ~ 10). On the contrary, during the summer, all the solutions equally triggered recruitment (5 out of 7 samples), irrespective of their concentrations, and no discernible effect of pruning was detected. In fact, the lowest AICc value was obtained from the null model and ΔAICc with respect to other models were always lower than 3.

During the spring no recruitment was ever observed on amino acid solutions. During the summer, the model with the lowest AICc was the one including only the variable PRUN. Similarly to what was observed in individual responses, less recruitment occurred on pruned than on intact trees, where recruitment was observed in average on 5 out of 7 and in 3 out of 7 trees, respectively.

3.3.3. SUPPLEMENTATION

Results of supplementation experiments are shown in Table 3.2 and Figure 3.2. Overall, MANOVA was highly significant, indicating that supplementation of various resources affected the choice of ants. Multivariate linear contrast showed that amino acids supplementation was the least effective in altering feeding preferences and no significant difference from controls was observed. On the contrary, a significant differences with respect to controls were evident after both water and sucrose supplementation. In particular, the acceptance of the least concentrated solutions was influenced by both water and sucrose supplementation (Figure 3.2). A similar response was observed for the higher aminoacid solution. On the contrary, the uptake of the highest sucrose solution was only affected by previous supplementation of sugars.

Source	**Pillai Trace**	**df**	**F**	***P***
MANOVA	1.4357	12,45	3.44	<0.01
A vs C	0.2792	4,13	1.26	n.s.
S vs C	0.8068	4,13	13.57	<0.001
W vs C	0.6924	4,13	7.32	<0.01

Table 3.2. Results of MANOVA and contrast analysis. C = controls, W = water, S = sucrose, A = amino acid supplementation. df = degrees of freedom (numerator, denominator).

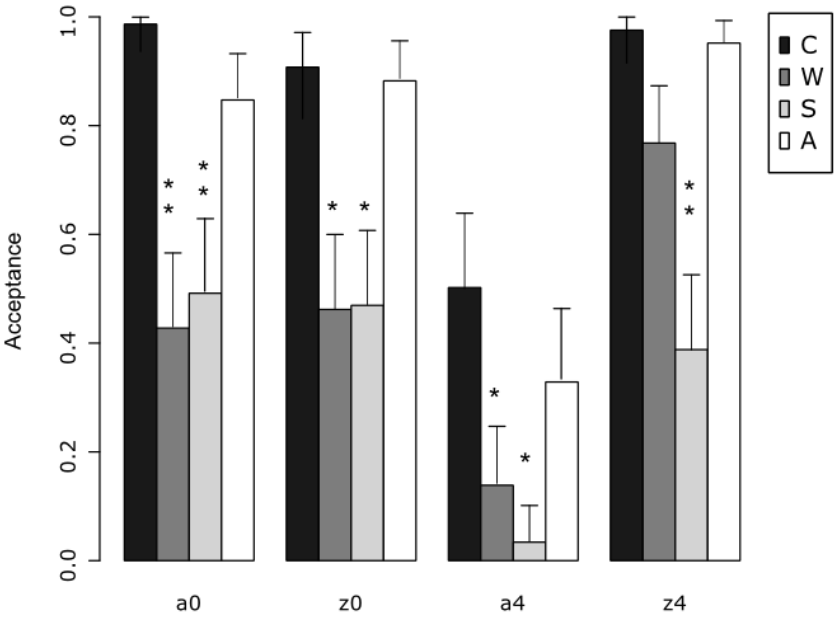

Figure 3.2. Results of supplementation experiments. C = controls, W = water, S = sucrose, A = amino acid supplementation. a0 and z0 are the lower test solutions used (0.031%) for amino acids and sucrose, respectively; a4 and z4 are the higher test solutions (4%).

3.3.4. HOMOPTERAN TENDING

Overall, supplementing the colonies had a significant effect on the degree of ant attendance to homopterans (F3,16=13.24, P<0.0001). Excess carbohydrate availability had the greatest effect (Figure 3.3) and after 3 days, the AR of experimental colonies were significantly lower than that of controls (Dunnett test P<0.01). Decrease of attendance was evident after only one day since the beginning of the experiment (Figure 3.4), and difference with controls increased in the successive days. A significant, although much less pronounced effect was also observed for water supplemented colonies (Dunnett test P<0.05), while, on the contrary, no effect was detectable for amino acids supplemented colonies (Dunnett test P>0.1).

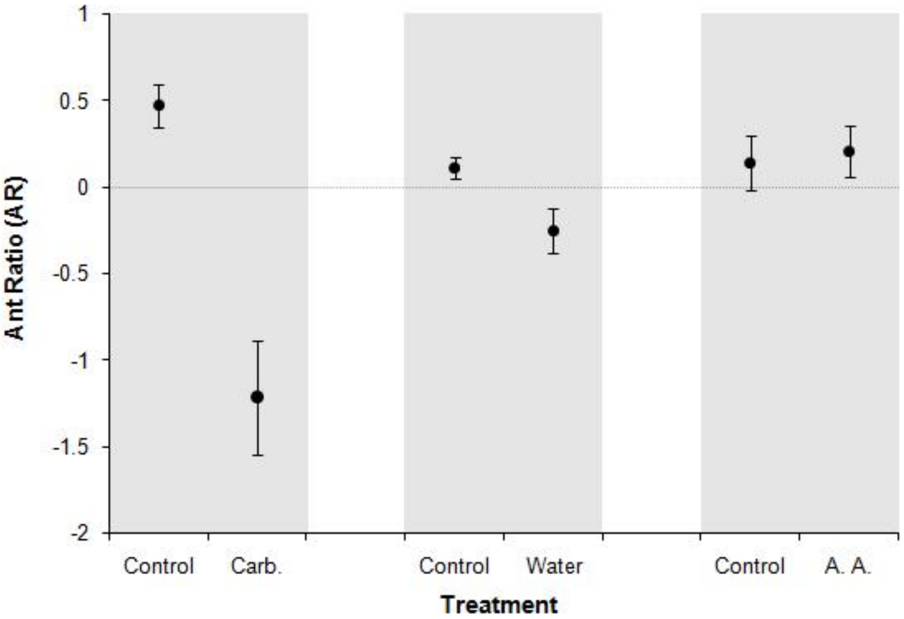

Figure 3.3. Results of experiments on homopteran tending after supplementation of carbohydrates, water and amino acids.

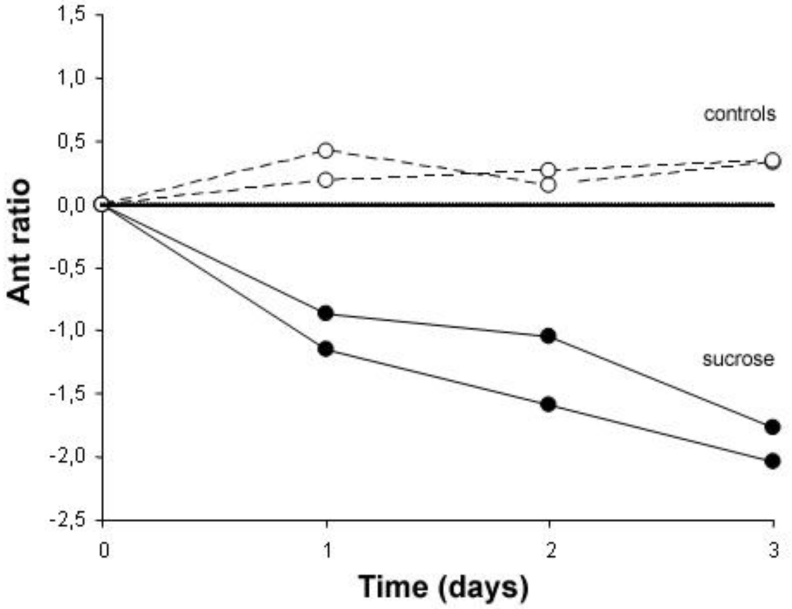

Figure 3.4. Examples of change in ant attendance on sucrose supplemented and control trees.

3.4. DISCUSSION

Crematogaster scutellaris is a generalist ant, which feed opportunistically on multiple sources of energy (Morris et al. 2002; Ottonetti et al. 2008). The results of these experiments confirmed the generalist feeding habits of this species but highlighted differences in the use of aminoacidic and carbohydrate foods. Furthermore a seasonal variation in the use of resources was observed (Hooper & Rust, 1997; Abril et al., 2007).

During the spring, the acceptance of carbohydrate solutions by individual ants increased with increasing concentration of the solutions, similarly to what observed e.g. by Kay (2002) in *Dorymyrmex smithi*. However, a different pattern was clearly observed during the summer when on the contrary, all the solutions were equally taken, irrespective of their sugar content. Interestingly also water was taken at a same rate, suggesting that water scarcity experienced by ants during the summer, makes less concentrated solutions and water itself valuable resources. Interestingly, on pruned trees, where resource/water availability was lower and heat/desiccation stress higher, the uptake of carbohydrate solutions is not different from that of intact trees, whereas water uptake was greater. Uptake of less concentrated solutions (but not water) could in principle be also due to a lower availability of carbohydrates during the summer, which seems to be unlikely given that during the summer there is a peak in the availability of homopteran parasites, which provide the principal source of carbohydrates to *C. scutellaris* in the study area (Bagnoli, 2003, Ottonetti et al., 2008). The acceptance level of higher sucrose concentrations was not affected neither by season nor by local (pruned vs non-pruned trees) and was still high (accepted by about 8 ants out of 10 tested) suggesting that carbohydrate were always valuable to *C. scutellaris*, even during the summer months when colonies have access to a rich carbohydrate supply from homopterans.

A different trend was clearly evident when considering amino acids. During the spring, aminoacid acceptance by individual ants was always very low with a weak negative concentration effect. During the summer uptake was much higher than during the spring and a negatively related to concentration. However, the uptake differed considerably between intact and pruned trees. In the latter, in fact, water uptake was greater than in the former, while aminoacid uptake was always lower.

Several causes are likely to contribute to this spring-summer difference in aminoacid acceptance. First, during the summer lower insect preys are available due to insect phenology in olive groves (e.g. see Morris et al. 1999 and personal observation), making thus aminoacid richer solutions more valuable. Secondly, July corresponds to maximal colony growth and activity (Tucci 2006) and thus more larvae, which require proteins for their growth (Cornelius & Grace, 1997; Judd, 2005), are presents in the nests.

That water uptake on pruned trees was slightly higher than on intact ones, confirmed our expectations about the heat stress and desiccation. Less clear is however the observed lower acceptance of amino acids on on pruned than on intact trees. Reduction of crown biomass and hence of global resources available to ants may have delayed colony development. On some of the pruned trees, part of the nest and the colony were removed when some major branches (diameter >40 cm) were cut (exposed galleries were well evident on the remaining cross sections) and greater number of ants were actively engaged in excavating new galleries than in foraging. Reduction in brood presence may have therefore lowered the protein requirements of the colonies. A second factor could be seen in a change of foraging behaviour. It is known that in tree-dwelling ants, ground foraging can increase if the resources present on the trees decrease (Levings, 1983; Byrne, 1994). Yet, the reduction of the number of branches and stems caused by pruning affect forced the ants to forage over the the ground, which is likely to increase their predatory behaviour and hence the intake of proteinaceous sources.

