The Ecology and Population structure of the invasive Yellow Crazy Ant *Anoplolepis gracilipes*

Dissertation zur Erlangung des

naturwissenschaftlichen Doktorgrades der

Julius-Maximillians-Universität Würzburg

vorgelegt von

Jochen Drescher

aus Köln

Würzburg 2011

Eingereicht am:

Mitglieder des Promotionskolloqiums

Vorsitzender:

Gutachter: PD Dr. Nico Blüthgen, Julius-Maximillians-Universität Würzburg

Gutachter: PD Dr. Thomas Schmitt, Albert-Ludwigs-Universität Freiburg

Tag des Promotionskolloqiums:

Doktorurkunde ausgehändigt am:

Ants are everywhere, but only occasionally noticed. They run much of the terrestrial world as the premier soil turners, channelers of energy, dominatrices of the insect fauna – yet receive only passing mention in textbooks on ecology. [...] The neglect of ants in science and natural history is a shortcoming that should be remedied, for they represent the culmination of insect evolution, in the same sense that human beings represent the summit of vertebrate evolution.

Bert Hölldobler & Edward O. Wilson, 'The Ants', 1990

ERKLÄRUNGEN

gemäß §4, Abs. 3, S. 3, 5 und 8 der Promotionsordnung vom 15. März 1999

(zuletzt geändert durch Satzung vom 12. August 2009)

Hiermit erkläre ich ehrenwörtlich, daß ich die vorliegende Dissertation selbständig angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.

Die Dissertation hat weder in gleicher noch in ähnlicher Form einem anderen Prüfungsverfahren vorgelegen.

Am 04. September 2006 hat mir die Universität Würzburg den akademischen Grad „Diplom-Biologe Univ." verliehen. Weitere akademische Grade habe ich weder erworben noch versucht zu erwerben.

Würzburg, 18. März 2011

Jochen Drescher

This thesis is based on the following manuscripts:

Drescher J, Blüthgen N, Feldhaar H (in prep) Mitochondrial DNA diversity of the Yellow Crazy Ant *Anoplolepis gracilipes* in Southeast Asia and the Indopacific

Drescher J, Blüthgen N, Schmitt T, Bühler J, Feldhaar H (2010) Societies drifting apart? Behavioural, genetic and chemical differentiation between supercolonies in the Yellow Crazy Ant *Anoplolepis gracilipes*. ***PLoS ONE*** 5(10): e13581

Drescher J, Blüthgen N, Schmitt T, Bühler J, Babis M, Feldhaar H (in prep) Worker aggression towards allocolonial reproductives facilitates speciation between supercolonies in the Yellow Crazy Ant *Anoplolepis gracilipes*

Drescher J, Feldhaar H, Blüthgen N (2011) Interspecific aggression and resource monopolization of the invasive ant *Anoplolepis gracilipes* in Malaysian Borneo. ***Biotropica*** 43: 93-99

Drescher J, Rinke S, Schmitt T, Feldhaar H, Blüthgen N (in prep) Catch me if you can: Agility of the ant guest *Myrmecophilus pallidithorax* as a major factor determining survival in colonies of the Yellow Crazy Ant *Anoplolepis gracilipes*

TABLE OF CONTENTS

I. ZUSAMMENFASSUNG

Die anthropogene Verschleppung und anschließende Etablierung invasiver Arten in neuen Habitaten ist einer der Hauptfaktoren des globalen Rückganges biologischer Vielfalt. Invasive Ameisen gehören zu den besonders schädlichen Invasoren und stellen fünf der 100 weltweit problematischsten invasiven Organismen. Ungeachtet einer Vielzahl von Studien über lokale ökologische Konsequenzen invasiver Ameisen sind die Mechanismen, die den Invasionen zugrunde liegen, kaum verstanden und weitestgehend auf die zwei Ameisenarten *Linepithema humile* und *Solenopsis invicta* beschränkt. Das Hauptziel der vorliegenden Dissertation war durch Untersuchung der ebenfalls invasiven Ameisenart *Anoplolepis gracilipes* zu einem besseren Verständnis der Mechanismen biologischer Invasionen und deren ökologischen Konsequenzen beizutragen.

Anoplolepis gracilipes ist eine in den Tropen weit verbreitete invasive Ameisenart, die in anthropogen gestörten Habitaten Südostasiens und des indopazifischen Raumes sehr häufig vorzufinden ist. Während detaillierte Informationen bezüglich ihres derzeitigen Verbreitungsgebietes vorliegen, ist ihre geographische Herkunft immer noch unbekannt. Weiterhin ist unklar, in welchem Maße die Sozialstruktur von *A. gracilipes* zu ihrer ökologischen Dominanz beiträgt und wie sich diese wiederum in einem potentiellen Herkunftsgebiet (Südostasien) darstellt.

Mitochondriale DNA-Sequenzen legen nahe, dass die überwiegende Mehrheit der im indopazifischen Raum vorkommenden Kolonien von südostasiatischen Populationen eingeführt wurde. Die südostasiatischen Kolonien entstammen möglicherweise einem bislang unbekanntem Ursprungsgebiet.

Verhaltenstests und genetische Analysen ergaben, dass Superkolonien von *A. gracilipes* aus sehr nah verwandten Individuen bestehen, womit sie stark vergrößerten Varianten monogyner, polydomer Kolonien anderer Ameisenarten ähneln. Ausserdem wiesen sowohl genetische Daten sowie Profile epikutikulärer Kohlenwasserstoffe auf eine erhebliche Differenzierung zwischen verschiedenen Superkolonien hin. Das Ausmaß der genetischen und chemischen Differenzierung deutet darauf hin, dass Genfluss zwischen Superkolonien stark reduziert oder sogar unterbrochen ist. Da die Paarung bei *A. gracilipes* wahrscheinlich nur im eigenen Nest stattfindet (Hochzeitsflüge wurden bislang noch nicht beobachtet), könnte eine positive Rückkopplung zwischen Aggression, Verwandtschaftsgrad und epikutikulärer Chemie dazu führen, dass die Differenzierung zwischen Superkolonien durch eine Kombination aus genetischer Drift und neutraler Evolution weiter verstärkt wird. Superkolonien, die nicht durch Genfluss miteinander im Austausch stehen, könnten sich also konsequenterweise in unterschiedliche evolutive Richtungen entwickeln. Eine der Möglichkeiten, durch die Genfluss zwischen verschiedenen Superkolonien aufrecht erhalten werden könnte, wäre deshalb die Einwanderung reproduktiver Individuen (Königinnen und Männchen) in fremde Superkolonien. Meine Untersuchungen ergaben, dass die Migration von Männchen und Königinnen zwische verschiedenen Superkolonien jedoch durch die Arbeiterinnen unterbunden wird, welche in erhöhten Maße aggressiv gegenüber Geschlechtstieren anderer Superkolonien waren. Weiterhin deuteten Kreuzungsexperimente zwischen koloniefremden Männchen und Königinnen darauf hin, dass Superkolonien von *A. gracilipes* unter Umständen schon reproduktiv isoliert sind, welches konsequenterweise zur Diversifizierung von *A. gracilipes* in verschiedene Arten führen sollte.

Bezüglich ihrer potentiellen ökologischen Dominanz in Nordost-Borneo konnte gezeigt werden, dass *A. gracilipes* die lokale Ameisenfauna erheblichen beeinflusst. Innerhalb der Superkolonien von *A. gracilipes* fanden sich sowohl weniger Ameisenarten als auch eine andere Artzusammensetzung als ausserhalb. Die Ergebnisse deuteten darauf hin, dass die ökologische Dominanz von *A. gracilipes* maßgeblich auf der Monopolisierung von Nahrungsquellen beruht. Diese wird ermöglicht durch eine Kombination aus schneller Rekrutierung von Nestgenossinnen, zahlenmäßiger Überlegenheit und ausgeprägter interspezifischer Aggression. *A. gracilipes* kommt fast ausschließlich in anthropogen gestörten Habitaten wie Wohngebieten oder landwirtschaftlich genutzten Flächen vor. Die zunehmende Habitatkonversion in Nordost-Borneo führt zu einem enormen Anstieg der von *A. gracilipes* besiedelbaren Habitate, so dass mit einem signifikanten Populationswachstum von *A. gracilipes* zu rechnen sein wird. Ein schnelles Populationswachstum sowie ökologische Dominanz sind jedoch nicht allein auf invasive Arten geprägte Charakteristika, sondern können auch bei nativen Arten zu beobachten sein, welche durch zunehmende Verfügbarkeit anthropogen veränderten Habitats zu Schädlingen werden können.

Abschließend wurden mehrere Arten potentieller Sozialparasiten in Nestern von *A. gracilipes* aufgefunden (mehrheitlich neue, unbeschriebene Arten), von denen die Grille *Myrmecophilus pallidithorax* eingehender untersucht wurde. Verhaltenstests und die Analyse kutikulärer Kohlenwasserstoffe zeigten, dass *M. pallidithorax* von ihrem Wirt bei jeder sich bietender

Gelegenheit angegriffen und sogar verzehrt wird. Jedoch kann sie den Aggressionen ihres Wirtes weitestgehend ausweichen dank schneller Fluchtreflexe sowie, möglicherweise, chemischer Tarnung.

Die vorliegende Dissertation zeigt, dass lang zurückliegende Invasionen die Unterscheidung zwischen eingeführten oder nativen Schädlingen erschweren, da beide tiefgreifende ökologische Einflüsse auf native Artengemeinschaften haben können. Es wurde weiterhin deutlich, dass die außergewöhnliche Sozialstruktur von invasiven Ameisen wie *A. gracilipes* ihre ökologische Dominanz begründet. Die Bildung von Superkolonien bei invasiven Ameisen stellt zudem nicht notwendigerweise eine evolutive Sackgasse dar, sondern kann im Gegenzug sogar zur Artbildung führen, begünstigt durch ungewöhnliche Paarungs- und Verbreitungsstrategien. Abschließend zeigte sich, daß die Untersuchung von Sozialparasiten invasiver Ameisen unter Umständen Rückschlüsse erlaubt bezüglich des historischen Ablaufes einer Invasion, der Biogeographie des invasiven Organismus sowie potentiellen Gegenmaßnahmen gegen die beteiligten Invasoren.

II. SUMMARY

The anthropogenic introduction and subsequent establishment of invasive alien species is a major factor contributing to decline of biodiversity in ecosystems worldwide. Invasive ants are particularly damaging invaders, constituting five of the 100 world's worst invasive alien organisms. While there are numerous reports describing the local ecological consequences of ant invasions are abundant, general insights on the underlying mechanisms are scarce and largely restricted to two ant species, i.e. the Argentine Ant *Linepithema humile* and the Red Imported Fire Ant *Solenopsis invicta*. The primary objective of this thesis was to broaden the spectrum of studied invasive ant species in order to facilitate a more general understanding of the mechanisms and ecological consequences of ant invasions.

The invasive Yellow Crazy Ant *Anoplolepis gracilipes* is a widespread tropical ant species which is particularly common in anthropogenically disturbed habitats in South-East Asia and the Indopacific region. Despite detailed information on its current distribution, its native range is unknown, and there is little information concerning its social structure as a potential mechanism facilitating invasion as well as its ecology in one of the putative native ranges, South-East Asia.

Using mitochondrial DNA sequences (mtDNA haplotypes), I demonstrated that the majority of the current Indopacific colonies were likely introduced from South-East Asian populations, which in turn may have been introduced much earlier from a yet unidentified native range.