The results of recruitment tests broadly paralleled those of individual choices. The main difference was in the degree of the behaviour observed. As for example whereas for individual choice of carbohydrates during the spring the acceptance steadily increased with the concentration of the solutions recruitment was evident only on the richer solutions. This difference with individual responses seems to be attributable to actual differences between colony-level and individual-level behaviours (Beugnon et al., 2001; Edwards & Pratt, 2009).

Supplementation experiments showed that amino acid availability had no influence on feeding behaviour and no difference with controls was observed. On the contrary, greater influences were observed after both water and sucrose supplementation, since both determined a decrease in the uptake of other solutions. Although an effect of abundant sucrose supplementation was expected (see e.g. Kay 2002) the effect of water was rather new. That water supplementation causes a generalised reduction of the uptake of other solutions (less pronounced for the highest sucrose concentration) confirms the role of water in affecting foraging decision of ants in this ecosystem. Further, this lead support to the observation that the difference in the spring-summer sucrose uptake is due to water lack but also suggests that this may contribute to explain part of the increase in amino acid uptake observed during the summer.

The interaction between *C. scutellaris* and its main homopteran counterpart, the scale *Saissetia oleae*, was also affected by previous availability of different resources. Again, amino acids availability did not caused any discernible effect with respect to controls, at least on the time scale examined. As expected the clearest effect was caused by sucrose supplementation which caused a constant decline in homopteran tending. This confirm that the relationship with homopterans is subject to several trade-offs and its value is continuously assessed by ants (Offemberg 2001). No direct attempt of ants to predate homopterans after bein supplemented with carbohydrates was observed, although no specific observation on this aspect was planned. The more unexpected result was the slight but significant decrease in homopteran tending was observed after the supplementation of water. Overall, these findings agree with the results from the supplementation experiments, where an increase of water availability caused a decrease in the acceptance of all the test solution including carbohydrates, although less pronounced than that caused by sucrose. This result led support to the idea that in Mediterranean agroecosystem, and more generally in arid ecosystems, water is a valuable resource by itself, which fully take part in trade-offs with other resources. Although there were other studies which reported an ant-attending decreasing after a large carbohydrates supplying (e.g. Offenberg, 2001), to our knowledge, no similar results have been obtained for water. The outcome that *C. scutellaris* uses the scales also for the water contents of their honeydew, is a new item that has to be confirmed by future studies.

Before concluding, a few caveat are needed here concerning the methodology employed. First, following previous investigations (Davidson 2004; Kay 2002, 2004), resources were always provided in a liquid form. Use of liquid sources allows a more direct control over the amount of resource tested than solid food. However this may introduce a bias. If from one side carbohydrates are often taken by ants in liquid form both as insect exudates or plant secretions, protein come more often as solid food from predation. The effect of this bias has not been explicitly tested, although observations shows that in the study sites ants readily recruit on solid (tuna) baits.

A second potential limitation is related to the concentration of solutions. In the present study no amino acid concentration greater than 4% was possible to be obtained (see also Davidson 2005), whereas, as for example, Kay (2002) obtained richer concentrations using a single protein (casein) solution. This results therefore apply to these particular concentrations and cannot be extended simply to higher concentrations of other proteins.

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4. INTRASPECIFIC AGGRESSION RELATED TO SPATIAL DISTANCE IN *CREMATOGASTER SCUTELLARIS* (HYMENOPTERA: FORMICIDAE)

4.1 INTRODUCTION

Nestmate recognition is one of the most important feature affecting ecology of many organisms, from bacteria to eucariotes with complex social structures. In ants, the ability to properly recognize conspecific individuals is fundamental to preserve colony integrity, which heavily depends on privileged relationship between individuals of the social group (Crozier e Pamilo, 1996). Mechanisms to discriminate among nestmate and non-nestmate colony members are higly variable according to species and social systems. They can be endogenous and have a strong genetic component (Greenberg 1979; Adams 1991; Crozier & Pamilo 1996; Beye et al. 1998), or be exogenous and open to the acquisition of information from the environment (Downs & Ratnieks 1999; Liang & Silverman 2000; Buczkowski & Silverman 2006). Signals are usually transferred among nestmates by trophallaxis, allogrooming or simple contact, which result in a common colony odour. This is known as ‘Gestalt theory’ (Crozier & Dix 1979; Soroker et al. 1995; Lahav et al. 1999; Boulay et al. 2000; Lenoir et al. 2001; Chapuisat et al. 2005). This flux of signals among workers generate a so called ‘gestalt’ odour in the colony, which enable the phenomenon known as ‘phenotype matching model’ (Waldman et al. 1988; Crozier & Pamilo 1996; Sherman et al. 1997). This provides that workers compare odour profiles of other individuals to a learned internal template, that is their own ‘gestalt’ colony odour (Breed & Bennett 1987; Crozier & Pamilo 1996; Breed et al. 2004). The amount of mismatch between these two parameters leads to an appropriate response, and whereas this gap is great than some threshold, aggressive behaviour is elicited (Wilson 1971; Holldobler & Michener 1980; Reeve 1989).

The Gestalt theory and the consecutive phenotype matching model explain how nestmate recognition can exist in colonies where individuals have a different genetic origin, such as in polygynous species (i.e., where more than a single reproducting queen is contained in the colony). In polydomous species, where nests belong to the same colony are phsically separated and individuals may live in different environments, a continous recycling of workers is fundamental to maintain the colony odour.

In their natural range, mature societies often defend an exclusive area around their nest where they attack alien ants to prevent the establishment of conspecific colonies (Ryti & Case 1986; Cushman et al. 1988; Breed et al. 1990; Nichols & Sites 1991; Wiernasz & Cole 1995). To reduce the cost of territorial defence, some species delineate areas around their nest with scent marks that repel aliens (Jaffe & Puche 1984; Cammaerts & Cammaerts 1996; Devigne & Detrain 2002; Devigne et al. 2004). The physical fight is sometimes replaced with ritual displays (Holldobler 1976; Mercier et al. 1997; Pfeiffer &

Linsenmair 2001; van Wilgenburg et al. 2005), and aggressiveness towards neighbours is lower than allopatric conspecific workers. This process follow the so-called 'dear enemy' effect described in several vertebrates and invertebrates (Fisher 1954; Rosell & Bjorkoyli 2002; Husak & Fox 2003; Leiser 2003). For ants, reduced aggression between neighbours was shown in some species, e.g. *Acromyrmex octospinosus* (Jutsum et al. 1979) and *Formica pratensis* (Pirk et al. 2001). In theory, two mechanisms could explain this effect. First, this mechanism may derive from habituation following repeated encounters (Langen et al., 2000), even if, in some cases, repeated encounters between neighbours may led to increased aggression, as in *Pogonomyrmex barbatus* (Gordon 1989), *Iridomyrmex purpureus* (Thomas et al. 1999), *Pristomyrmex pungens* (Sanada-Morimura et al. 2003) and *Cataglyphis fortis* (Knaden & Wehner, 2003). Second, the recognition cues of colonies living in the same area comprising a mixture of cuticular hydrocarbons (Vander Meer & Morel 1998; Lahav et al. 1999; Lenoir et al. 1999; Wagner et al. 2000; Ozaki et al. 2005), which is influenced by environment and genetic underground, may be more similar than those of allopatric colonies (e.g. *F. pratensis*, Pirk et al. 2001).

In this study I analyse how the spatial distance and the environmetal features affect nestmate recognition and intraspecific aggression in *Crematogaster scutellaris*, a dominant ant species widely distributed in the mediterranean basin, where it may form large colonies consisting of up to several thousand specimens in tree trunks or dead logs (Bernard, 1968; Casevitz-Weulersse, 1970, 1972, 1991). Its colony organisation is not clear, although some studies suggest a polydomous structure (Schatz and Hossaert-McKey, 2003, see also Ch.2 of this thesis). The study was carried out in two different habitats, an olive orchard and a degraded wood. On the first, the *C. scutellaris* distribution is highly dependent on the spatial arrangement of trees, which represent the only available nesting sites. Moreover, olive orchards are a peculiar agro-ecosystem since most of the resources are highly packed in well defined and regularly spaced patches (trees) embedded in a poorer habitat matrix (grasses or bare ground) characterised by much harsher abiotic conditions (extreme heat, water scarcity). A similar spatial packing of both resources and shade may reduce the encounter rate between neighbours, although *C. scutellaris* is potentially able to perform long forays from the nest to feeding sites. On the second, resources are scattered along the wood while nest sites are various and dispersed. This create the conditions for a large colonies overlap, which may increase the encounter frequency of workers. Comparing the responses coming from the aggression test performed in this two habitats may help to focus the weight of environmental features on intraspecific dynamics of *C. scutellaris.*

4.2 MATERIALS AND METHODS

The study was carried out between May 2007 and October 2008 in an olive orchard (northern Tuscany, Italy – 43° 53' 8" N, 11° 9' 40" E) and in a degraded wood in the same area . The plantation extended over about 40 hectares on the slopes of a hill (maximum elevation 140 m above sea level). The wood was about 200 m far from orchard (maximum elevation 180 m above sea level), and its extension was about 2 hectares embedded in a forest matrix mainly formed by turkey oaks (*Quercus cerris*) and cluster-pines (*Pinus pinaster*). The climate is Mediterranean, with hot summers and mild wet winters. The olive trees were arranged in a regular fashion and the average distance among trees was about 5-6 m, while wood trees were irregularly distributed. Management practices in the orchard were limited and mainly consisted in mowing (between June and July every year) and pruning (about once every 2-3 years). Neither fertilizers nor irrigation were used. The last wood managing were performed about in 2003 and it did not provides regular arrangement of trees. Many cut trunks and branches are still present. The presence of *Crematogaster* on each tree both in orchard and in wood was assessed by repeatedly hammering on the trunk. A trunk was scored as hosting a nest only if the hammering elicited defensive swarming of ants from nest holes. The occasional presence of isolated ants scouting on trees was not considered.

4.2.1. AGGRESSION TESTS

Confrontation tests in an arena were used to assess aggressive responses between pairs of nests. Ants from a nest were induced to forage on a tuna bait placed in an open transparent plastic container (diameter 10 cm) pinned over a tree trunk. Each container was closed after 20±2 ants were recruited on the bait and was then used for a confrontation test within the next 15 minutes. Each group of ants and container was used only once. The test consisted in joining two containers to allow the mixing of ants. The ant behaviour was followed for three minutes and the type of interaction was noted. Interactions were scored as: a) no aggression, when the transferred ants mixed with resident ants with no evident reaction; b) aggression, involving biting, charging or using chemical weaponry from the gaster. In the wood, six types of tests were carried out:

i) Controls: both groups of ants were taken from the same tree. These tests were used as controls to remove any possible effect on ant behaviour due to manipulative stress.
ii) 5 meters: the two groups of confronted ants came from two trees about 5 m apart.
iii) 10 meters: the two groups of confronted ants came from two trees about 10 m apart.
iv) 20 meters: the two groups of ants came from nests on trees about 20 m apart.
v) 40 meters: the two groups of ants came from nests on trees 40 m apart.
vi) 80 meters: ants from a randomly chosen nest inside the study area were mixed with ants from a nest located outside the study area (distance between nests > 500 m).