By conducting behavioural, genetic and chemical analyses, I found that *A. gracilipes* supercolonies contain closely related individuals, thus genetically resembling enlarged versions of monogynous, polydomous colonies of other ant species. Furthermore, the data showed that mutually aggressive *A. gracilipes* supercolonies were highly differentiated both genetically and chemically, suggesting limited or even absent gene flow between supercolonies. Intranidal mating and colony-budding are most likely the predominant, if not the exclusive mode of reproduction and dispersal strategy of *A. gracilipes*. Consequently, a positive feedback between genetic, chemical and behavioural traits may further enhance supercolony differentiation though genetic drift and neutral evolution. This potential scenario led to the hypothesis that absent gene flow between different *A. gracilipes* supercolonies may drive them towards different evolutionary pathways, possibly including speciation. Thus, I examined one potential way by which gene flow between supercolonies of an ant species without nuptial flights may be maintained, i.e. the immigration of sexuals into foreign supercolonies. The results suggest that this option of maintaining gene flow between different supercolonies is likely impaired by severe aggression of workers towards allocolonial sexuals. Moreover, breeding experiments involving males and queens from different supercolonies suggest that different *A. gracilipes* supercolonies may already be on the verge of reproductive isolation, which might ultimately lead to the diversification of *A. gracilipes* into different species.

Regarding the ecological consequences of its potential introduction to NE-Borneo, I could show that *A. gracilipes* supercolonies may seriously affect the local ant fauna. The ant community within supercolonies was significantly less diverse and differed in species composition from areas outside supercolonies. My data suggest that the ecological dominance of *A. gracilipes* within local ant communities was facilitated by monopolization of food sources within its supercolony territory, achieved by a combination of rapid recruitment, numerical dominance and pronounced interspecific aggression. *A. gracilipes*' distribution is almost exclusively limited to anthropogenically altered habitat, such as residential and agricultural areas. The rate at which habitat conversion takes place in NE-Borneo will provide *A. gracilipes* with a rapidly increasing abundance of suitable habitats, thus potentially entailing significant population growth. An potentially increasing population size and ecological dominance, however, are not features that are limited to invasive alien species, but may also occur in native species that become 'pests' in an increasing abundance of anthropogenically altered habitat.

Lastly, I repeatedly detected several ant guests (most of them being new species) in supercolonies of *A. gracilipes*. I subsequently describe the relationship between one of them (the cricket *Myrmecophilus pallidithorax*) and its ant host. By conducting behavioural bioassays and analyses of cuticular hydrocarbon (CHC) profiles, I revealed that although *M. pallidithorax* is ferociously attacked and even consumed by *A. gracilipes* whenever possible, it may evade aggression from its host by a combination of supreme agility and, possibly, chemical deception.

Overall, this thesis adds to our general understanding of biological invasions by contributing species-specific data on a previously understudied invasive organism, the Yellow Crazy Ant *Anoplolepis gracilipes*. It demonstrates that introductions which may have occurred a considerably long time ago may make it difficult to determine whether a given species is an

introduced invader or a native pest species, as both may have pronounced ecological effects in native species communities. Furthermore, this thesis suggests that supercolonialism in invasive ants may not necessarily be an evolutionary dead end, but that it may possibly give rise to new species due to reproductive boundaries between supercolonies evoked by peculiar mating and dispersal strategies. Lastly, it shows that invasive species may be subject to parasitism, either by native or likewise introduced species, and that the nature of these interspecific associations may hold important information concerning invasion history, biogeography or even countermeasures against the respective alien invader.

II. Summary

III. GENERAL INTRODUCTION

The increasing human population with its growing demands for resources is globally altering natural ecosystems. Many of these changes are purposeful and intended to benefit society, such as agriculture or stock farming. Others, albeit intentional, have negative side effects that may threaten the goods and services delivered to society by natural ecosystems (Mooney 2005). These benefits to humankind include provisioning services (e.g. food, water, pharmaceuticals, energy), regulating services (e.g. disease, climate, decomposition, pollination) and supporting services (e.g. nutrient dispersal and circulation, seed dispersal, soil formation, primary production) (Millennium Ecosystem Assessment, MA, 2003). Many ecosystem services are positively associated to biodiversity, i.e. high levels of biodiversity are linked to reliable delivery of ecosystem services and vice versa (Worm et al. 2006; Nelson et al. 2009). Biodiversity in both terrestrial and marine ecosystems, however, is in worldwide decline (Whitmore and Sayer 1992; Jones et al. 2004; Sodhi et al. 2004; Clausen and York 2008), thus threatening the supply of ecosystem services to human society. There are several reasons for the massive decline of global biodiversity (see Swanson 1995) and among the most important threats ranks the establishment of invasive alien species (Mack et al. 2000a; Sala et al. 2000; Mooney and Cleland 2001).

Biological Invasions

Invasive organisms are found in a vast variety of taxa, including marine and terrestrial vertebrates and invertebrates, plants and fungi. The term ‘biological invasion’ usually refers to the ecologically harmful establishment of anthropogenically introduced organisms, regardless whether the organisms were introduced intentionally or accidentally. An important part of this definition is that alien species have to cause ecological damages to be termed ‘invasive’, as not all introduced organisms become established and not all established intruders become ecological problems (Williamson 1996). However, given the high frequency by which alien organisms are introduced into ecosystems worldwide, the number of species that become established and cause ecological problems can be quite large. For instance, of the ca. 1500 exotic insects that have become part of the U.S. insect fauna over the past 20 years, 16% are regarded invasive pests (i.e. 235 species, Pimentel 1993). The observation that only a fraction of introduced species turn out to be ecologically harmful (thus invasive) has led Williamson and Fritter (1996) to propose the ‘tens rule’, by which 10% of the introduced species become established, of which 10% become invasive. While some reports support the predictions made by the tens rule (e.g. Pimentel 1993; Boudouresque and Verlaque 2002), others show that the proportion of established species that become invasive can be much greater, often exceeding 50% (Kiritani and Yamamura 2003; Jeschke and Strayer 2005).

One of the most-cited hypotheses explaining the higher success of invasive species compared to native species is the ‘enemy-release hypothesis’ (‘ERH’) (Keane and Crawley 2002; Colautti et al. 2004; Liu and Stiling 2006; Liu et al. 2006). The ERH states that the success of an invading organism – often measured as abundance, population density or capacity to displace native species – is due to the scarcity of natural enemies (pathogens, parasites, predators) in its introduced range compared to its native range. Since its original proposition (Williamson 1996; Crawley 1997), various experimental studies have revealed supportive evidence verifying the ERH (Mitchell and Power 2003; Torchin et al. 2003; White et al. 2008; Adams et al. 2009; Cincotta et al. 2009). However, using the ERH as the sole explanation biological invasions is inappropriate, as a multitude of other factors may contribute to the success of individual invasive alien species (Colautti et al. 2004).

Just like invasive species, native organisms can also have negative ecological and economical impacts and are predominantly referred to as ‘pests’ (as opposed to ‘invasive alien species’). In the USA, approx. a third of the major crop weeds, about half of the pasture weeds and three quarters of the major forest pests are from within the country (Pimentel 1993; Mooney 2005). However, invasive alien species appear to be cause more serious problems than native species, at least in terms of weeds (Pimentel et al. 2005).

Consequences of biological invasions

Biological invasions may entail severe ecological consequences, ranging from increased resource competition for native species, regional displacement and even extirpation of individual native species to the alteration of entire ecosystems (reviewed by Crooks 2002). Well-known examples of ecologically devastating invaders include the Brown Tree Snake *Boiga irregularis*, which is held responsible for the extinction of 13 of the 22 native breeding birds as well as several bat and lizard species on Guam (Savidge 1987; Rodda and Savidge 2007), or the Nile perch *Lates niloticus* which contributed to the demise of dozens of cichlid species in the Great East-African Lakes (Witte et al. 2000; Verschuren et al. 2002; Aloo 2003). As a consequence, invasive alien species are often regarded as belonging to the most important drivers of the globally progressing environmental change (Vitousek et al. 1996;

Mack et al. 2000a; Sala et al. 2000; Mooney et al. 2005; Perrings et al. 2010). Moreover, invasive alien species often cause severe economic damage (Perrings et al. 2002; Born et al. 2005; Pimentel et al. 2005), either indirectly by the disruption of ecological processes (and thus a lowered supply of ecosystem services, see above) or directly by the infestation of agricultural facilities or other commercial structures. For instance, the costs for cleaning intake pipes of US-American waterworks from colonies of the invasive European zebra mussel *Dreissena polymorpha* have amounted to approx. 3.1 billion USD between 1993 and 2003 (Vitousek et al. 1996), and the combined costs for its economic damage and eradication measures are currently estimated to be as high as one billion USD per year (Pimentel et al. 2005). Overall, Pimentel et al. (2005) estimated the costs associated with the economic damages and control measures of more than 50,000 invasive alien species in the USA to be as high as 120 billion USD per year (Pimentel et al. 2005).

Vectors of biological invasions and susceptibility to invasion

There are various means by which invasive organisms reach previously uninhabited ecosystems (via ‘invasion vectors’). The vast majority of motorized vehicles used in both private and commercial traffic today (i.e. ships, planes, automobiles) act as invasion vectors, introducing up to dozens of alien species into foreign habitats in one go (reviewed by Ruiz and Carlton 2003). Terrestrial ecosystems that evolved in isolation, constrained by biogeographic barriers such as oceans or mountain ranges, have now become functionally connected due to the global human traffic which moves biological material biological material over long distances in relatively short amounts of time (Mooney 2005). Similarly, coastal ecosystems of different continents, though likewise separated by oceans, have become linked by the increasing amount of intercontinental cargo ships carrying a multitude of potentially invasive species and exotic pathogens in their ballast water tanks (Ruiz et al. 2000; Baier et al. 2003; Drake and Lodge 2004; Drake et al. 2007).

The vulnerability of ecosystems to this global exchange of biota, however, may differ between regions, with islands seeming particularly prone to biotic invasions (Loope and Mueller-Dombois 1989; Atkinson and Cameron 1993; McDonald and Cooper 1995). The susceptibility of island ecosystems to biotic invasions is reflected by the large proportion of non-indigenous species inhabiting their flora and fauna, e.g. up to 65% of all the vascular plant species found on Bermuda (Vitousek et al. 1996), nine out of 20 species in the Auckland islands avifauna (Case 1996) or more than 20% of all the biota on Hawaii (across various taxa, Eldredge and Miller 1997). The reasons for the higher susceptibility of islands to biotic invasions relative to comparable mainland areas has been subject to much debate, and is often attributed to the differences in biotic communities between islands and continents. Compared to continental ecosystems, biological islands usually support communities of low diversity and high differentiation (Sax and Brown 2000) which are often unsaturated (Moulton and Pimm 1986) and thus may contain wide or open ecological niches (e.g. Olesen et al. 2002). Diversity, in turn, may facilitate biotic resistance to invasion (Tilman 1997; Stachowicz et al. 2002), thus possibly explaining the lower invasion rates observed on continents compared to insular ecosystems.