For each of the six test types, ten replicate tests involving independent pairs of nests were performed. Furthermore, in the wood, for each encounter the time occurred from the begin of the test and the first aggressive event was measured.

In the orchard, aggression test involving pairs of all occupied trees were performed. When two nests were not aggressive, that was considered as a 'cluster', a group of unaggressive nests, evauated as a single unit in the following encounters. Between two values of maximum and minimum distance (160m and 0m), 6 random distances were chosen, and for each of these the percentage of aggression between nests separated by that distance +/-1 meter were recorded.

4.2.2. DATA ANALYSIS

To assess whether spatial distance affects on aggressive behaviour, 4 logistic models were built, both on orchard and wood data. To model the probability (p) of an aggressive encounter, the proportion of aggression events at different distances was fitted to the following set of candidate models:

$$p \sim 1 \qquad \text{(model\_1)}$$
$$p \sim DIST \qquad \text{(model\_2)}$$
$$p \sim DIST + DIST^2 \qquad \text{(model\_3)}$$
$$p \sim \log(DIST) + \log(DIST^2) \qquad \text{(model\_4)}$$

where DIST is the distance between nests. Model 1 is the null model, model 2 is probability of aggressive behaviour linearly related to the distance, models 3 and 4 provide two different bell-shaped trend (Fig. 4.1). Models were ranked on the basis of the Akaike's Information Criterion (AIC, Burnham & Anderson, 2002):

$$AIC = D + 2K$$

where D is an index estimated on the basis of the model unfitting, and K is the number of parameters, which is directly related to the complexity of the model. The lower is AIC, the better is the model, that is the simplest and the most informative.

In the wood, to assess whether the distance between nests affects the time of first aggression, two models were built on log-transformed data:

$$\log(TIME) \sim 1 \qquad \text{(model 1)}$$
$$\log(TIME) \sim DIST+(DIST^2) \qquad \text{(model 2)}$$

where TIME is the time of first aggression and DIST is the distance. The first is the null model, and the second fits a bell-shaped curve. As above, even these models were ranked on the basis of AIC values.

4.3 RESULTS

No aggressive events were recorded in the encounters between workers belong to the same nest (tree), both in orchard and wood. In the orchard, the lowest proportion of aggressive events was recorded at low distance (10m), while the highest value was assessed at medium distances (20-40m). At high distances (>40), the proportion decrease, with a minimum measured at 50m (Fig. 4.1A). A similar trend was recorded for wood data (Fig. 4.1B). The best model (i.e. that with the lower AIC) was always model 4, which provides fitting of an asymmetrical bell-shaped curve (Tab. 4.1).

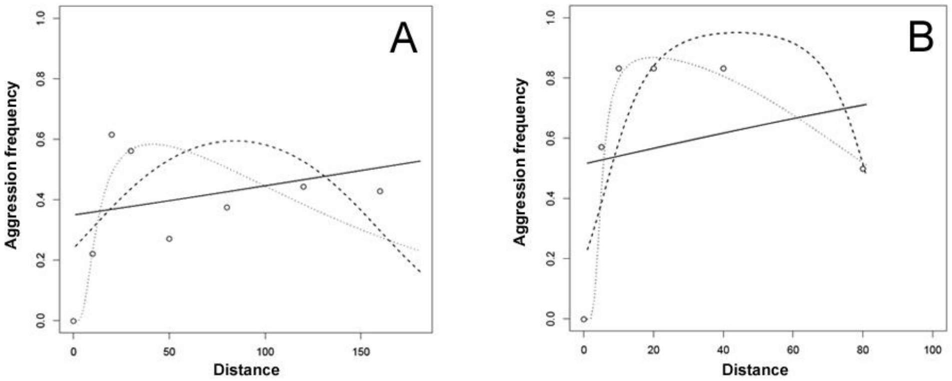

Figure 4.1. Frequency of aggression behaviours, both in orchard (A) and in wood (B). Continuous, dashed and dotted lines represent models number 2, 3 and 4, respectively.

In the wood, in cases of hostile behaviour, the average time preceding the first aggressive event is low (25”) for near nests (5m), increase with the distance till a maximum at 40m (93”) and then decrease again (80m, 46”)(Fig. 4.2).

Model	Model formula	AIC	
		Orchard	Wood
1	$p \sim 1$	196,43	53,83
2	$p \sim DIST$	197,53	55,36
3	$p \sim DIST + DIST^2$	194,59	47,45
4	$p \sim \log(DIST) + \log(DIST^2)$	**177,78**	**40,91**

Table 4.1. Results of the analysis about frequency of aggression related to the distance, AIC values of the 4 models built are shown. In bold, the lowest values. DIST=Distance, p=aggression frequency.

The best of the two models built to assess the dependency of the time of first aggression with the distance is the model 2. Even if the gap between AIC values from the two model ranked (AIC model 1=65.248, AIC model 2=61.581, see Table 4.2) was not high, the anlysis of deviance shows that this difference was significative (F=3.956, p<0.05).

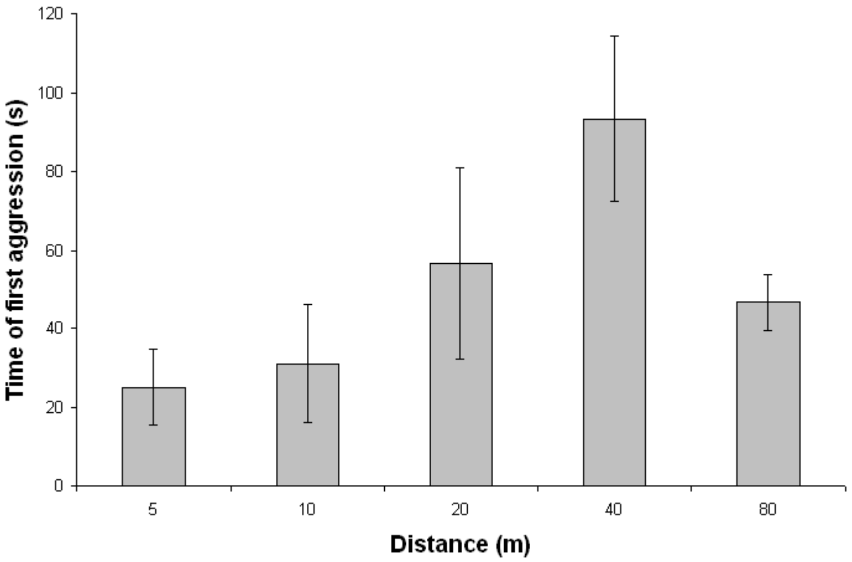

Figure 4.2. Average time of first aggression per distance in the wood, Error bars display the S.E.

Model	Model formula	AIC
1	log (TIME) ~ 1	65,24
2	log (TIME) ~ DIST+(DIST^2)	**61,58**

Table 4.2. AIC values of the two models built to assess the relationship between distance and time of first aggression. In bold, the lowest value. TIME=time of first aggression, DIST=distance.

4.4. DISCUSSION

According to polydomy definition, an ant colony could occupy several nests at the same time, despite the absence in some of them of a reproductive female (Debout et al., 2007). This colony structure can confer numerous ecological advantages, such as wider foraging areas and higher resources availability, reducing the costs of central place foraging (McIver, 1991). However, a part of this vantages might be countered by strong ecological costs, in terms of brood loss during internest transport or increased risk of predation. Because this strategy evolves, the benefits must be higher than costs. Then, resources distribution, concern about nesting sites and food availability, is a central environmental feature which could leads to a different colony structures. When resources are distributed in patches, for example as a consequence of habitat fragmentation or, as in one of these cases, trees dipped in a dried matrix, polydomy can help the colony to cover a wide area and exploit the more food resources as possible. Moreover, habitat structure and resources availability can have strong effects even on competition and dominance hierarchies (Holway et al.,2002; Ribas et al., 2003; Sorvari & Hakkarainen, 2004).

The results of this study, further support the idea that *C. scutellaris* colonies can adopt a polydomic arrangement of colonies. Indeed, in the olive orchard, it was possible to define clusters boundaries on the basis of aggression tests (see also Cap 2). Moreover, the low proportion of aggressive events between workers collected from close nests, in both habitats, is likely due to the high probability of two randomly chosen nests separate by few distances to belong to the same colony.

In the wood, the irregular arrangement of trees did not gave the chance to define any boundaries. However, the proportion of aggressive events with the distance follow a similar path as in the olive orchard. The results about the time of first aggression, show that when they are aggressive, neighbour workers deliver the first attack in a short time, increasing with the distance, since about 80 meters. Than, it decrease again. This behaviour, assessed for other species as *Pogonomyrmex barbatus* (Gordon, 1989) or *Catagliphis fortis* (Knaden & Wehner, 2003), derive from a neighbour conspecific recognition. In many other species, of both ants and other taxa, neighbours recognition may lead to a behaviour knows as ‘dear enemy phenomenon’, whereby competing animals respond less aggressively to threats by neighbours than strangers (Temeles, 1994; Heinze et al., 1996; Langen et al., 2000). In some ant species, this process might be inverted, that is aggressiveness higher to nearby than remote nests. In principle, there are at least two proximate factors that could be responsible for recognizing familiar neighbors. (i) Environmental cues are known to affect the colony odour (Obin, 1986; Heinze et al., 1996). By comparing the “environmental” part of a non-nestmate’s label, ants might be able to distinguish conspecific neighbors from conspecific strangers. (ii) Ants are known to adjust their internal olfactory template to changing colony odors (Obin, 1986; Errard and Hefetz, 1997). This flexibility might enable them to learn a second enemy-specific template. In the case of *C. scutellaris*, both hypothesis could be effective, because the wood can offer several cues which affect colony odour, since different types of materials may be encountered during nest construction by different colonies (Heinze et al., 1996). Moreover, the frequent encounters due to the widespread dispersion of resources, can promote internal odour adjusting. A short time of aggression, could increase competition ability of the colony.

The reason that yielded to the decreasing of the average time of first aggression at 80 meters is unclear. Probably several factors could be responsible. First, is it possible that some of the colonies examined were extremely large, and because of the interconnected structure of the wood, they got in contact by some of their nests. This could lead to a certain level of reciprocal learning of the odour patterns. Morover, an eventual polygyny (and/or polyandry) can affects nestmate recognition increasing aggressiveness, as a consequence of the decreased genetic relationship (Stuart, 1988; Starks et al., 1998; Stuart and Herbers, 2000; Brown et al., 2003). Whichever the reason, the proportion of aggressive events decreased at higher distances, underlining that in this particular case the aggressive behaviour is similar between low and high distances. This preliminmary results claim for further studies involving genetic analysis to determine workers' genotypes, and chemical analysis of cuticolar hydrocarbons on ants from different nests. In conclusion, following the results, *C. scutellaris* seems to have a polydomic colony structure, and the spatial area covered by the colony can be highly wide, even if the boundaries are not clearly defined. Furthermore, the spatial distance has an effect on the intraspecific aggression, and in particular *Crematogaster* seems to be able to learn chemical signals of workers belong to alien colonies. It will be also interesting to better analyse the kin structure (polygyny and polyandry) for this species, and whether it affects on colony aggression level.

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5. CHARACTERIZATION OF POLYMORPHIC MICROSATELLITE LOCI IN THE ANT *CREMATOGASTER SCUTELLARIS*

The ant *Crematogaster scutellaris* is found in both natural and man-managed ecosystems throughout the Mediterranean basin, where several thousand individuals may assemble in large colonies inside tree trunks or logs (Bernard 1968). Previous investigations have shown that this species is a strong competitor among Mediterranean ant communities and its presence may severely affect the performance and behaviour of more submissive species (Cammell et al. 1996; Santini et al. 2007). *C. scutellaris* is an active predator as well as an homopteran tender, providing protection from predators and parasitoids (Morris et al. 1998; Ottonetti et al. 2008; Schatz and Hossaert-McKey 2003). This often facilitates proliferation of homopteran pests (Addicott 1979; Breton and Addicott 1992) and may results in serious damages to plants, particularly relevant in permanent crops such as olive and citrus groves (Daane et al. 2004). It is also known that *C. scutellaris* can delay or prevent the spread of invasive ants, one of the major threats to autoctone ant communities (Gotelli and Arnett 2000; Way et al. 1997). Description of population genetic structure, dispersal strategies and intraspecific interactions using informative microsatellite markers can help in a deeper understanding of the ecology of *C. scutellaris* and arthropod communities in Mediterranean habitats.

Ants were collected from an olive grove in Tuscany, Italy, using a suction device and stored at -80 C. Whole DNA was extracted from 20 workers of two colonies following a standard phenol-chloroform protocol. Approximately 5 µg of DNA was partially digested with two blunt-end enzymes (RsaI and SmaI) and ligated to a double-stranded adaptor made of Oligo A (5' – GGC CAG AGA CCC CAA GCT TCG – 3') and Oligo B (5' PO4 – GAT CCG AAG CTT GGG GTC TCT GGC C – 3') oligonucleotides (Edwards 1996). DNA fragments between 500 bp and 1,500 bp were eluted from a 1.5% agarose gel and hybridized to a mixture of (CA)14 and (GA)14 biotinylated probes at a final concentration of 4 µM each. Hybrid molecules were captured using 1 mg of streptavidin-coated magnetic beads (Roche, Basel, Switzerland).

Microsatellite enriched DNA was recovered by PCR using an Applied Biosystems (Foster City, California, USA) 2720 thermal cycler in 50 µl total volume reaction with 1X Expand Long Template buffer 1 (Roche), 4 U of Expand Long Template Enzyme mix (Roche) and 6 µM of Oligo A as PCR primer with a 3' end tail of 14 bases (5' – CCT TCG CCG ACT GA – 3') to allow for selection of recombinants during the cloning process. Thermal profiles consisted of an initial denaturation step at 92 °C for 2 min, followed by 35 cycles of 10 s at 92 °C, 30 s at 56 °C annealing and 2 min at 68 °C extension temperature, with a final extension step of 7 min at 68 °C. PCR products were purified using cellulose membrane filters (MF-Millipore, Billerica, Massachus-

Table 5.1. Characteristics of nine microsatellite loci for the ant *Crematogaster scutellaris*

Locus	Repeat	Genbank accession number	Primer sequence ($5' \rightarrow 3'$)	Number of alleles	Allele size range (bp)	H_O	H_E	P_{HW}	F_{IS}
Crem12	$(GA)_{27}$	GQ853668	F: FAM-CCAATGACGCCACACACAG R: CCGGTTAGCAATTCCCTCG	17	172–220	0.885	0.867	0.147	−0.020
Crem16	$CT_{12}CA(CT)_{14}$	GQ853669	F: HEX-AGTCACTCTTCAGATTCTTGTTTCC R: TCTCGACGGTGTTCCTTCC	13	191–233	0.923	0.869	0.715	−0.062
Crem19	$(CT)_{26}$	GQ853670	F: NED-ACCCTCTTCTCGATCGCAC R: AAGGTGGGTATCACGCCTC	15	158–216	0.769	0.851	0.038	0.096
Crem21	$CA_5TA(CA)_{17}TA_6$	GQ853671	F: FAM-ACGAAACAATCGAACGTACTTG R: GGATCTTGTTGGCGCAGTC	13	230–276	0.800	0.861	0.156	0.071
Crem22	$(TC)_{27}$	GQ853672	F: HEX-GAAAGACGTCCTAGTCTTCCAAA R: TCTTCATCCCGCTTTGATTC	10	137–171	0.760	0.799	0.458	0.049
Crem23	$GA_{14}GG(GA)_{13}$	GQ853673	F: NED-TGATAGTCCCGCGAGAACG R: TCAGCGTCGTTTTCCATGC	14	165–219	0.885	0.786	0.734	−0.126
Crem24	$(CA)_{14}$	GQ853674	F: FAM-TCTCGTGTTTGCGCATCTG R: CTTCCCGATTCCGGCTCTG	12	232–266	0.846	0.859	0.150	0.015
Crem45	$(TG)_{24}$	GQ853675	F: HEX-CGAGACGGGAGTGAAATGAG R: ACACTGTGGTGTAAGTAATCGTC	10	237–261	0.769	0.837	0.299	0.081
Crem46	$(GA)_{20}$	GQ853676	F: NED-CGCGCGGAATGAAAATCTG R: CTCCTTTCTCTCTCCCGCC	8	224–256	0.800	0.736	0.466	−0.087

Dye phosporamidites are reported for each forward primer sequence

H_O observed heterozygosity, H_E expected heterozygosity, P_{HW} probability values of Hardy–Weinberg equilibrium test etts, USA) and cloned using the StabyCloning kit (Delphi Genetics, Charleroi, Belgium). A total of 336 clones were collected, boiled for 10 min in DNase/Rnase-free distilled water and cycle-sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing products were resolved by capillary electrophoresis in an Applied Biosystems 3100 genetic analyzer and screened for the presence of microsatellites using OLIGOFACTORY (Schretter and Milinkovitch 2006). Sequences were edited using CODONCODE ALIGNER (CodonCode Corporation, Dedham, Massachusetts, USA). Seventy-six sequenced clones had dinucleotide repeats with sufficient flanking sequences for primer design. Primers for microsatellite PCR amplification were designed for 26 loci using PRIMER3PLUS (Untergasser et al. 2007) and MSATCOMMANDER (Faircloth 2008). Microsatellite loci were screened for polymorphism on 1.5% agarose gel for 15 ants from different colonies. PCR amplification was performed in 10 µl total volume containing 10 ng of DNA, 1X reaction buffer, 1.5 mM MgCl2, 100 µM of each dNTP, 0.2 µM of each primer and 0.2 U of Taq DNA polymerase (Invitrogen, Carlsbad, California, USA). Thermal profiles consisted of an initial denaturation step at 94 ˚C for 5 min, followed by 35 cycles of 40 s at 94 ˚C, annealing for 30 s at locus-specific temperature and extension of 60 s at 72 ˚C, with a final extension step of 5 min at 72 ˚C.

Allelic variation was assessed at nine polymorphic loci for 26 workers, each collected from a different colony. Multiplex PCRs were performed each for three loci using the QIAGEN (Hilden, Germany) Multiplex PCR Kit in 10 µl total volume containing 10 ng of DNA, 0.5X Multiple PCR Master Mix and 0.2 µM of each primer. Thermal profiles followed the manufacturer's protocol with annealing at 55°C for all primer pairs reported in Table 1. PCR products were resolved on an Applied Biosystems 3100 genetic analyser and allele sizes were determined using GENEMAPPER version 4.0 with GeneScan 500 ROX size standard (Applied Biosystems).

Scoring error due to stuttering, allele dropout and evidence for null alleles were tested using MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004). A sequential Bonferroni correction was performed to adjust critical probability values for multiple tests in order to minimize type-I errors (Sokal and Rohlf 1994). Observed and expected heterozygosity, departure from Hardy-Weinberg equilibrium and genotypic linkage disequilibrium were assessed using GENEPOP version 4.0 (Rousset 2008). Statistical significance of the fixation index (FIS) was tested by comparing observed values to a frequency distribution of FIS indices obtained after 10,000 permutations of alleles performed with GENETIX 4.01 (Belkhir et al. 2004). Allelic diversity was 12.4 and the number of alleles per locus ranged from 8 to 17 (Table 5.1). Mean observed and expected heterozygosity were 0.826 and 0.829, respectively. FIS values were low and not statistically significant ($P>0.05$). No significant linkage disequilibrium or presence of null alleles was observed. Departure from Hardy-

Weinberg equilibrium was detected at locus Crem19 ($P<0.05$). This deviation was not significant after applying a sequential Bonferroni correction.

This novel set of polymorphic microsatellite loci will be used to assess population genetic structure, dispersal strategies and mating system of C. scutellaris. Of particular importance will be a first assessment of genetic relationships among individuals engaged in intraspecific conflicts under varying environmental conditions and resource distribution.

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6. PRELIMINARY ANALYSIS OF POPULATION SUBDIVISION IN THE ANT *CREMATOGASTER SCUTELLARIS* USING MICROSATELLITE DNA MARKERS

6.1. INTRODUCTION

Following the kin selection theory (Hamilton 1964a,b), in eusocial Hymenoptera workers the probability of survival of the copies of their genes is higher when they help in the rearing of their sisters than if they were to produce their own offspring. Workers are indeed closely related to the brood they rear because of their haplo-diploid sex-determining system (Crozier 1971). Two factors mainly influence relatedness among individuals within a colony: multiple mating (polyandry) and multiple reproductive queens (polygyny) (Ross 2001). Polyandry and polygyny yield a decrease in the mean intracolonial relatedness and, hence, reduce the mean indirect fitness (Bourke & Franks 1995; Crozier & Pamilo 1996).

The increase of genetic diversity within colonies, provides multiple selective benefits, such as increasing resistance against pathogens (Sherman et al. 1988; Schmid-Hempel & Crozier 1999) or reducing queen–worker conflicts (over reproduction) by reduction of relatedness asymmetries (Ratnieks & Boomsma 1995). An hypothesis suggests that multiple-mate could help queens to store enough sperm to fertilize all their eggs (Cole 1983; Fjerdingstad & Boomsma 1998). The most species are monoandry, but polygyny is common in many ants and affect greatly the colony kin structure (Crozier & Pamilo 1996; Herbers & Stuart 1996; Ross et al. 1997). Polygynous colonies may occur because of low survival of solitary colonies, but it is also possible that the productivity of the colonies increase with polygyny (Keller 1993, 1995; Bourke & Heinze 1994). The number of queens can greatly vary among colonies, populations and species (Bourke & Franks 1995; Keller 1995; Crozier & Pamilo 1996). Moreover, colony structure may vary over time as a consequence of queen turnover, i.e. replacement of old queens by newly inseminated immigrant queens (Evans 1996; Goodisman & Ross 1999; Foitzik & Heinze 2000). Polygyny is associated with specific reproductive and social traits such as loss or limitation of mating flights, colony budding, and polydomy (Bourke & Franks 1995; Pamilo et al. 1997).