Biological invasions by ants

Social insects, especially ants, rank among the most devastating groups of invaders (Moller 1996). More than 12,500 ant species have been described to date (*http://www.antbase.org/*; assessed in March 2011) of which approx. 150 have been introduced into new habitats by humans (McGlynn 1999a, b). The majority of introduced ant species remain confined to anthropogenically disturbed habitats. Several introduced ant species, however, have the capacity to invade natural ecosystems and are thus termed 'invasive' (Holway et al. 2002). The Global Invasive Species Database (GISD, managed by the Invasive Species Specialist Group ISSG of the IUCN Species Survival Commission) currently lists 19 ant species as invasive, five of which have been rated as belonging to the 100 worst invasive species (Lowe et al. 2000). These five ant species are the Yellow Crazy Ant *Anoplolepis gracilipes*, the Argentine Ant *Linepithema humile*, the Big-Headed Ant *Pheidole megacephala*, the Red Imported Fire Ant *Solenopsis invicta* and the Little Fire Ant *Wasmannia auropunctata*. Until recently, insights on the causes and consequences of ant invasions were mainly derived from studies on two focal species, i.e. *L. humile* and *S. invicta* (Holway et al. 2002). Like biological invasions by other taxa, successful establishment of invasive ant populations involve both economic and ecological damages. For instance, the establishment of *S. invicta* in the USA is estimated to cause crop losses and general damages amounting to 600 million USD p.a., followed by 400 million UDS p.a. for control and eradication measures (Pimentel et al. 2005). Ecologically, ant invasions have repeatedly been shown to entail significant decreases in diversity and abundance of both vertebrate and invertebrate populations (Holway 1998; Suarez and Case 2002; Suarez et al. 2005; Wetterer and Moore 2005; Davis et al. 2008; Hoffmann and Parr 2008). Occasionally, invasive ants may even alter the structure of entire ecosystems, e.g. by displacement of key-stone species (O'Dowd et al. 2003), competitive advantages over native invertebrates (McNatty et al. 2009), reduction of fruit and seed production of native plants (Lach et al. 2010), or by disruption of essential mutualisms such as seed dispersal (Christian 2001; Davis et al. 2010) or pollination (Blancafort and Gomez 2005). Moreover, invasive ants regularly affect native ant communities (Holway 1999; Human and Gordon 1999; Sanders et al. 2003; Lessard et al. 2009), which may lead to severe community-level changes given the diversity of roles that ants play in terrestrial ecosystems (Hölldobler and Wilson 1990; Holway et al. 2002). Invasive ants share several characteristics that facilitate their superiority over native ant species, both in terms of direct competition as well as indirect competition for resources. Among these features are pronounced interspecific aggression (Holway 1999; Human and Gordon 1999), superiority at resource discovery and monopolization (Davidson 1998; Holway 1999) and numerical dominance within the invaded habitat (Walters and Mackay 2005). Furthermore, the majority of invasive ant species forms large, polygynous and polydomous ‘supercolonies’ (see below), in which males and queens often mate within the nest (‘intranidal mating’) and which grow by ‘budding’, i.e. the establishment of additional nests sites at the supercolony fringe by supercolony subsets (‘propagules’) containing one or several queens, brood and workers (reviewed by Holway et al. 2002; Helanterä et al. 2009). This social structure may likely contribute to the ecological success of invasive ant species through positive feedback, i.e. the larger a supercolony gets, the more likely it is to outcompete other ant species and thus grow further (e.g. Helanterä et al. 2009). And finally, invasive ants may have a further advantage over native ant species in that they have repeatedly been shown to possess unusual reproductive systems, e.g. clonal reproduction of males and queens in the invasive longhorn ant *Paratrechina longicornis* (Pearcy et al. 2011) and little fire ant *Wasmannia auropunctata* (Fournier et al. 2005; Foucaud et al. 2009), which may further contribute to increased supercolony growth. All of these characteristics are, to a certain degree, connected to the special population structure that is almost exclusively limited to invasive ant species.

Population structure of invasive ant species

Most ant species show a multicolonial population structure, in which a population is subdivided into individual colonies which aggressively compete for resources such as territory and food. Such colonies are closed family units, usually containing one egg-laying queen and her often sterile daughter workers. Some ant species, however, show a unicolonial population structure, where local populations are characterized by weak or nonexistent colony boundaries and in which intraspecific aggression is reduced or absent (Wilson 1971; Hölldobler and Wilson 1977). Local populations of intraspecifically tolerant colonies are referred to as supercolonies (Giraud et al. 2002; Buczkowski et al. 2004; Jaquiery et al. 2005), where aggression between nests is absent and workers, queens, brood and food items are exchanged freely between nests. While the same may apply to polygynous (more than one queen) and polydomous colonies (more than one nest) of 'normal' ant species, established supercolonies contain "[…] such a large number of nests that direct cooperative actions are impossible between individuals in distant nests" (Pedersen et al. 2006). Invasive ant species have repeatedly been reported to form polydomous and polygynous supercolonies in their introduced range (Suarez et al. 2002; Espadaler et al. 2004; Errard et al. 2005; Pedersen et al. 2006; Thomas et al. 2006; Cremer et al. 2008; Ugelvig et al. 2008; Thomas et al. 2010a) and sometimes even in their native ranges (Pedersen et al. 2006; Vogel et al. 2009). Supercolonies of invasive ant species may be extremely large, as demonstrated by the main European supercolony of *L. humile* which covers thousands of kilometres along the coast of the Iberian Peninsular (Giraud et al. 2002; Jaquiery et al. 2005) and which may even span across several continents (Brandt et al. 2009b). While different supercolonies exhibit aggression between each other, the lack of aggression between nests from the same supercolony may be energetically advantageous, as costs associated with aggression are reduced (Reeve 1989; Steiner et al. 2007). This, in turn, may allow invasive ant supercolonies to sustain worker densities exceeding those of native ants, thus potentially entailing numerical superiority (Holway et al. 1998; Giraud et al. 2002; Suarez et al. 2008) and ecological dominance over native ants within the invaded habitat (Holway 1999; Morrison 2000; Holway et al. 2002).

Unicolonial populations and supercolonies have been reported from both invasive and non-invasive ant species (reviewed by Helanterä et al. 2009) as well as termites (Leniaud et al. 2009; Perdereau et al. 2010; Vargo and Husseneder 2011). The origins of supercolonialism (i.e. the ability to form supercolonies), however, are not entirely clear and several mechanisms have been proposed (e.g. Keller and Ross 1999; Tsutsui et al. 2000; Giraud et al. 2002; Steiner et al. 2007). In the Red Imported Fire Ant *Solenopsis invicta*, the formation of supercolonies seems to be exclusively linked to a single locus, General Protein Gp-9 (Keller and Ross 1999). Colonies of *S. invicta* can be either monogynous or polygynous (Glancey et al. 1987) according to the alleles that queens bear on the Gp-9 locus (Keller and Ross 1999). Gp-9 affects the expression of a fertility signal, which in turn affects aggression between *S.*

invicta colonies (Krieger 2005). Queens with a homozygote dominant genotype on this locus are independent colony foundresses, while queens with a heterozygote genotype jointly form polygynous colonies which are referred to as supercolonies (Macom and Porter 1996; Vander Meer and Porter 2001). Polygynous *S. invicta* supercolonies may be up to two times larger than monogyne colonies (Balas and Adams 1996; Macom and Porter 1996) and food is exchanged between different nests of the same supercolony, depending on the distance between nests, the amount of food reserves and the type of food being collected (Weeks et al. 2004). This type of supercolony formation, however, may be exclusively limited to *S. invicta*.

In other ant species, the situation is much less clear and a number of hypotheses, mainly derived from studies on the Argentine Ant *Linepithema humile*, are currently being discussed. One hypothesis is that supercolonies could be formed by the fusion of smaller colonies, as was observed in the strictly subterranean, non-invasive ant species *Lasius austriacus* (Steiner et al. 2007). Colony fusion has also been observed in *Linepithema humile* colonies under laboratory conditions (Vasquez and Silverman 2008; Vasquez et al. 2009) but empirical data supporting this scenario in wild supercolonies is lacking. Theoretically, fusion of small colonies to large supercolonies might be facilitated by two mechanisms, i.e. genetic similarity (and thus similarity in heritable recognition cues) of introduced colony subsets (proposed by Tsutsui et al. 2000, among others) or similarity of recognition cues irrespective of genetic similarity, e.g. as a result of frequency-dependent selection against colonies bearing rare recognition alleles (proposed by Giraud et al. 2002). In particular, Tsutsui et al. (2000) suggested that supercolonies in *Linepithema humile* may likely be the consequence of severe genetic bottlenecks accompanying the introduction and establishment of subsets of native populations (termed 'propagules') into previously uninhabited territory (supported by data from Brandt et al. 2009b). The reduced overall genetic variability, may likely be reflected by limited phenotypic variability in the heritable cues involved in nestmate recognition (Adams 1991; Pennanec'h et al. 1997; Takahashi et al. 2001; Brandt et al. 2009b; van Zweden et al. 2009) which are often cuticular hydrocarbons (CHC's)(Vander Meer and Morel 1998; Lahav et al. 1999; Akino et al. 2004; Torres et al. 2007; Brandt et al. 2009a). The reduced variability in CHC profiles, in turn, might thus facilitate the fusion of smaller, genetically and chemically similar propagules into large supercolonies (Tsutsui et al. 2000; Payne et al. 2004; Vasquez and Silverman 2008; Vasquez et al. 2009). However, since they detected low levels of relatedness in the very same supercolonies that Tsutsui et al. (2000) studied, Giraud et al. (2002) suggested that *L. humile* supercolonies might not have experienced a genetic bottleneck. Alternatively, Giraud et al. (2002) suggested that a reduced genetic variability at alleles involved in the biosynthesis of CHC’s (a potential prerequisite for tolerance in supercolonies) may have been the result of a ‘genetic cleansing’ process in which frequency-dependent selection against less common alleles after introduction might lead to genetically heterogeneous but chemically homogenous supercolonies. Both scenarios, however, seem to be incorrect. If *L. humile* supercolonies resulted from the fusion of unrelated small colonies, large supercolonies should be genetically more diverse than small supercolonies, which is not the case (e.g. Jaquiery et al. 2005). Furthermore, Pedersen et al. (2006) were able to show that in its native range, *L. humile* also forms supercolonies comprising unrelated individuals, implying that the formation of supercolonies in *L. humile* may not a phenomenon that follows upon introduction into a new habitat.

Thus, the emergence of large *L. humile* supercolonies in its introduced range may be the result of a developmental transformation rather than an evolutionary one (Helanterä et al. 2009). Assuming that colony-fusion is an inappropriate hypothesis, supercolonies inevitably start small, i.e. from small colony fragments (propagules) or single mated queens dispersing on the wing. As a consequence, genetic bottlenecks are likely to be a common starting-point among supercolonies (Helanterä et al. 2009). Like in other biotic invasions, relaxed ecological constraints such as release from predators or pathogens may facilitate supercolony growth (Holway et al. 2002; Cremer et al. 2008) until it is hampered by rivalling supercolonies or unsuitable habitat. The mode by which supercolonies usually grow (i.e. intranidal mating and colony budding) is a mix of reproduction and dispersal strategies that should favour family-based supercolonies, as is supported by high relatedness values within supercolonies of various invasive and non-invasive ant species (Helanterä et al. 2009).

Focal species and aim of the study

Anoplolepis gracilipes (Smith)(Formicidae, Formicinae), formerly known as *Anoplolepis longipes* (Emery 1925), *Formica longipes* (Jerdon 1851) and *Plagiolepis longipes* (Emery 1887), is an invasive ant species that occurs over large areas in South-East Asia and the Indopacific islands and archipelagos (reviewed in Wetterer 2005). This ant species has received worldwide attention due to its local displacement of a key-stone omnivore, the endemic red land crab *Gecarcoidea natalis* on Christmas Island, Indian Ocean (O'Dowd et al. 2003), leading to structural changes in the island forest ecosystem (Davis et al. 2008; Davis et al. 2010). Apart from its negative impact on individual invertebrate species (McNatty et al.

2009) and even entire invertebrate communities (Holway et al. 2002; Hill et al. 2003; Gerlach 2004; Lester and Tavite 2004), infestations of *A. gracilipes* may also affect vertebrate species such as reptiles (Feare 1999) and birds (Gerlach 2004; Davis et al. 2008; Matsui et al. 2009; Davis et al. 2010; Lach and Hooper-Bùi 2010).

Despite detailed information on its current distribution, its origin remains elusive (Wetterer 2005). While some authors suggested that *A. gracilipes* may be native to some parts of tropical Asia, especially India (due to the oldest records), others considered East Africa as the origin of this species as the genus *Anoplolepis* is almost exclusively African (e.g. Bolton 1995; reviewed by Wetterer 2005). Historical records, ecological data and the repeated detection of new incursions demonstrate that *A. gracilipes* is introduced and invasive in Japan (Matsui et al. 2009), Australia (Hoffmann and Saul 2010), New Zealand (Ward et al. 2006) and the Indopacific region, e.g. on Hawaii (Krushelnycky et al. 2005; Kirschenbaum and Grace 2007), the Seychelles (Gerlach 2004), Tokelau (Lester and Tavite 2004; Abbott et al. 2007) and Christmas Island (O'Dowd et al. 2003; Thomas et al. 2010a). In South-East Asia, *A. gracilipes* thrives especially in disturbed habitats such as private orchards and corporate oil palm plantations on Borneo (Pfeiffer et al. 2008; Brühl and Eltz 2009; Drescher et al. 2011), cacao agroforests on Sulawesi (Bos et al. 2008; Wanger et al. in press) and secondary forests on Papua New Guinea (Klimes et al. 2011).

Like other invasive ant species such as *Lasius neglectus* (Ugelvig et al. 2008), *Linepithema humile* (Jaquiery et al. 2005), *Pheidole megacephala* (Fournier et al. 2009), *Solenopsis invicta* (Vander Meer and Porter 2001) and *Wasmannia auropunctata* (Le Breton et al. 2004), *A. gracilipes* forms large, polygynous (multi-queens) and polydomous (multiple nests) supercolonies of extremely high worker densities (Abbott 2005; Abbott 2006; Lester et al. 2009). Queens usually mate within the nest (intranidal mating) and small-scale dispersal occurs via budding, i.e. occupation of a suitable nest at the supercolony fringe by one or several queens accompanied by workers and sometimes brood (‘propagule’) from the queens’ maternal nest (Holway et al. 2002). Despite the capture of winged males and females in malaise traps on Christmas Island (Abbott 2006), active long-range dispersal by *A. gracilipes* has yet to be observed. In contrast, passive long-range dispersal via private and commercial human traffic (termed ‘human-mediated jump-dispersal’) is well documented (i.e. Harris et al. 2005) and may potentially represent *A. gracilipes*’ only form of dispersal over long distances.

Despite the numerous scientific publications describing its ecological impact in the Indopacific region, *A. gracilipes* belongs to the lesser studied invasive ant species, particularly with regard to its origin, its ecology in the native range and the way by which supercolonies are structured. With this thesis I aim to fill some of these gaps in order to contribute to the general insights on the mechanisms of biological invasions and its consequences for biodiversity and community composition. In particular, I studied genetic relationships between populations in one of *A. gracilipes*' putative native ranges (South-East Asia) and its introduced range (Indopacific region) to shed light on *A. gracilipes*' origin. If *A. gracilipes'* origin were South-East Asia, its population there ought to be characterized by considerably higher genetic diversity than the population in the Indopacific region, and individual matrilines might be found in both regions, indicating rather recent transfer. Based on the observations from other invasive ant species (see above), however, I did not expect to find a difference in social structure between both regions. I furthermore studied *A. gracilipes'* role in local ant communities as well as its interactions with ant guests in North-East Borneo to learn more about its ecology in a putative native range. If South-East Asia was *A. gracilipes'* native region, I would expect to find comparably small supercolonies (as in *Linepithema humile's* native region, Pedersen et al. 2006) and potentially many mutualistic and antagonistic interspecific relationships, such as species-specific trophobioses or coevolved parasites. In turn, if *A. gracilipes* had been introduced to South-East Asia, I would expect larger and ecologically destructive supercolonies similar to those in the Indopacific. However, large populations of *A. gracilipes* and ecological damage alone would be too little evidence to infer that *A. gracilipes'* was introduced to South-East Asia, as native species adapted to naturally disturbed ecosystems may become serious pests if anthropogenic land use results in dramatically increased availability of suitable habitat. Thus, I measured behavioural, genetic and chemical properties of different *A. gracilipes* supercolonies to gain insights into its population structure in a potential native region. Furthermore, detailed information on the structure of *A. gracilipes* may hold important information on the transferability of mechanisms of supercolony-formation identified in a few focal species (above all *L. humile* and *S. invicta*) to other invasive and non-invasive supercolonial ant species. And finally, as supercolonialism in ants may be an evolutionary dead end (Helanterä et al. 2009), revealing the structure of supercolonies and the interactions between them may provide insights into their evolutionary future.

IV. THE ECOLOGY AND POPULATION STRUCTURE OF THE INVASIVE YELLOW CRAZY ANT *ANOPLOLEPIS GRACILIPES* – A SYNOPSIS

The global spread and establishment of invasive ant species poses a serious thread to ecosystems worldwide. While numerous reports describe local ecological impacts of many invasive ant species, most of our knowledge on the mechanisms behind ant invasions is derived from research on two focal species, the Argentine Ant *Linepithema humile* and the Red Imported Fire Ant *Solenopsis invicta* (Holway et al. 2002). However, in order to understand biological invasions in general and invasive ants in particular, studies on other invasive ant species are required.

The primary objective of this study was to contribute to the insights on biological invasions by studying the invasive Yellow Crazy Ant *Anoplolepis gracilipes*. In particular, I explored the genetic relationship between *A. gracilipes* populations from its introduced range (Indopacific) and one of its putative native ranges (South-East Asia), providing first genetic evidence of *A. gracilipes'* potential introduction history I further examined behavioural, genetic and chemical properties of different *A. gracilipes* supercolonies, thus revealing *A. gracilipes'* mode of supercolony formation as well as the evolutionary potential of supercolonialism in an invasive ant. And finally, I studied the ecology of *A. gracilipes* in South-East Asia, in particular its interactions with the local ant fauna and its association with several ant guest species, and discuss my findings with regard to the study area as a potential native region of this species.

In CHAPTER I, I present data on the genetic diversity of *Anoplolepis gracilipes* populations in South-East Asia and the Indopacific region. The current distribution of *A. gracilipes* stretches across tropical regions worldwide, with the majority of records stemming from South-East Asia and the Indopacific (Fig. 1). While there is no doubt that *A. gracilipes* has been introduced to large parts of the Indopacific, its native region in general and its status in South-East Asia in particular remain unclear (Wetterer 2005). Mitochondrial DNA sequences (mtDNA 'haplotypes') of workers from 118 locations in 15 countries revealed considerably high haplotype diversity but very low nucleotide diversity in both the South-East Asian and the Indopacific population, suggesting that *A. gracilipes* may have experienced severe genetic bottlenecks in both regions followed by rapid population growth. Despite being low in both

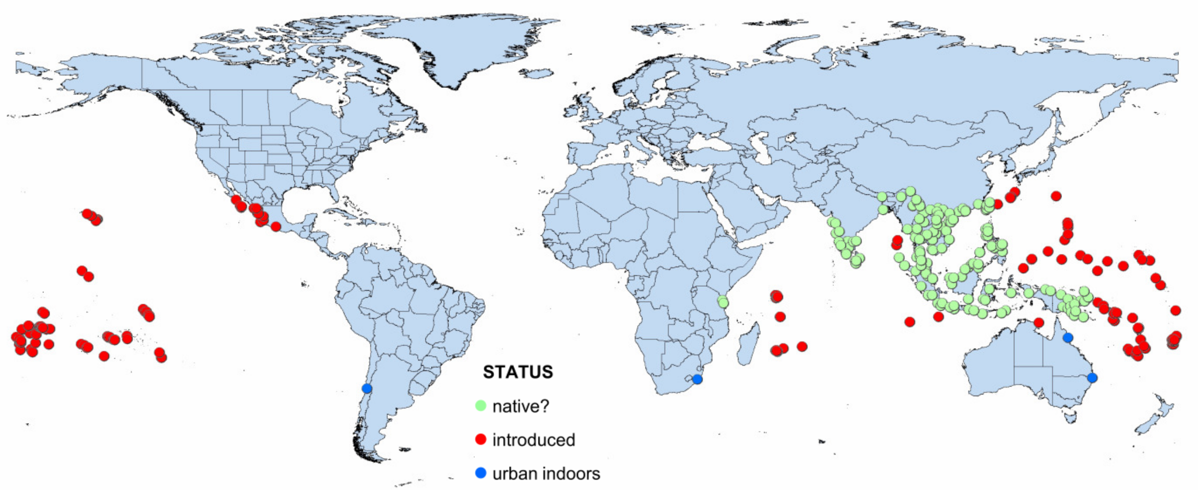


Figure 1. Global distribution of the invasive Yellow Crazy Ant *Anoplolepis gracilipes*. The colour coding represents *Anoplolepis gracilipes'* invasional status in tropical regions worldwide with South-East Asia indicated as one of the potential native ranges (with kind permission from Darren Ward, Landcare Research, New Zealand, 2005).

regions, nucleotide diversity in the Indopacific was half the nucleotide diversity of the South-East Asian population, implying that the genetic bottleneck experienced by the Indopacific population may have been more severe and may have occurred more recently. Taking into account the detection of matching haplotypes between the South-East Asian and the Indopacific population, I suggest that *A. gracilipes* may have been first introduced to South-East Asia from a yet unidentified native region (possibly from India in the early 19th century) and subsequently to the Indopacific islands from South-East Asian source populations.

In CHAPTERS II and III, I explore the population structure of *Anoplolepis gracilipes* as well as its evolutionary implications based on behavioural, genetic and chemical analyses of several supercolonies. CHAPTER II contains a detailed investigation of six *A. gracilipes* supercolonies from Poring Hot Springs in North-East Borneo, combining data from behavioural bioassays, analysis of nuclear microsatellite markers and GC-MS analyses of cuticular hydrocarbon (CHC) profiles (Drescher et al. 2010). The study indicated that *A. gracilipes* supercolonies contain very closely related individuals, with relatedness values between workers even exceeding those that would be expected in monogynous colonies of singly mated queens. Supercolonies of *A. gracilipes* thus differ substantially from supercolonies of *Linepithema humile* and *Solenopsis invicta*, which may contain unrelated individuals (Bernasconi and Strassmann 1999; Pedersen et al. 2006). The extreme genetic similarity between individuals in *A. gracilipes* supercolonies is likely due to almost exclusive intranidal mating (i.e. inbreeding in the case of *A. gracilipes*) and an unusual reproductive system that may involve clonal reproduction of queens (Drescher et al. 2007). Two pairs of the six spatially separated supercolonies were closely related, showed high cuticular hydrocarbon (CHC) profile similarity and tolerated each other in bioassays, suggesting that they represented established, anthropogenically dispersed fragments of the same supercolony. With the exception of these two pairs of mutually tolerant supercolony fragments, workers from different supercolonies were highly aggressive towards each other (Fig. 2) and were profoundly differentiated both genetically and chemically. In particular, several supercolonies contained private microsatellite alleles, and CHC profiles of mutually aggressive supercolonies showed qualitative differences, i.e. the profiles partially comprised different compounds. The extent of qualitative differences between CHC profiles of different *A. gracilipes* supercolonies is remarkable, because in most ants, CHC profiles differ quantitatively between conspecific colonies and qualitatively between species (Vander Meer and Morel 1998). Overall, the data suggested that gene flow between mutually aggressive *A. gracilipes* supercolonies may be extremely limited or even absent. A positive feedback between genetic, chemical and behavioural traits may further enhance supercolony differentiation through genetic drift and neutral evolution.