Using microsatellite markers (see Ch.5), we analysed the relationship among workers of the ant *Crematogaster scutellaris*, collected in different trees (nests) which were gathered in several clusters according to a large number of interindividual aggression tests (see Chs. 2 and 4). All aggression tests performed between workers from these clusters resolved in aggressive behaviour. Nine loci (Crem12, Crem16, Crem19, Crem21, Crem22, Crem23, Crem24, Crem45, Crem46)(Frizzi et al., 2009, see also Ch.5) were used to genotype workers.

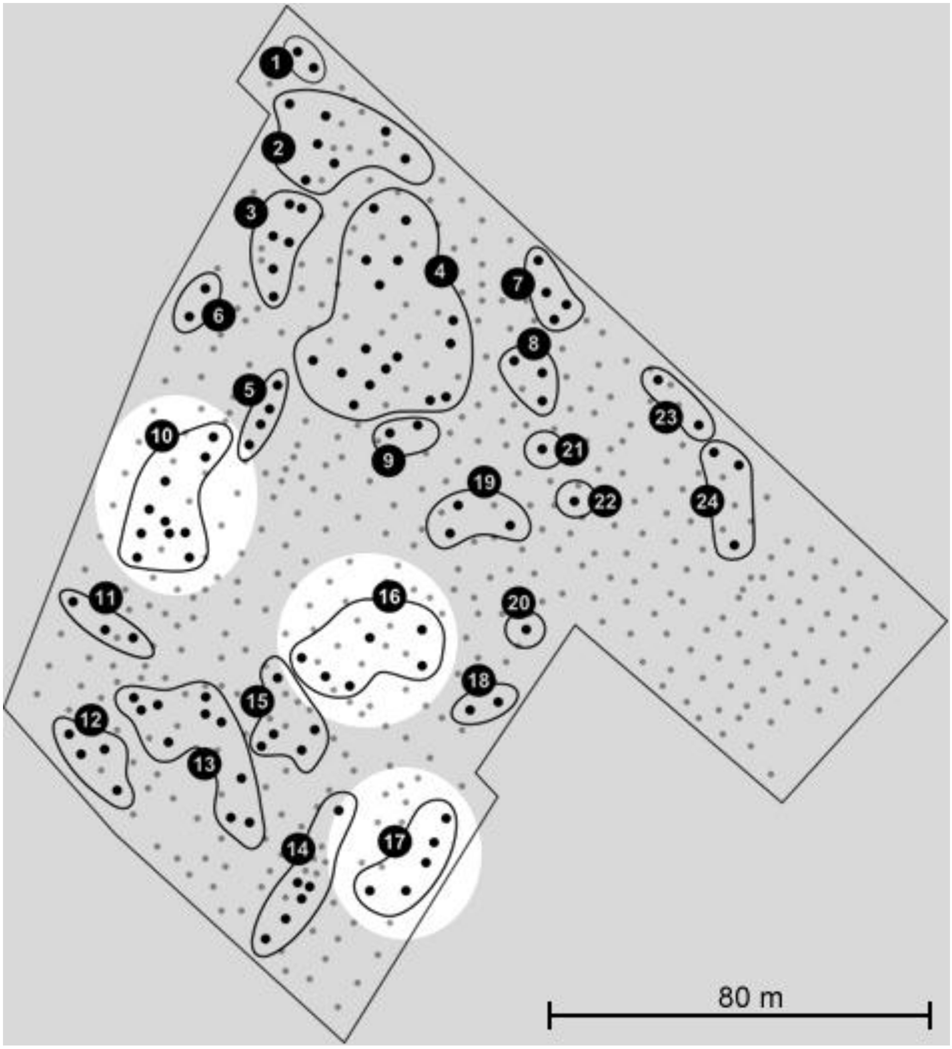

Figure 6.1. Clusters built on the basis of aggression tests (see Ch. 2). The clusters examinated are evidenced in white.

6.2. METHODS

In the olive orchard, 6 workers from 12 trees belong to 3 different clusters (4 trees per cluster) were taken. Cluster were arranged along an about straight line, where cluster one (C10) is nearest to cluster two (C16) than cluster three (C17)(Fig. 6.1). The distance between the approximated geometrical centre of C10-C16 and C16-C17 is about the same, 45-50m. In aggression tests, each of these cluster were aggressive with each other.

6.2.1. GENETIC ANALYSIS

DNA was extracted from ants using Chelex® resin, following UCLA (University of California, Los Angeles) Department of Ecology and Evolutionary Biology protocol (http://www.eeb.ucla.edu/Faculty/Barber/Web%20Protocols/Protocol2.pdf). Polymerase Chain Reaction (PCR) were performed to amplify extracted DNA, using Multiplex amplification method. Forward primers were tagged with universal fluorescent tags (named FAM, HEX and NED). Three loci were simultaneously amplified per well. Each well was filled with 5µl of Multiplex mix, 1µl of Primers mix, 1µl of Q-Solution, 1µl of extracted DNA and 2µl of DNase/RNase free distilled water, for a final volume of 10µl. After an initial denaturing step of 15 min at 95°C, the PCR consisted of 40 cycles of 30 s at 94 ° C, 90 s at the annealing temperature (63°C for Multiplex mix comprising loci Crem12, Crem16 and Crem19, 59°C for each other), and 60 s at 72 ° C, followed by a final extension step of 10 min at 72 ° C. PCR products were resolved by capillary electrophoresis in an ABI®3100 automated sequencer (Applied Biosystems). Allele size were been assessed using GENEMAPPER® v. 4.0 software (Applied Biosystems). Software attribute allele sizes for each sample with reference to GeneScan® -500 ROX® Size Standard (Applied Biosystems), which is designed for sizing DNA fragments in the 35-500bp range and provides 16 single-stranded labelled fragments. The use of an internal lane size standard during electrophoresis enables automated data analysis and precise DNA fragment size comparisons between electrophoresis runs.

6.2.2. DATA ANALYSIS

Tests for linkage disequilibrium over the entire dataset and for deviations from Hardy–Weinberg genotype proportions (HW) among individuals within nests were performed with KINGROUP software (Konovalov et. al., 2004) applying 10,000 randomizations.

Relationship between workers was inferred using Queller & Goodnight (1989) estimate methods, as implemented in KINGROUP software (Konovalov et. al., 2004). The relatedness coefficient estimated in the analysis is *r*, defined as (using *x* as a reference individual, Queller & Goodnight, 1989):

$$r = \frac{\sum_{l} \sum_{m=a,b} (p_{ym} - \bar{p}_m)}{\sum_{l} \sum_{m=a,b} (p_{xm} - \bar{p}_m)}$$

where *x* and *y* are the individuals in the pair, *l* is the number of loci and *m* index alleles per locus; *a* and *b* indicate alleles at allelic position 1 of *x*, allelic position 2 of *x*, respectively; p_{xm} is the frequency (0.5 or 1) of the allele at allelic position m in individual *x*, p_{ym} is its frequency (0, 0.5 or 1) in *x*'s partner, and $\overline{p}_m$ is the population frequency of the allele under consideration. A null distribution of *r* was created by randomly reshuffling sample alleles (Guo & Thompson, 1992). 17000 null-hypothesis of non-relation simulated dyads were performed.

6.3. RESULTS

There was no significant (P >0.05) linkage disequilibrium between any pair of loci. Relatedness pairwise analysis shows that workers from trees belonging to different cluster were never significantly (p<0.05) related. Comparisons between workers from trees belong to the same cluster, revealed a variable proportion of significantly related pairs of individuals. Average frequency of significative comparisons were (Fig. 6.2):0.923 for C10 (SE 0.0184), 0.489 for C16 (SE 0.009), and 1 for C17.

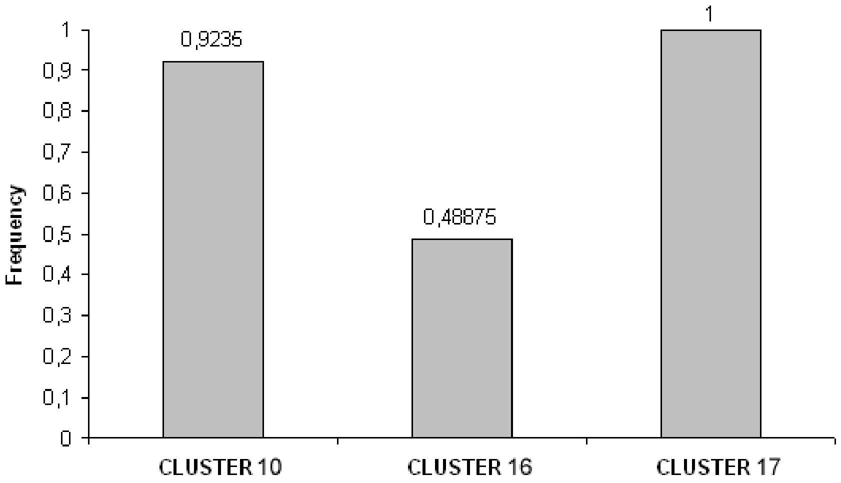

Figure 6.2. The figure shows the proportion of significantly related pairs of individuals in comparisons within the clusters.

6.4. DISCUSSION

The first important output showed by results, is that clusters identified on the basis of aggression tests (see Chs. 2 and 4) really belong to different colonies. On the contrary, results of intracluster comparisons revealed a variable level of relatedness between workers belonging to a same cluster. This may mean that within each cluster, but also within each nest, specimens not completely related may coexist. The reduction of intracolony genetic relatedness might be caused both from polyandry and polygyny, although this last has a much greater potential effect (Sündstrom, 1993; Schlüns et al., 2009). Further, data are needed to clarify this point. If confirmed, the data from cluster 3, which showed a full relatedness among the 18 specimens tested, suggests that a polydomic arrangenment of nests was associated with monoginy. On the contrary, results from cluster 2 and particularly from cluster 1, where also specimens from the same tree (nest) were not fully related, suggest that polydomy was also probably associated with polyginy. Whichever the cause the results show that the genetic organization of this species is by far more complex than previously hupotesized by Baroni Urbani and Soulié (1962).

A second central point resulted from this work, is that high polymorphic microsatellites designed for this species (Frizzi et al., 2009, Ch. 5 of this thesis), are able to discriminate colonies on the basis of workers genotypes at a very low scale. This could be useful to develop future genetic population analysis involving this species.

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7. LANCHESTER MODELS OF ATTRITION IN ANTS

7.1. INTRODUCTION

Lanchester (1916) proposed two attrition models of opposing armies, known as ‘linear law’ and ‘square law’, respectively. Given two groups of opponents say m and n, the death rate in each group according to the linear and the square law can be written as

$$\begin{cases} \dfrac{dm}{dt} = -\alpha_n \cdot n \cdot m \\ \dfrac{dn}{dt} = -\alpha_m \cdot m \cdot n \end{cases} \qquad \text{eq.1}$$

$$\begin{cases} \dfrac{dm}{dt} = -\alpha_n \cdot n \\ \dfrac{dn}{dt} = -\alpha_m \cdot m \end{cases} \qquad \text{eq.2}$$

where α_n and α_m are the fighting ability of group n and m, respectively.