Figure 2.
Intercolonial aggression between workers of *Anoplolepis gracilipes*. In an initial phase of aggression between *A. gracilipes* workers from different super-colonies, workers will often grasp the intruder by its legs and antennae. (Photo: J. Drescher)

CHAPTER III contains a separate study which confirmed the results and implications of the aforementioned study (CHAPTER II). The study was conducted in a different NE-Bornean region (Sepilok Forest Reserve) and comprised behavioural, genetic and chemical analyses of eleven local supercolonies as well as three supercolonies from Poring Hot Springs. In addition to the analysis of nuclear microsatellites (CHAPTER II), I examined mtDNA sequences of the 14 supercolonies (see CHAPTER I) to study the extent of genetic differentiation between supercolonies. The study revealed that intercolonial differentiation in mitochondrial DNA corresponded to microsatellite and CHC profile differentiation as well as patterns of aggression between different supercolonies. Gene flow between different *A. gracilipes* supercolonies is extremely limited or even absent (Drescher et al. 2010; Thomas et al. 2010a), which may be largely due to strict intranidal mating and absence of nuptial mating flights (Drescher et al. 2007; Drescher et al. 2010). Hence, the emigration of males and queens from their own supercolony to foreign supercolonies may represent the only way by which gene flow between different supercolonies may be maintained. Behavioural assays between workers and sexuals (males/queens) from mutually aggressive supercolonies, however, revealed pronounced aggression of workers towards males and queens from foreign supercolonies (Fig. 3A, 3B), thus possibly impairing gene flow between different supercolonies by means of inhibiting the migration of sexuals between supercolonies. As gene flow between different supercolonies may have been reduced or even been absent for quite some time, *A. gracilipes* supercolonies may already be genetically and chemically sufficiently different to hamper mating and colony founding by males and queens from different supercolonies. In a series of preliminary breeding experiments using laboratory colonies, I tested whether queens still retained the capability to mate with males from different supercolonies and whether queens fertilized in such a way were able to start new colonies. The experiments demonstrated that despite mating between allocolonial sexuals occurred (as confirmed by filled spermathecae of queens that were raised as virgins) and despite the fact that the queens laid eggs, no viable worker offspring was produced within six months, which eventually led to the demise of the experimental subcolonies. Thus, reproductive isolation between different *A. gracilipes* supercolonies may possibly already impede gene flow between them, which should consequently lead to the evolutionary divergence of different *A. gracilipes* supercolonies into different species.

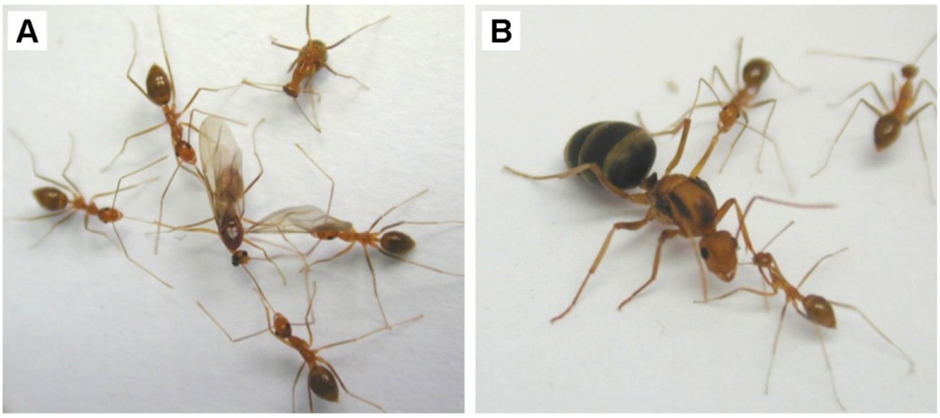


Figure 3. Aggression of *Anoplolepis gracilipes* workers towards sexuals from different supercolonies. (A) Worker aggression towards allocolonial males and (B) worker aggression towards allocolonial queens (Photos: J. Drescher)

In CHAPTERS IV and V, I focus on *A. gracilipes'* ecology. CHAPTER IV describes the ecological dominance of *Anoplolepis gracilipes* within an ant community in North-East Borneo (Drescher et al. 2011). Like most invasive organisms, *A. gracilipes* is generalistic and opportunistic in both feeding and nest site preferences. This ant is omnivorous and can frequently be observed foraging honeydew (Fig. 4A) and both live and dead arthropods (Fig. 4B). Mimicking sugar- and protein-based food resources with honey and tuna baits, I could demonstrate that within supercolonies, ant diversity was much lower than outside supercolonies. Moreover, *A. gracilipes* detected food resources quicker than other ants and aggressively defended them until they were fully exploited. This combination of early detection and subsequent monopolization of food items has also been described in the invasive ant *Linepithema humile* (Davidson 1998; Holway 1999) and may trigger the release from the dominance-discovery trade-off, which describes that ants are subject to a trade-off between rapid resource discovery and subsequent domination of resource exploitation. The dominance-discovery trade-off is perceived as a mechanism facilitating species coexistence, thus breaking the trade-off may promote *A. gracilipes'* ecological dominance in ant communities and the potential displacement of individual ant species within its supercolony boundaries. However, the worker density in an ant colony and its competitive abilities in resource discovery and monopolization are linked (Oliver et al. 2008), thus making it difficult to disentangle whether breaking the trade-off is a cause or rather a consequence of high worker densities in *A. gracilipes* supercolonies. The study further suggested that although *A. gracilipes*' potential capability to displace native ants and its superiority in resource acquisition may be intertwined, its ecological success in NE-Borneo may be fundamentally based to its elevated interspecific aggression. In arena bioassays, *A. gracilipes* readily engaged in mortal encounters with seven native ant species (including *Dolichoderus thoracicus*, Fig. 4C) and survived significantly longer than the majority of the species tested, occasionally even when being outnumbered tenfold (e.g. in bioassays against *Crematogaster coriaria*). The weaver ant *Oecophylla smaragdina* was the only ant species being able to

Figure 4. Feeding preferences and interspecific aggression of *Anoplolepis gracilipes*. As a generalist feeder, *A. gracilipes* gathers nutrients from various sources such as (A) sugar-rich honeydew from hemipteran trophobionts and (B) protein from prey items or carrion. *A. gracilipes* is highly aggressive towards other ants such as (C) *Dolichoderus thoracicus*, and dead ants from other species can frequently be found on refuse piles inside *A. gracilipes* nests. (Photos by J. Drescher)

withstand *A. gracilipes'* elevated interspecific aggression, potentially due to its size and its increased aggressive behaviour as a dominant ant species. However, other large, feisty ant species such as *Diacamma* sp. or *Odontomachus* sp. were frequently attacked and pursued by individual *A. gracilipes* workers, overall suggesting that *A. gracilipes* may dispose of superior fighting skills compared to many ant species of comparable size.

CHAPTER V deals with the association between *Anoplolepis gracilipes* and a cleptoparasitic ant guest, the myrmecophilous cricket *Myrmecophilus pallidithorax* (Fig. 5A, 5B). Among several ant guests (such as the Handsome Fungus Beetle *Trochoideus desjardinsi*, two unidentified species of tropical log beetles of the genus *Aphanocephalus* and two species of gnat bugs, *Oncylocotis spp.*, see Štys et al. 2010), *Myrmecophilus pallidithorax* was found in the majority of *A. gracilipes* supercolonies in NE-Borneo that were screened for ant guests. Behavioural experiments involving the exchange of intact and manipulated crickets (manipulated = hind legs removed by induced autotomy to reduce mobility) between experimental subcolonies from different *A. gracilipes* supercolonies indicated that *A. gracilipes* was capable of detecting the cricket, but failed to catch and kill it due to the supreme agility of the cricket. The experiments further suggested that chemical deception may play an important role in the infestation of *A. gracilipes* supercolonies by *M. pallidithorax*, as the mortality of manipulated crickets was slightly lower when ants and crickets were sampled from the same supercolony than when sampled from different supercolonies. Finally, preliminary data of cuticular hydrocarbon (CHC) profiles revealed considerable congruency between CHC profiles of *A. gracilipes* and *M. pallidithorax*. The lack of replication across several *A. gracilipes* supercolonies, however, does to allow conclusions as to whether *M. pallidithorax* uses chemical mimicry or chemical camouflage to reduce aggression from its host.

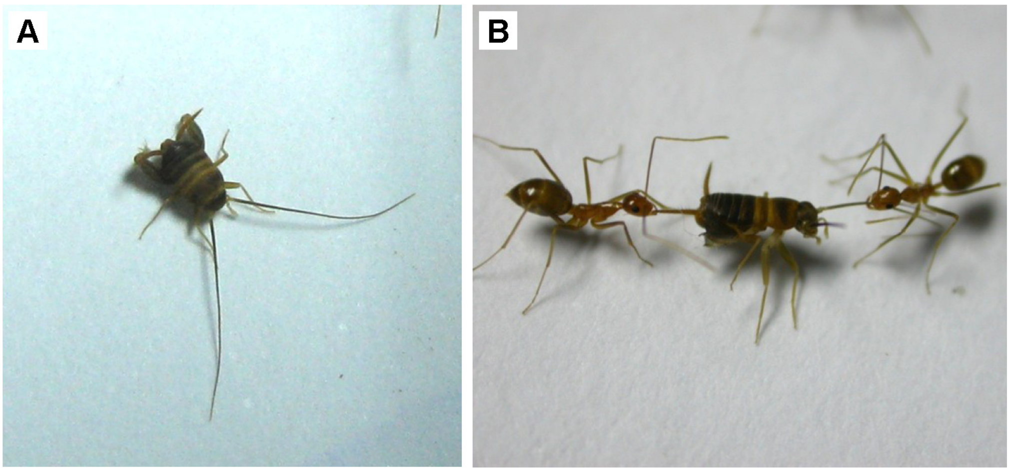


Figure 5. *Myrmecophilus pallidithorax*, one of the social parasites of *Anoplolepis gracilipes*. (A) Intact cricket from top view and (B) manipulated cricket being attacked by *A. gracilipes* workers. (Photos: J. Drescher)

V. MITOCHONDRIAL DNA DIVERSITY OF THE YELLOW CRAZY ANT *ANOPLOLEPIS GRACILIPES* IN SOUTHEAST ASIA AND THE INDOPACIFIC

ABSTRACT

Identifying the native ranges and invasional pathways of invasive organisms is crucial for the development of management strategies and preventive measures. This study focused on the invasive Yellow Crazy Ant *Anoplolepis gracilipes*, of which the native range is unclear. We compared samples from the introduced range (Indopacific region) to samples from one of its putative native ranges (South-East Asia) to resolve whether *A. gracilipes* might be native to SE-Asia and to identify the origin of the Indopacific colonies. We analyzed mtDNA sequences of 118 individuals obtained from different nests. After genotyping with two mitochondrial DNA markers, we calculated genetic diversity using standard indices and constructed a haplotype network. Haplotype diversities in the SE-Asian and the Indopacific samples were similarly high. Nucleotide diversity, however, was low SE-Asia and even lower in the Indopacific, indicating recent genetic bottlenecks followed by rapid population growth in both regions. Overall, we detected 29 haplotypes, of which three were shared by samples from both SE-Asia and the Indopacific. Both regions contained large proportions of private haplotypes as well as several singletons (haplotypes found in only one sample). Our data indicate that both the SE-Asian and the Indopacific population of *A. gracilipes* may have experienced a genetic bottleneck, which may have been more severe in the Indopacific. This suggests that *A. gracilipes* may have initially been introduced to SE-Asia followed by subsequent introduction to the Indopacific from SE-Asian populations, which is supported by matching haplotypes in samples from both regions. Furthermore, *A. gracilipes* populations in both regions may be subject to diversification processes, leading to the evolution of novel mtDNA lineages. We suggest sampling and genotyping *A. gracilipes* samples from other putative native ranges (e.g. southern India) to confirm these findings.

INTRODUCTION

Biological invasions threaten local biodiversity in many habitats worldwide through negatively affecting native taxa (e.g. Kenis et al. 2009), thus frequently entailing both economic and environmental damage in the regions affected (Pimentel et al. 2005; Ehrenfeld 2010; Perrings et al. 2010). In order to reduce the impact of biotic invasions, studying invading species within their home ranges is crucial to develop biocontrol strategies through identifying potential antagonistic organisms (Messing and Wright 2006). Likewise, locating the origin of an invasive organism is imperative for devising preventive measures such as intercepting invasional pathways by targeting vectors of invasion (Mack et al. 2000a; Myers et al. 2000; Carlton and Ruiz 2005; Geller et al. 2010). Hence, knowing where an invader comes from, which community it evolved in and how it was introduced into the invaded habitat may enable us not only to understand the mechanism of its invasion, but further facilitates the development of both management strategies and preventive measures. Yet there are numerous invasive species whose native range or introduction pathway is unknown (Geller et al. 2010). Similarly, there is a vast number of cryptogenic species (*sensu* Carlton 1996), i.e. species that cannot be labeled as being either native or introduced in a given habitat due to lack of sufficient biogeographic, historical, systematic or ecologic data (Hewitt et al. 2004; Neves and da Rocha 2008; Geller et al. 2010; Ignacio et al. 2010). The recent progress in molecular markers, however, has provided adequate genetic tools to target these questions in invasion biology, as has repeatedly been shown by retrospectively constructing individual invasion histories and introduction pathways of invasive organisms (Jousson et al. 2000; Durka et al. 2005; Grapputo et al. 2005; Corin et al. 2007; Caldera et al. 2008; Ugelvig et al. 2008; Iriarte et al. 2009; Foucaud et al. 2010).

In this study we analyzed the genetic relationship between supercolonies of the invasive Yellow Crazy Ant *Anoplolepis gracilipes* from Southeast Asia and the Indopacific region. This invasive ant species has been brought to worldwide attention due to its local displacement of a key-stone omnivore, the endemic red land crab *Gecarcoidea natalis* on Christmas Island, Indian Ocean (O'Dowd et al. 2003), leading to structural changes in the island forest ecosystem (Davis et al. 2008; Davis et al. 2010). Apart from its negative impact on individual invertebrate species (McNatty et al. 2009) and even entire invertebrate communities (Holway et al. 2002; Hill et al. 2003; Gerlach 2004; Lester and Tavite 2004), infestations of *A. gracilipes* may also affect vertebrate species such as reptiles (Feare 1999) and birds (Gerlach 2004; Davis et al. 2008; Matsui et al. 2009; Davis et al. 2010; reviewed in Lach and Hooper-Bùi 2010). Like other invasive ant species such as *Lasius neglectus* (Ugelvig et al. 2008), *Linepithema humile* (Jaquiery et al. 2005), *Pheidole megacephala* (Fournier et al. 2009) and *Wasmannia auropunctata* (Le Breton et al. 2004), *A. gracilipes* forms large, polygynous (multi-queens) and polydomous (multiple nests) supercolonies of extremely high worker densities (Abbott 2005; Abbott 2006; Lester et al. 2009). Queens usually mate within the nest (intranidal mating) and small-scale dispersal occurs via budding, i.e. occupation of a suitable nest at the supercolony fringe by one or several queens accompanied by workers and sometimes brood (termed propagule) from the queens' aboriginal nest (Holway et al. 2002; Drescher et al. 2010). Active long-range dispersal is unknown in this species. However, passive long-range dispersal occurs nonetheless as propagules of this species are inadvertently introduced into new habitats via human-mediated dispersal, e.g. in soil, agricultural products, timber, packaging material, sea containers or automobiles (both local traffic and shipments)(Harris et al. 2005, pers. obs.). The current distribution of *A. gracilipes* is almost exclusively limited to tropical regions worldwide, with the highest density of populations found in Southeast Asia and the Indopacific region (Wetterer 2005). However, the origin of this species, which was first described as *Formica longipes* in India (Jerdon 1851), remains unclear. Several authors suggested that *A. gracilipes* may be native to some parts of tropical Asia, especially India (due to the oldest records), while others considered East Africa as the origin of this species as the genus *Anoplolepis* is known to be almost exclusively African (see Wetterer 2005 for references). Historical records, ecological data and the repeated detection of new incursions demonstrate that *A. gracilipes* is introduced and invasive in Japan (Matsui et al. 2009), Australia (Hoffmann and Saul 2010), New Zealand (Ward et al. 2006) and the Indopacific region, e.g. on Hawaii (Krushelnycky et al. 2005; Kirschenbaum and Grace 2007), the Seychelles (Gerlach 2004), Tokelau (Lester and Tavite 2004; Abbott et al. 2007) and Christmas Island (O'Dowd et al. 2003; Thomas et al. 2010a).

In all regions including the potential native range of SE-Asia, A. gracilipes thrives especially in disturbed habitats. High-density supercolonies have been reported from orchards and oil palm plantations on Borneo (Pfeiffer et al. 2008; Brühl and Eltz 2009; Drescher et al. 2011), cacao agroforests on Sulawesi (Bos et al. 2008; Wanger et al. in press) and secondary forests on Papua New Guinea (Klimes et al. 2011). This almost exclusive limitation of its distribution to anthropogenically disturbed habitats and its ecological dominance may indicate that *A.*

gracilipes is invasive in SE-Asia. Alternatively, *A. gracilipes* may potentially be a native species adapted to naturally disturbed habitats (e.g. annually flooded swamp forests along the river Kinabatangan in Borneo (Drescher et al. 2007), which has become increasingly abundant due to an increasing availability of suitable, anthropogenically disturbed habitat.

Thus, in this study, we investigated whether *A. gracilipes* may have originated in Southeast Asia by analyzing mitochondrial DNA diversity in samples from both the introduced and the putative native range. In particular, based on the repeated observation that invasive populations experience severe genetic bottlenecks upon introduction (e.g. Tsutsui et al. 2000; Suarez et al. 2008; Geller et al. 2010), we should expect to detect higher genetic variability in the native range (potentially SE-Asia) than in the introduced range (Indopacific). Taking into account that the introduction of *A. gracilipes* to some Indopacific habitats has occurred within the past 100 years (O'Dowd et al. 1999; O'Dowd et al. 2003) and still occurs up to date (Harris et al. 2005), we should furthermore expect to find matching haplotypes between SE-Asia and the Indopacific, thus enabling us to identify pathways of introduction. Finally, provided there are populations that have been established long enough to acquire novel mutations *in situ* (as may be the case on Christmas Island, Thomas et al. 2010a), we should expect to find unique haplotypes in the introduced range that cannot be found in the putative native range.

METHODS

Anoplolepis gracilipes workers were sampled from a total of 118 locations in 15 countries throughout Southeast Asia and the Indopacific region (Fig. 6). Malaysian Borneo is overrepresented in this data set, as detailed studies focusing on the population structure, behaviour and ecology of *A. gracilipes* were conducted in this area (Drescher et al. 2007; Drescher et al. 2010; Stys et al. 2010; Drescher et al. 2011). Individuals were collected near the nest entrance to ensure supercolony affiliation and immediately transferred into 99.8% EtOH p.A..

As preliminary surveys revealed that there was never more than one mtDNA haplotype to be found per supercolony, we extracted DNA from a single worker per location (putative supercolony) using the PUREGENE® DNA purification kit. The obtained DNA pellet was resuspended in 50µl double distilled water and stored at -20°C. Mitochondrial DNA was

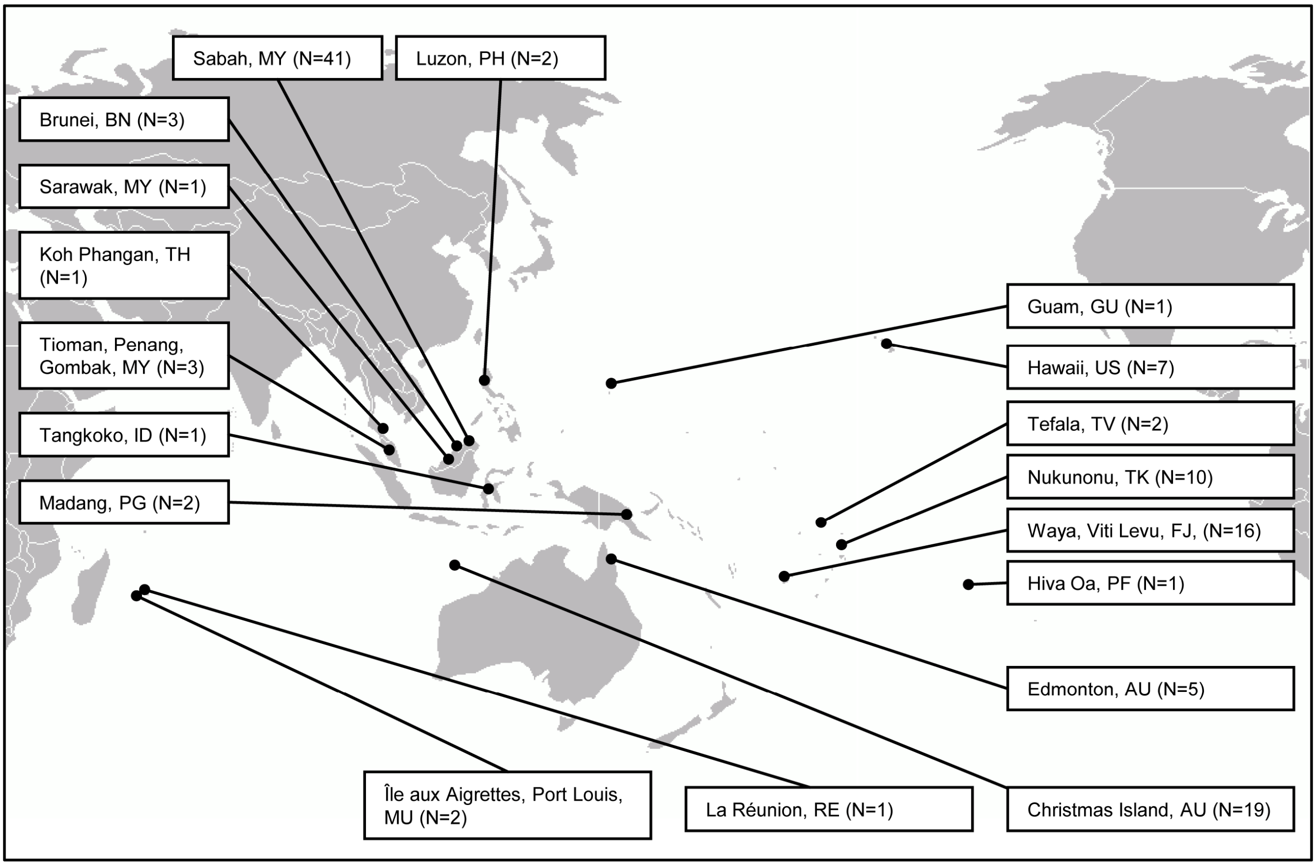


Figure 6. Locations of *Anoplolepis gracilipes* sampling sites throughout Southeast Asia and the Indopacific region. Sampling site labels are arranged according to the invasional status of *A. gracilipes* in its known range (Wetterer 2005), i.e. invasive in the Indopacific (lower right) and potentially native in Southeast Asia (upper left).