In words, the linear law states that the mortality within each group depends equally on the number of opponents and their fighting ability. On the contrary, in Lanchester square law there is a greater effect of the size of opponent group that of their fighting ability. This effect can be mechanistically explained assuming that under the square law members of the more numerous group gangs together up on individual opponents. On the contrary, under the linear law it is assumed that a battle is represented by a series of individual duels, so that outnumbering individuals on the more numerous group remain unengaged until an opponent become available.

Since their early formulation, Lanchester models have been extensively used to model human warfare (e.g. Bracken, 1995; Fricker, 1998; Johnson and MacKay, 2008). More recently, these models have been applied to animal contests too (e.g. Franks and Partridge, 1993; Wilson et al. 2002; Adams and Mesterton-Gibbons, 2003; Plowes and Adams, 2005).

In this paper we applied the Lanchester linear and square laws to ant combats. In particular, combats between a focal species, *Lasius neglectus*, against three other common species were considered. *Lasius neglectus* is an invasive ant which has recently spread its range throughout Europe, causing serious concern for the effects on autochthonous ant assemblages (Ugelvig et al., 2008). This species was confronted with three species fairly common in the area recently invaded in Central Italy. These were: *Lasius paralienus, Lasius emarginatus* and *Crematogaster scutellaris*. In particular, *L. emarginatus* is an highly aggressive ant, which form large colonies with up to several thousand specimens and share several features in common with *L. neglectus* (Bernard, 1968; Seifert, 1992). *C. scutellaris* is a Myrmicine ant widely distributed throughout the Mediterranean basin. This species form large colonies within tree trunks and dead logs and ranks high in ant competitive hierarchies (Cammell et al. 1996; Way et al., 1997; Santini et al., 2007).

The aim of the paper was first to clarify if and how combats between these ants could be better described by linear or a square law. Secondly, we tested if the numerical assessment of its own group dimension affects the willingness of ants to enter an interspecific combat.

7.2. MATERIALS AND METHODS

7.2.1. ANT SAMPLING AND SITE

Ants were collected during June/July 2009 in Prato (Northern Tuscany, Italy, 43° 52' 46"N, 11° 05' 50" E), where abundant populations of *Lasius neglectus* have been recently discovered. Collection took place during the morning in urban gardens, where the four studied species were common. For each species, ants from at least three different nests were used and their order of use randomized to avoid nest-related effects. All the four species are monomorphic with reduced intra-specific differences in the sizes of ants. Care was taken, however, to select specimens of same sizes. Samples of 30 ants for each species were collected and their dry weight measured. Average dry weights were: *L. neglectus*, 0.23 mg; *L. paralienus*, 0.34 mg; *L. emarginatus*, 0.33; *C. scutellaris*, 0.77 mg.

7.2.2. LANCHESTER BATTLES

Ants collected from the field were carried to the laboratory and stored without food (but with water available) into 10 cm Petri dishes with Fluon coated walls prior to a battle. Each container hosted about 100 ants, which were left to acclimatize to laboratory conditions for at least for 50 min (T= 27°C).

To estimate the α_m and α_n coefficients (enemies killed h-1 ant-1) a first set of 10 battles involving 1 *L.neglectus* and 1 opponent species were realised. Two ants were simultaneously dropped within a 5 cm Petri dish with Fluon coated walls and their behaviour recorded at 20 minutes intervals for up to 7 hours. At the end of the experiment the ant still alive was assessed. The hourly mortality rate for each species, say δ_n and δ_m for *L.neglectus* and the opponent respectively, were computed from the fraction of surviving ants at the end of the experiments. Given that during the time of the experiments the only cause of death for an ant can be reasonably attributed to the fight the it is reasonably to assume that the following equalities hold:

$\alpha_m = \delta_n$ and
$\alpha_n = \delta_m$

Groups of ants of different species were pitted against one another in the following combination:

i) 30 FS vs. 10 OS

ii) 20 FS vs. 20 OS

iii) 10 FS vs. 30 OS

were FS (= focal species) is *Lasius neglectus* and OS (=opponent species) is one of *L. paralienus*, *L. emarginatus* or *C. scutellaris*. Five independent replicates were obtained for each combination for battles of *L. neglectus* against *L. paralienus* and *C. scutellaris*. Six replicates were instead obtained for battles involving *L. emarginatus*.

Battles took part in a neutral arena (10 cm Petri dish with Fluon coated walls) where the two groups were dropped simultaneously. Dead ants were counted at 1hr intervals and experiment ended after 7 hours. Control tests using groups of homospecific ants were also run in parallel, to control for mortality not related to interspecific fights. The time series of the two species were obtained numerically solving the Lanchester linear (eq. 1) and Lanchester square laws (eq. 2), using the αm and αn values obtained from the 1vs1 experiments described above. The predicted time series were compared to observed data and the using the library FME (Soetaert & Petzoldt, 2009) under the R statistical software package (R Development Core Team, 2008). Models' fit was assessed computing the corresponding AIC values. The 'best' model was chosen as the one having the lowest AIC value (Burnham & Anderson 2002). In short, a difference in AIC values between two models (ΔAIC) less than 2 indicates that the two models are indistinguishable, while ΔAIC =4–7 indicates that the poorer model has considerably less support than the better one and ΔAIC>10 shows that the poorer model has essentially no support (Burnham & Anderson 2002).

7.2.3. NUMERICAL ASSESSMENT AND WILLINGNESS TO FIGHT

Ants were carried into the laboratory and stored without food (but with water available) into 10 cm Petri dishes with Fluon coated walls for 50 min prior to a contest. Each container had only ants from one nest. Ants were randomly assigned to one of the following density treatment D: i) low density (L): 5 ants per dish; ii) high density (H): 50 ants per dish.

After acclimation (50 min, T = 27 °C) ants from different treatments were used in aggression tests. Two ants were simultaneously dropped into a neutral arena (5 cm dish with Fluon coated walls) and their behaviour observed until the first attack from one of the two ants and up to a maximum of 6 minutes. The following series of tests was carried out:

i) FSL vs OSH

ii) FSH vs OSH

iii) FSH vs OSL

were FS (= focal species) is *Lasius neglectus* and OS (=opponent species) is one of *L. paralienus*, *L. emarginatus* and *C. scutellaris*). L and H subscripts refer to the acclimation density (low and high, respectively). For each test 20 independent replicates were obtained. The species attacking first and the time of first attack, if any, were recorded. During each experiment the following outcomes were possible: i) FS attack first, ii) OS attack first, iii) simultaneous attack by both and iv) no aggression. In the majority of cases it was easy to recognize an attack. In the three *Lasius* species an attack usually consisted in actively pursuing and biting the enemy. After a combat had begun, gaster flexion and venom spraying also occurred. On the contrary, attacks by *Crematogaster* usually consisted in raising the gaster and venom spraying (see Marlier et. al, 2004, for a full ethogram of *C.scutellaris*).

Results were analyzed using logistic multinomial regression technique (Agresti, 2007) using as a dependent variable the counts of the different outcomes described above and as explanatory variables the treatment (high density or low density conditioning) of both the focal (DFS) and the opponent species (DOS). Five different models of increasing complexity were fitted starting from a null model up to a full factorial model including the two main terms DFS and DOS and their interaction. Model were ranked on the basis of their AICs and the best model chosen as the on with the lowest AIC (Burnham and Anderson, 2002). Time of aggression was analyzed using ANOVA, after log transform of the data to cope with non homogeneity of variances (Sokal and Rohlf, 1995). All the analysis were carried out using the R statistical package (R Development Core Team, 2008).

7.3. RESULTS

7.3.1. LANCHESTER BATTLES

Results of Lanchester analysis are shown in Table 7.1. In general, *L. neglectus* suffered a greater mortality than all the other species and mortality increased as the number of opponent increased. The lower mortality levels were observed during battles against *L. paralienus*, when percent mortality ranged from a minimum of 8.7% to a maximum of 48%. When facing the other two species, *L. neglectus* mortality was greater and reached a maximum of 97% in battles against *L. emarginatus*. The opponent species suffering the greatest mortality was *C. scutellaris*.

Opponent Species	**Type of test**	**Mean % mortality**		**Type of combat**	**ΔAIC**
		L. neglectus	Opponent		
L. paralienus	30 FS vs 10 OS	8.7	14.0	2	154.07
	20 FS vs 20 OS	32.0	10.0	2	103.51
	10 FS vs 30 OS	48.0	6.7	2	24.1
L. emarginatus	30 FS vs 10 OS	33.9	36.7	2	93.63
	20 FS vs 20 OS	71.7	33.3	2	3.73
	10 FS vs 30 OS	96.7	11.3	2	67.17
C. scutellaris	30 FS vs 10 OS	49.3	80.0	2	27.82
	20 FS vs 20 OS	82.8	36.5	2	12.31
	10 FS vs 30 OS	90.0	18.7	1	2.72

Table 7.1. Results of Lanchester battles. Type of combat shows model (L= linear, S= square) receiving the greater support (lower AIC) from the data. ΔAIC is the difference in AIC value between the two models. Mean % mortalities suffered by each species for the different numerical combination are also shown.

In general, the square model was superior in explaining the outcome of the experiments. As for the battles against *L. paralienus* (Figure 7.1), the square model considerably outperformed the linear law in all the three experiments. However, in the last one (10FS vs 30 OS) despite the model tended to overestimate the mortality of *L. neglectus*.

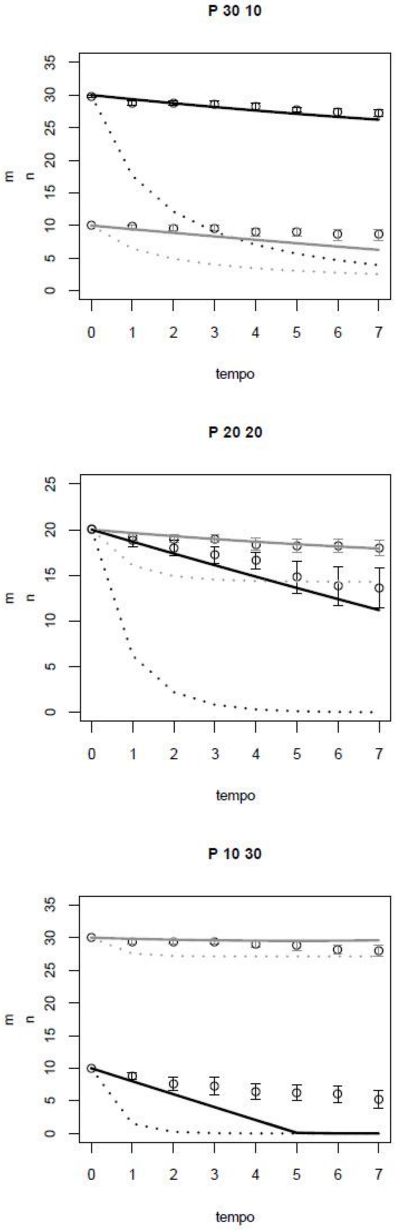

Figure 7.1. Results of Lanchester battles against *L. paralienus*. Starting numbers of the two species are reported above each graph. The first number is the abundance of *L.neglectus* and the second is the abundance of the opponent species. Grey line = opponent species; black line = *L.neglectus*. Dotted line =Linear law, continuous line = square law.