amplified using the primer pairs Horst (5′-AC(TC)-ATACTTTTAACTGATCG-3′) designed by D. Kronauer (unpublished)/Ben (5′-C(AT)AC(AT)AC(AG)TAATA(GT)GTATCATG-3′) (Moreau et al. 2006) for partial Cytochromeoxidase I (COI) (Genbank Accession nos DQ888821–DQ888825) corresponding to positions 2407–2427 (Horst) and 2891–2914 (BEN) relative to the mitochondrial genome of *Apis mellifera* (Crozier and Crozier 1993) and CBI/CBII for partial cytochrome b (cyt*b*) (Genbank Accession nos DQ888817–DQ888820) (Crozier et al. 1991).

PCR was performed in a total reaction volume of 25 µl containing approximately 10 ng of template DNA, 1x PCR buffer, 2 mM $MgCl_2$, 240 µM dNTP's, 800 µM of both forward and reverse primer and 1.2 U of Taq DNA polymerase (Mol*Taq* byMolzym GmbH or Genaxxon BioScience Taq-Polymerase). Amplification via PCR of both fragments of mtDNA was performed either in an Eppendorf or Biometra thermocycler at the following conditions: 3 min at 94°C, followed by 30 cycles of 94°C for 1 min, 1 min for the annealing step at 45°C, 1.5 min at 72°C and a final extension of 3 min at 72°C.

The purified mtDNA fragments were sequenced by SEQLAB sequence laboratories (Göttingen, Germany), analyzed and aligned using BioEdit version 7.0.5.3 (Hall 1999). Genetic diversity within each region (SE-Asia and Indopacific) was estimated by calculating haplotype diversity *h* (probability that two randomly chosen haplotypes are different) and nucleotide diversity π (mean proportion of nucleotides differing in all pairwise combinations) using DnaSP ver. 5.10.01 (Librado and Rozas 2009). High h and high π values indicate large, stable populations with long evolutionary histories or secondary contact between population subsets, high h and low π suggest a recent bottleneck followed by rapid growth and accumulation of mutations and low h and low π suggest a recent bottleneck or founder event by a single or few lineages (Grant and Bowen 1998; Quek et al. 2007). Additionally, a haplotype network was constructed based on the concatenated sequence of 904 bp of mitochondrial DNA (consisting of a 460 bp fragment of the COI gene and a 444 bp fragment of cyt*b*) using the 95% parsimony algorithm implemented in TCS 1.21 (Clement et al. 2000b).

RESULTS

We detected 29 mtDNA haplotypes in the 118 *Anoplolepis gracilipes* samples from SE-Asia and the Indopacific (Fig. 7). Sixteen of the 29 haplotypes were singletons, i.e. were found in just a single sample, while the other 13 haplotypes were shared by two to 26 samples. The SE-Asian population contained 11 of these singletons, while only five singletons were found

Table 1. Genetic diversity measures of *Anoplolepis gracilipes* based on mtDNA haplotypes of samples from Southeast Asia (putative native range) and the Indopacific region (introduced range). *N*, number of samples per region; N_{ht}, number of haplotypes per region; N_{ph}, number of private haplotypes per region; N_{ps}, number of polymorphic sites; *h*, haplotype diversity; SD_h, standard deviation; π, nucleotide diversity (in %).

Sampling region	*N*	N_{ht}	N_{ph}	N_{ps}	*h*	SD_h	π (%)
SE-Asia	56	18	15	28	0.847	±0.033	0.69
Indopacific	62	14	11	15	0.867	±0.018	0.37
Total	118	29	-	30	0.966	±0.013	0.66

in the Indopacific population. Overall genetic diversity was high ($h = 9.966 \pm 0.013$, $\pi = 0.66$ %, Tab. 1). Samples from the SE-Asian population contained more private haplotypes (15 in SE-Asia vs. 11 in the Indopacific) and polymorphic sites (28 in SE-Asia vs. 15 in the Indopacific) as the Indopacific population and showed almost twice the nuclear diversity ($\pi_{\text{SE-Asia}} = 0.69$ % vs. $\pi_{\text{Indopacific}} = 0.37$ %). Haplotype diversity, however, was similarly high in both the Indopacific and the SE-Asian population (Tab. 1). Three of the 29 haplotypes were shared by samples from both the Indopacific and the SE-Asian population. In detail, these three haplotypes contained samples from (1) Malaysia/Brunei/Philippines and Tokelau/Fiji/Tuvalu/Australia, (2) Malaysia and Hawaii, USA and (3) Thailand and Australia (Fig. 7, 8).

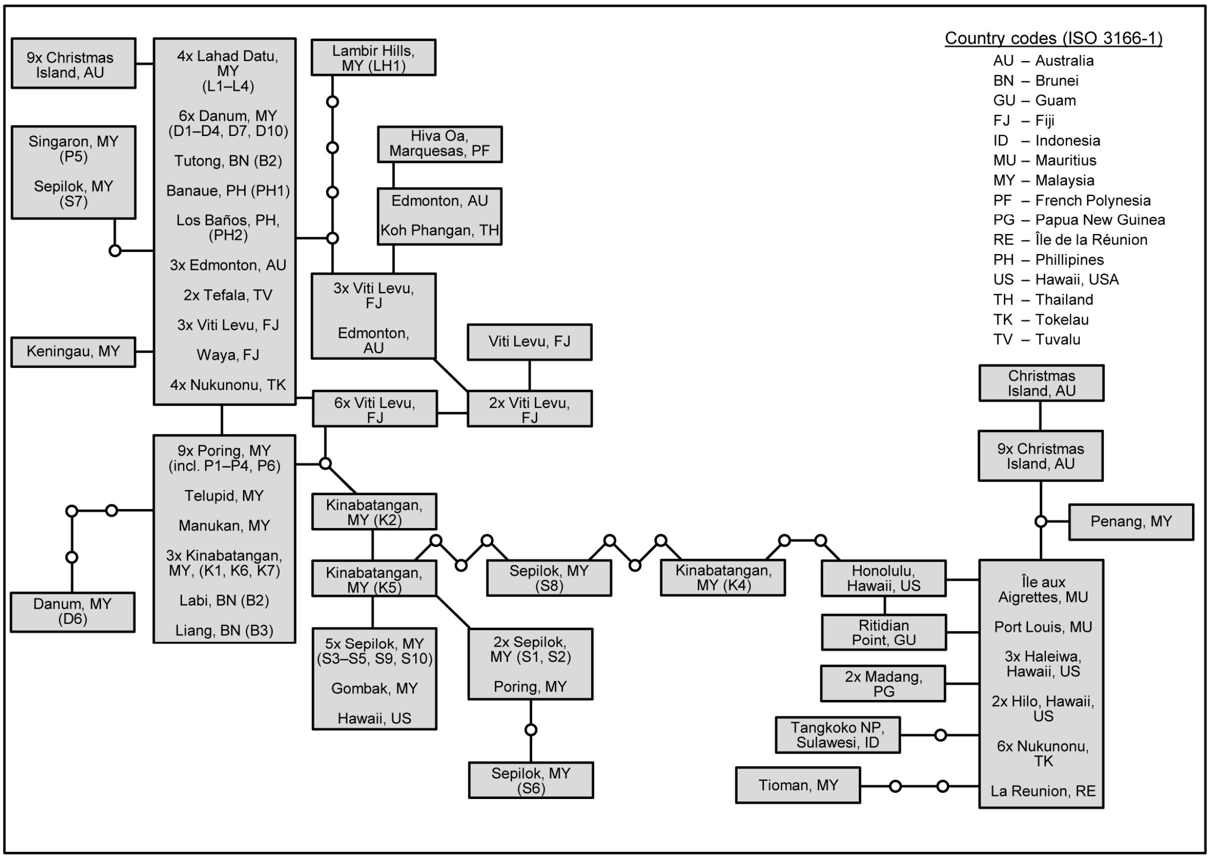


Figure 7. **95% parsimony haplotype network of *Anoplolepis gracilipes* mtDNA sequences from SE-Asia and the Indopacific region.** The concatenated mtDNA sequence comprised 904 bp (460 bp of COI, 444bp of cyt*b*) and was constructed using TCS 1.21 (Clement et al. 2000b). Samples within the same box share a common haplotype. The number of internodes separating different boxes indicates the number of mutations needed to transform one haplotype into the other. Country abbreviations are given according to ISO standard 3166-1. The size of the circles is equivalent to the number of samples sharing a haplotype.

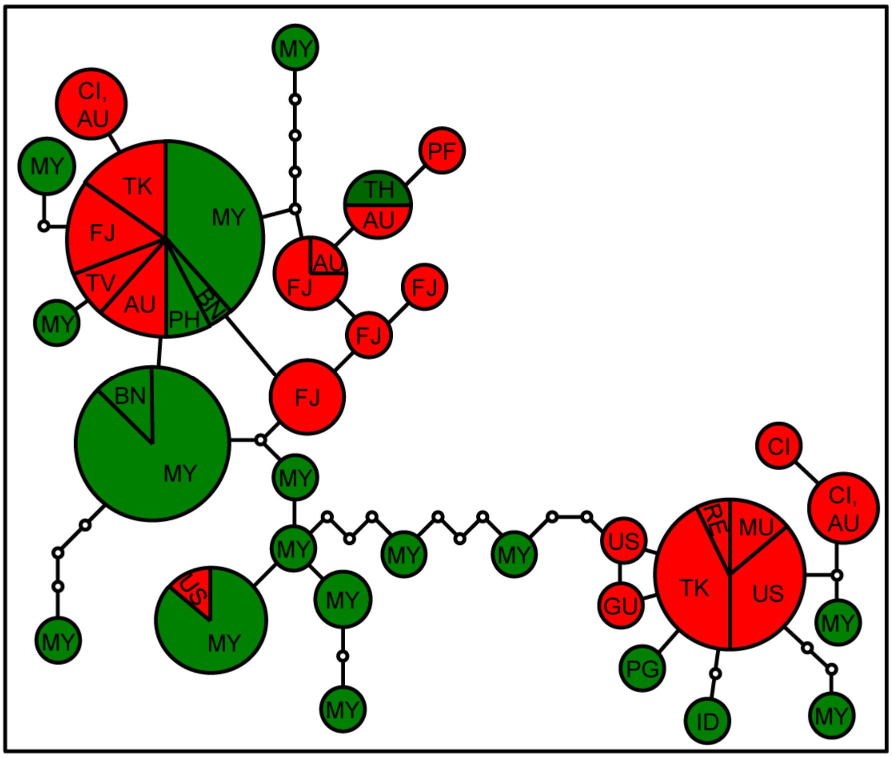
DISCUSSION

This study explored the genetic relationship between *Anoplolepis gracilipes* populations from its introduced range in the Indopacific and one of its putative native ranges, Southeast Asia, using two mitochondrial DNA markers. The genetic analyses could not unambiguously identify SE-Asia as the native range of *A. gracilipes*, but strongly suggested that many of the *A. gracilipes* supercolonies found in the Indopacific today may have been introduced from there. Sampling from other potential native ranges of *A. gracilipes*, especially southern India (Wetterer 2005) is thus crucial in revealing the origin of this ant, as well as clarifying its status (invasive vs. native) in SE-Asia.