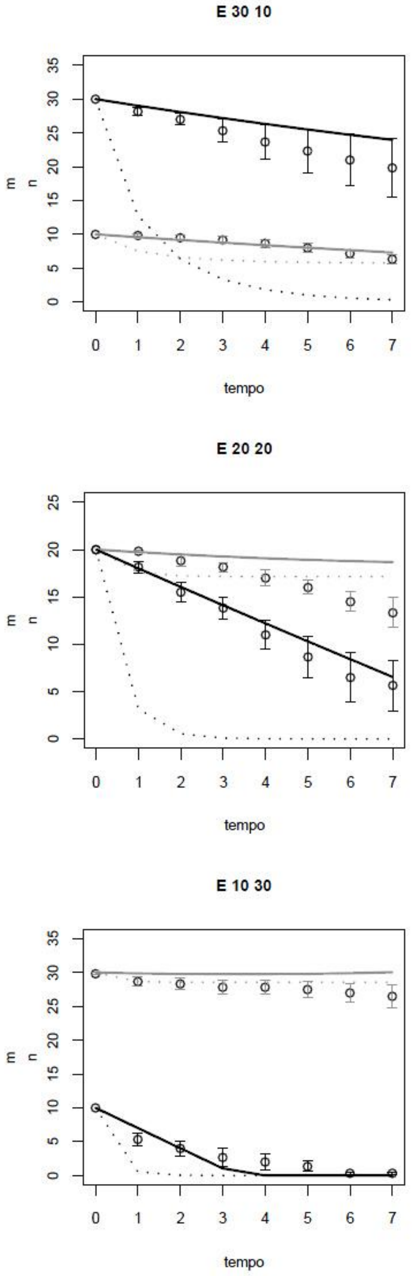

Figure 7.2. Results of Lanchester battles against *L. emarginatus*. Starting numbers of the two species are reported above each graph. The first number is the abundance of *L.neglectus* and the second is the abundance of the opponent species. Grey line = opponent species; black line = *L.neglectus*. Dotted line =Linear law, continuous line = square law.

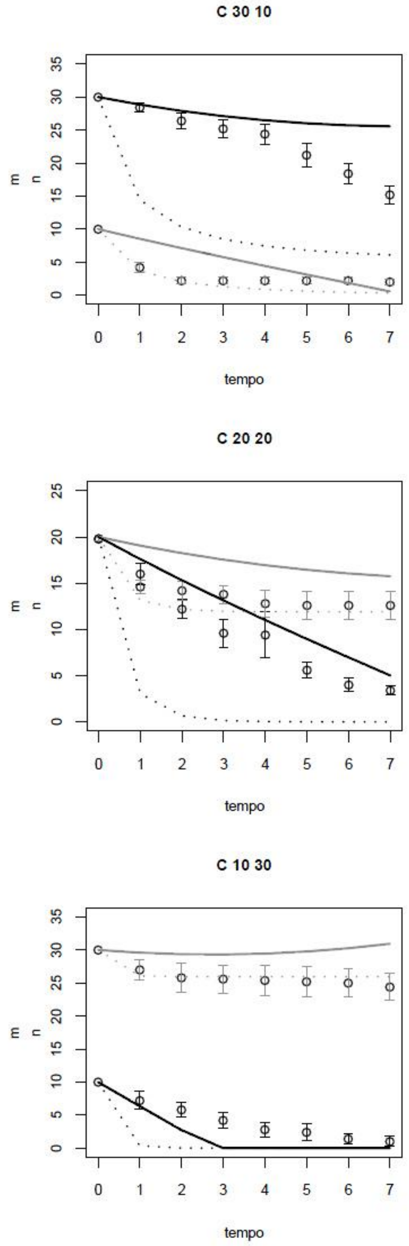

Figure 7.3. Results of Lanchester battles against *C. scutellaris*. Starting numbers of the two species are reported above each graph. The first number is the abundance of *L.neglectus* and the second is the abundance of the opponent species. Grey line = opponent species; black line = *L.neglectus*. Dotted line =Linear law, continuous line = square law.

A similar pattern emerged when battles against *L. emarginatus* were considered (Figure 7.2). Again in all the three cases the square law outperformed the linear law, particularly in the 30FS vs 10OP and 10FS vs 30OS tests. A lower agreement was found in the 20vs20 tests where both the effects of the two species appeared to be underestimated. Finally, a lower agreement between models and data was observed for the battles involving *C.scutellaris* (Figure 7.3). In general the square model outperformed the linear in the first series of experiments (30FS vs 10OP and 20FS vs 20OS) but the two models had very close performances in the latter (10FS vs 30OP). However a closer visual examination of the predicted and observed time series revealed that none of the two models satisfactorily explained the observed patterns.

7.3.2. NUMERICAL ASSESSMENT AND WILLINGNESS TO FIGHT

Results of aggression tests for the pair *L. neglectus* vs *L. paralienus* are shown in Table 7.2. The model with the lower AIC was the one containing only the explanatory variable D_{OS}.

Opponent Species	**Type of Model**	**AIC**	**W**
L. paralienus	Null model	170.45	0.01
	D_{FS}	175.10	0.00
	D_{OS}	**161.22**	**0.87**
	$D_{FS}+D_{OS}$	166.16	0.07
	$D_{FS}+D_{OS}+(D_{FS}*D_{OS})$	167.22	0.05
L. emarginatus	Null model	200.57	0.00
	D_{FS}	203.16	0.00
	D_{OS}	**169.95**	**0.76**
	$D_{FS}+D_{OS}$	172.54	0.21
	$D_{FS}+D_{OS}+(D_{FS}*D_{OS})$	176.18	0.03
C. scutellaris	Null model	**189.87**	**0.38**
	D_{FS}	191.44	0.17
	D_{OS}	194.08	0.05
	$D_{FS}+D_{OS}$	195.36	0.02
	$D_{FS}+D_{OS}+(D_{FS}*D_{OS})$	189.88	0.38

Table 7.2. Results of fitting multinomial models. AIC = Akaike information criterion, W=Akaike weigth. In bold the model with the lower AIC value. DFS is the acclimation density of L. neglectus, DOS is the acclimation density of the opponent species.

This indicates that the outcome of the contest was affected only by changes in the acclimation density of the opponent species. The results, separated by acclimation densities of *L. paralienus* (i.e. D_{OS}), are shown in Figure 7.4A. In both cases, the proportion of observed events significantly deviated from uniformity (χ_{23}=35.4, P<0.0001 and χ_{23}=35.6, P<0.0001 for the low and high density conditioning of *L. paralienus*, respectively). When *L. paralienus* came from the low density treatment, *L. neglectus* attached first in greater proportion of the tests (about 65%) and *L. paralienus* showed a much lower aggressiveness. On the contrary, when *L. paralienus* came from the high density treatment there was an increase in its aggressiveness and a reduction in that of *L. neglectus*, although the difference between the two was not statistically significant.

An effect of the sole acclimation density of the opponent species was also found for the pair *L. neglectus* vs *L. emarginatus* (Table 7.2 and Figure 7.4B). Even in this case the proportions of observed events significantly deviated from uniformity (χ_{23}=10.2, P<0.05 and χ_{23}=52.4, P<0.0001 for low and high acclimation density, respectively). However, differences from previous tests were evident. When *L. emarginatus* come from the high density treatment it was significantly more aggressive than *L. neglectus*, on the contrary, when coming from a low density treatment its aggressiveness was reduced, while that of L. *neglectus* increased. Furthermore, there was a significant number of trials where no aggressive event were recorded.

When analyzing the pair *L. neglectus* vs *C. scutellaris* the model with the lower AIC was the null model, indicating that the outcome of the tests did not changed neither with the density of the focal species nor with that of the opponent (Table 7.2). The results were thus pooled for subsequent analysis (Figure 7.1C). Also in this case, proportions of outcomes deviated from a uniform distribution (χ_{23}=45.7, P<0.0001) but *L. neglectus* proved to be always more aggressive than *C. scutellaris*.

Analysis of aggression times gave no significant results and did not differed among the species nor by specie*treament interaction.

7.4. DISCUSSION

Results of this study revealed that the Lanchester square model was superior in explaining fighting by ants with respect to the Linear one, with the only exception of the fights against *C.scutellaris*, where both models performed very badly. Previous experimental investigations on Lanchester battles in ants failed to find supporting evidences in favor of the square law. As for instance, Plowes and Adams (2005) which studied intraspecific combats in the fire ant Solenopsis invicta concluded that no clear support in favor of the square law could be found. Similar results have been found by Batchelor and Briffa (in prep.) in *Formica rufa*.

Lanchester models, originally proposed to describe human warfare, are however based on a number of restricting assumptions whose violations may

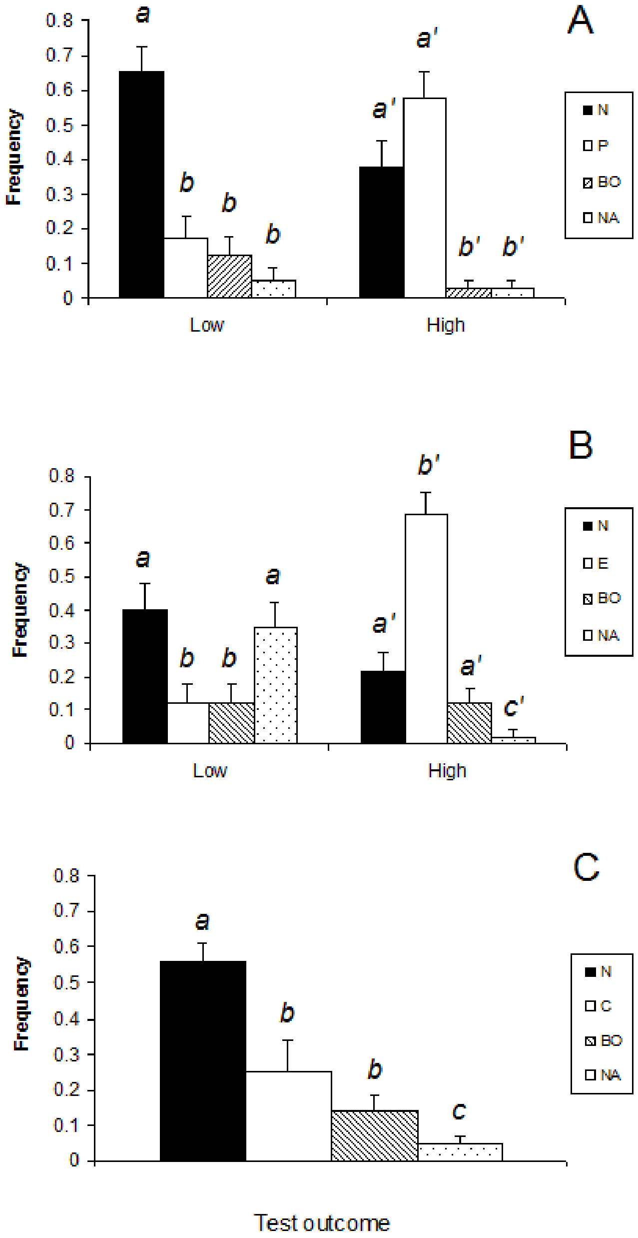

Figure 7.4. Results of individual fights. Bars represent the proportion of first attacks by each species. Black bars: *L. neglectus*; white bars: opponent species; dashed bars: simultaneous attack by both species; dotted bars: no aggression. In A) the opponent is *L.paralienus*, in B) is *L.emarginatus* and in C *is C. scutellaris*. In A and B results are separated for the two acclimation densities of the opponent species (low and high, respectively). In C pooled results are shown. Bars sharing the same letter were not significantly different at P =0.05, on the basis of Tukey post-hoc test for proportions.

partly explain the observed differences with observed data. Several of these limiting assumption have been carefully examined by Mesterton-Gibbons and Adams (2003), and yet more general equations could be written. As for example, the square law assumes that death rates for each group do not depend directly on the number of individuals within the group. The rationale is apparent here if one consider human armies firing projectiles. A force of 10 rifles may cause as many casualties per unit time when they face 100 or 1000 opponents, so long as they can acquire targets at the same rate. This assumption hardly apply to animal contests, where fighting require close contact and death rates should depend on the size of both groups. Similarly, the models assumes that the death rates suffered by a fighting group is not directly affected by the fighting abilities of its members. Again the rationale is clear here if one consider human warfare. Lanchester (1916) sought to describe the effect of modern weapons that are used to fire opponents. Such weapons are offensive but are not used directly in defence because they rarely intercept incoming projectiles. Clearly this assumption hardly applies to animal combats were opponents grapple in immediate contact with one another and kill by biting, stinging, poisoning. It seems likely in many cases that animals that are better in injuring the opponents are also better to defend themselves from injuries.

Apart from these other basilar assumption of the models seems challenging when willing to applying them to animal combats. First both the models assumes that the two opponent groups use a same fighting strategy. Yet this seems reasonable to occur in human warfare, where combat strategies of armies are more similar, or during intraspecific fights in animals. However, when considering interspecific battles this assumption is less likely to be satisfied, since combat modes heavily depends on a number of constraints (physical, physiological, behavioural and, ultimately, evolutionary) which may cause very different fight strategies to occur (i.e. smaller species are more likely to fight in groups that bigger ones, e.g. Franks & Partridge 1993). A second important point is that Lanchester models are "spatially and temporally homogeneous, allowing no variation in unit type, terrain or tactics, command or control, skill or doctrine" (Johnson & MacKay, 2008). Among other things, this means that i) the type of combat does change in time and ii) the battle is well mixed system, where every individual opponent enter the fight according to a given fixed strategy. These two points seems more critical and probably explains part of observed lack of fit. The results of the experiments on the willingness to fight clearly shows that the decision to enter a battle may change according to own perception of its numerical strength, which indeed may change during the course of the battle.

Furthermore, it was a common observation in our experiments that even in combats between equally sized groups (20FS vs 20OS) no all ants did actually fought and several remained unengaged at a side. Indeed, even if the αm and αn values don not change in time, if the number of ants actually participating the battle is different from the total number, the number of casualties may considerably differ from the predicted one.

The worst agreement among data and models was found during the battles against *Crematogaster scutellaris*. This point deserves some attention. Yet *C. scutellaris* adopt a fighting mode really different from *Lasius* spp. In general, *L. neglectus* was the first to attack, irrespectively of its numerical strength, using biting. On the contrary *C. scutellaris* attack more rarely and seems to rely more on a defensive strategy: whenever an incoming opponent approaches it is sprayed with venom from the gaster. Since it is known that a single *C. scutellaris* may produce up to 90 venom drops (Marlier et. al., 2004) it is conceivable that she is able to kill several opponents in a short time. Furthermore, the venom produced by *C. scutellaris* is known to cause delayed mortality (up to 48 hors later) and this may probably explain the apparent change in the steepness of the observed time series in *L.neglectus* mortality, between 4 and 5 hours.

In synthesis, the results obtained in these experiments claim first for further experimental analysis, to better clarify the details of the behavioural mechanisms involved in fights among ants. Secondly, these results prompt for further modelling effort to produce more and more realistic models.

Such factors may probably explain why in several cases the observed number of deaths was greater or lower than observed. To our knowledge our study is the first clear attempt to experimentally test Lanchester models in interspecific fights.

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8. CONCLUSIONI

I lavori sviluppati nella tesi, hanno contribuito ad aumentare il livello conoscitivo sia dell'ecologia che dell'etologia di *C. scutellaris*. Alcuni aspetti finora dibattuti o non ben definiti, come la struttura sociale o la distribuzione spaziale delle colonie, sono stati delineati con maggiore precisione.

L'analisi della distribuzione spaziale tramite test di aggressività ha permesso di formulare alcune ipotesi sui sistemi di strutturazione della colonia, prima su tutte l'ipotesi di polidomia. La variabilità temporale nel numero e nella distribuzione dei nidi attraverso le stagioni, può indurre a ipotizzare fenomeni di occupazione temporanea di alcuni nidi nei periodi di massima attività, nidi poi abbandonati nei periodi invernali. Questo fenomeno, conosciuto anche in altre specie di formiche, comporta i vantaggi della polidomia (maggiore copertura del territorio, maggiore sfruttamento delle risorse), riducendo allo stesso tempo i rischi dovuti ad una eccessiva frammentazione della colonia.

Lo sviluppo di 9 markers su DNA microsatellitare, hanno permesso di produrre un primo screening genetico della popolazione di *C. scutellaris* nell'oliveto. I markers hanno dimostrato di riuscire a discriminare gli individui appartenenti a colonie diverse in base al loro genotipo anche ad un livello di scala molto ridotto. In lavori di genetica di popolazione che prevedono gli stessi fattori di analisi, le distanze in gioco sono spesso molto più ampie, superiori al chilometro o alla decina di chilometri. L'elevato potere discriminatorio dei markers, incoraggia verso un loro utilizzo in molti altri contesti ambientali che prevedano la necessità di valutazioni raffinate delle diversità, come ad esempio criticità prodotte da inquinamento o da frammentazione dell'habitat.

Come per la gran parte delle specie di formicidi, alla polidomia è spesso associata la poliginia.Nel caso di *C. scutellaris*, i dati emersi dallo screening genetico, oltre a validare l'ipotesi di polidomia, portano proprio verso l'ipotesi di poliginia e/o di poliandria per questa specie. Infatti, è noto che la compresenza di più regine, unita a probabili accoppiamenti con più maschi, diminuisce il livello di parentela all'interno della colonia. La diversità nella proporzione di individui imparentati all'interno dei tre clusters in esame emersa dall'analisi dei coefficienti *r* di parentela, fa supporre che le tre colonie siano caratterizzate da diversi livelli di questo fenomeno. Gli scarsi dati fino ad oggi in nostro possesso, descrivevano invece una probabile monoginia per la specie.

I test di aggressività interspecifica, che oltre a *C. scutellaris* si sono focalizzati sulla specie invasiva *L. neglectus*, hanno permesso di evidenziare come le strategie di competizione attraverso comportamento aggressivo siano diverse tra le specie in esame. Infatti, soltanto due specie, *L. paralienus* e *L. emarginatus*, hanno mostrato di diversificare il loro comportamento in base all'entità del gruppo di appartenenza. Se il gruppo era ad elevata densità, l'aggressività del singolo individuo risultava più netta, e viceversa. Questo suggerisce la possibilità per queste due specie di effettuare una sorta di 'conteggio' del proprio gruppo, a seguito del quale attuare una strategia più aggressiva o più difensiva.

La strategia di *L. neglectus,* sembra invece prevedere un livello di aggressività stabile sia che la densità del gruppo di appartenenza sia elevata o scarsa. Probabilmente il fattore vincente che rende questa specie un così forte competitore è la straordinaria e costante abbondanza di individui che caratterizza le colonie, ed è altresì probabile che tale fattore sia importante nel determinare il tipo di espressione comportamentale di combattività rilevata dai test. Essendo infatti il fattore gruppo sempre molto presente, grazie anche alla scarsa aggressività tipica della specie nei confronti di altre colonie conspecifiche, tanto da parlare in *L. neglectus* di 'supercolonia', il continuo apporto dell'intera colonia negli scenari di competizione interspecifica può far sì che gli effetti del gruppo sui singoli individui siano costanti. In sostanza, anche variando sperimentalmente l'entità del gruppo di provenienza, gli individui si comporteranno ogni volta come se fossero parte di un gruppo estremamente ampio, come di fatto avviene in scenari naturali. Più approfonditi studi futuri dovranno chiarire con maggior precisione quali siano le risposte comportamentali dell'intera colonia a situazioni di competizione, così da poter definire in maniera più chiara quali siano i motivi di una così esplosiva espansione di *L. neglectus* a danno di altre specie dominati già presenti sul territorio europeo.

Attraverso lo sviluppo dei modelli Lanchester sui dati di mortalità/tempo estratti dai test di aggressività di gruppo, si può ipotizzare diverse strategie comportamentali nei conflitti interspecifici per le 4 specie analizzate. Il modello detto 'square', il quale prevede che l'esito di un conflitto sia dipendente dalla capacità individuale di combattimento e dall'entità del gruppo, è risultato essere spesso il modello migliore, trasversalmente a tutte le specie. Questo suggerisce come la strategia dell'aggressività di gruppo sia diffusamente sfruttata nelle formiche, e come questa ricopra un ruolo importante nelle dinamiche di competizione. E' da sottolineare come in alcuni casi, soprattutto in scontri *L.neglectus/C. scutellaris*, il modello 'linear', secondo il quale l'esito del conflitto è dipendente unicamente dalla capacità individuale di combattimento dei contendenti, è risultato il migliore. In altri casi ancora, nonostante il modello 'square' fosse il migliore, sono state registrate delle sovra o sottostime degli andamenti della mortalità nel tempo. Sicuramente i meccanismi comportamentali e biologici coinvolti nei conflitti tra formicidi sono molteplici ed è probabile che ulteriori fattori di analisi debbano essere introdotti per meglio calibrare i modelli. Eventuali futuri studi ecologici ed etologici su questi insetti che tengano preventivamente conto della possibilità di utilizzo dei modelli Lanchester nell'analisi degli esiti dei conflitti, potrebbero chiarire con maggiore precisione i fattori in gioco.

In conclusione, il lavoro della presente tesi ha in primo luogo risolto alcune questioni sull'ecologia e sull'etologia di *C. scutellaris,* mentre in secondo luogo ha permesso di aprire alcuni orizzonti di studio su specie diverse, come *L. neglectus*, di elevata rilevanza ambientale e conservazionistica, ponendo le basi per futuri studi che cerchino di rispondere alle domande sollevate e alle questioni ancora aperte.

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