Previous research revealed that *A. gracilipes* supercolonies never contain more than one haplotype (unpub. results, Abbott et al. 2007; Thomas et al. 2010a) and supercolonies bearing the same haplotype may often belong to different supercolonies (Drescher et al. 2007; Drescher et al. 2010). Thus, our finding of 29 haplotypes in 118 *A. gracilipes* samples represents the minimum number of supercolonies contained in the data set.

Despite the geographic bias in our data set and unequal sample sizes for both geographic subgroups, the haplotype diversity was similarly high in both the SE-Asian and the Indopacific range of *A. gracilipes* and was only slightly lower than the haplotype diversity in a worldwide sampling of the Argentine Ant *Linepithema humile* (h = 0.92, (Corin et al.

Figure 8. ***Anoplolepis gracilipes* mtDNA haplotypes in relation to their geographical origin.** Samples are color-coded as stemming from either *A. gracilipes*' introduced range (Indopacific region, red) or its putative native range (Southeast Asia, green) according to Wetterer (2005). Country abbreviations are given according to ISO 3166-1. The size of the circles is equivalent to the number of samples sharing a haplotype.



2007)). In turn, overall nucleotide diversity π was very low. In concert with high haplotype diversity *h*, low π values indicate a severely bottlenecked population after a phase of rapid growth (Grant and Bowen 1998; Quek et al. 2007). Moreover, the extremely low nucleotide diversity of the Indopacific population indicates that the genetic bottleneck upon introduction of *A. gracilipes* to the Indopacific may have been a lot more severe than upon the potential introduction to SE-Asia. Alternatively, the severe difference in nucleotide diversity between the SE-Asian and the Indopacific population may alternatively suggest a step-wise introduction history (first bottleneck upon introduction to SE-Asia, second bottleneck upon introduction to Indopacific from already bottlenecked SE-Asian sources).

Haplotype networks and phylogenetic approaches based on mtDNA have repeatedly been used to identify introduction pathways of other invasive ant species such as *Linepithema humile* (Corin et al. 2007), *Solenopsis invicta* (Caldera et al. 2008) or *Wasmannia auropunctata* (Mikheyev and Mueller 2007). In these studies, introduction pathways were identified by finding haplotypes or phylogenetic branches that were shared by samples from the native range and the introduced range, respectively. In the present study, we found three haplotypes that were shared by samples from *A. gracilipes*' putative native range (SE-Asia) and its introduced range (Indopacific)(Fig. 7, 8). This observation implies that the respective supercolonies were introduced to Hawaii, Australia, Fiji, Tokelau and Tuvalu from the same source supercolonies from *A. gracilipes* populations on Borneo, Thailand and the Philippines. This scenario is supported by interception records at Australian borders which show that roughly half of the *A. gracilipes* propagules that reached Australia between 1986 and 2003 were detected on cargo ships or planes coming from SE-Asia, while most other were from the Indopacific (Harris et al. 2005). This, however, does not necessarily mean that *A. gracilipes* is native to SE-Asia, as SE-Asia may have been colonized by *A. gracilipes* supercolonies from yet another potential native range such as India or East Africa (Wetterer 2005), thus constituting a transit region from where *A. gracilipes* is further introduced into habitats in the Indopacific. As both India as well as several regions in SE-Asia (incl. Malaysia, Brunei, Myanmar/Burma, Java) were connected by naval trade routes as parts of the British Empire from as early as the 19th century on, introductions of *A. gracilipes* from India to SE-Asia followed by local establishment of invasive populations in SE-Asia could have occurred frequently over the past 150 to 200 years. As trade and commerce increased both within SE-Asia as well as between SE-Asia and the Indopacific in the past decades, these potentially invasive SE-Asian populations could now have become the source of many of the introductions of *A. gracilipes* into Indopacific habitats.

Aside from haplotypes that were shared between samples from SE-Asia and the Indopacific, we also found unique haplotypes in both regions, including haplotypes that were found in just a single sample. Of these singletons, 11 were found in the SE-Asian population, while four were found in the Indopacific. Large numbers of unique haplotypes in both SE-Asia and the Indopacific may be due to incomplete sampling (i.e. the haplotypes have only been sampled in one of the regions), differences in the propagules that have been introduced into both regions from yet another unidentified native source population, or *in situ* mutations that differ among regions (e.g. on Christmas Island, Thomas et al., 2010). The latter may be facilitated by a combination of unusual mating and dispersal strategies. Intranidal mating seems to be the exclusive reproductive strategy of *A. gracilipes* and gene flow between supercolonies is largely absent (Drescher et al. 2010). As a result, *A. gracilipes* supercolonies may represent isolated units following independent evolutionary trajectories. As novel mutations are likely to spread more rapidly through small populations as opposed to large populations, independent colony founding by dispersing alate queens (as observed by Abbott 2006) or establishment of anthropogenically dispersed propagules may represent the two predominant processes facilitating the emergence of supercolonies bearing novel mtDNA haplotypes.

Conclusion

This study provides first insights into the invasional biogeography of the invasive Yellow Crazy Ant *Anoplolepis gracilipes* in SE-Asia and the Indopacific. Though failing in locating *A. gracilipes*' native region, our data imply that the SE-Asian population has experienced a less severe bottleneck than the Indopacific population, suggesting that *A. gracilipes* may have been introduced to the Indopacific from invasive populations in SE-Asia. Matching haplotypes between SE-Asia and the Indopacific further suggest that SE-Asia may be origin of many of the introduced supercolonies in the Indopacific. Finally, *A. gracilipes* populations in both regions may be subject to diversification processes, potentially leading to novel haplotypes that are maintained within supercolonies.

ACKNOWLEDGEMENTS

We thank Kirsti Abbott, Milan Janda, Robert Junker, Petr Klimes, Alain Lenoir, Juliane Mooz, Thomas Schmitt, Deborah Schweinfest, Andrew V. Suarez and Darren Ward for their contribution of *A. gracilipes* samples. This work was part of the Royal Society's Southeast Asia Rainforest Research Programme (SEARRP) and was funded by the Deutsche Forschungsgemeinschaft DFG (SFB 554 'Evolution and mechanisms of arthropod behaviour', project E2).

VI. GENETIC AND CHEMICAL DIFFERENTIATION BETWEEN *ANOPLOLEPIS GRACILIPES* SUPERCOLONIES

This chapter has been published as:

Drescher J, Blüthgen N, Schmitt T, Bühler J, Feldhaar H (2010) Societies drifting apart? Behavioural, genetic and chemical differentiation between supercolonies in the Yellow Crazy Ant *Anoplolepis gracilipes*. ***PLoS ONE*** 5(10): e13581

ABSTRACT

In populations of most social insects, gene flow is maintained through mating between reproductive individuals from different colonies in periodic nuptial flights followed by dispersal of the fertilized foundresses. Some ant species, however, form large polygynous supercolonies, in which mating takes place within the maternal nest (intranidal mating) and fertilized queens disperse within or along the boundary of the supercolony, leading to supercolony growth (colony budding). As a consequence, gene flow is largely confined within supercolonies. Over time, such supercolonies may diverge genetically and, thus, also in recognition cues (cuticular hydrocarbons, CHC's) by a combination of genetic drift and accumulation of colony-specific, neutral mutations. We tested this hypothesis for six supercolonies of the invasive ant *Anoplolepis gracilipes* in north-east Borneo. Within supercolonies, workers from different nests tolerated each other, were closely related and showed highly similar CHC profiles. Between supercolonies, aggression ranged from tolerance to mortal encounters and was negatively correlated with relatedness and CHC profile similarity. Supercolonies were genetically and chemically distinct, with mutually aggressive supercolony pairs sharing only 33.1% ± 17.5% (mean±SD) of their alleles across six microsatellite loci and 73.8% ± 11.6% of the compounds in their CHC profile. Moreover, the proportion of alleles that differed between supercolony pairs was positively correlated to the proportion of qualitatively different CHC compounds. These qualitatively differing CHC compounds were found across various substance classes including alkanes, alkenes and mono-, di- and trimethyl-branched alkanes. We conclude that positive feedback between genetic, chemical and behavioural traits may further enhance supercolony differentiation

through genetic drift and neutral evolution, and may drive colonies towards different evolutionary pathways, possibly including speciation.

INTRODUCTION

In most social insect species, a colony is a closed family unit that contains a queen and her daughter workers (Hölldobler and Wilson 2009). The queen lays eggs, which are cared for and reared by the workers. As the colony reaches maturity, males and virgin queens are produced which mate with reproductives from other colonies in periodic mating events. Soon after copulation, the males die while the fertilized queens disperse in an attempt to independently found new colonies. As a consequence of this strategy, gene flow among colonies is maintained within the population.

Social insects such as ants, however, show a large variety in social organization, reproductive systems, mating behaviour and dispersal modes. Some tramp ant species such as *Linepithema humile* (Jaquiery et al. 2005), *Pheidole megacephala* (Fournier et al. 2009), *Wasmannia auropunctata* (Le Breton et al. 2004), *Lasius neglectus* (Ugelvig et al. 2008) or *Anoplolepis gracilipes* (O'Dowd et al. 2003) share a similar set of strategies: They form large polydomous, highly polygynous supercolonies, within which aggression is absent and individuals move freely between nests (Holway et al. 2002). Supercolonies differ from other polygynous and polydomous ant colonies, in that they are usually too large to allow direct cooperative interactions of individuals from distant nests (Pedersen et al. 2006). In contrast to most other ant species, nuptial flights of supercolony-forming ant species are rare or even absent, and mating often takes place within the nest or the supercolony. After fertilization, queens stay within the maternal nest, move to other nests within the supercolony or disperse at the fringe of the supercolony through budding, i.e. the occupation of a suitable nest by one or several queens that are accompanied by workers and sometimes brood from the colony the queens themselves originated from. As a consequence of these mating and dispersal strategies, gene flow between supercolonies may be extremely limited or absent, as has been pointed out by studies on *L. humile* (Jaquiery et al. 2005; Pedersen et al. 2006; Thomas et al. 2006) and *A. gracilipes* (Thomas et al. 2010b). The study by Thomas *et al.* (2010b) strongly suggests that gene flow is absent between two sympatric and mutually aggressive *A. gracilipes* supercolonies on Christmas Island, Indian Ocean. The two supercolonies were genetically differentiated both in nuclear and mitochondrial loci. Furthermore, intranidal mating in *A. gracilipes* can be frequently observed (in laboratory colonies of *A. gracilipes*) and workers are highly aggressive towards virgin queens and males from other supercolonies (Drescher, Feldhaar, pers. obs.), suggesting that gene flow among supercolonies may be prevented by behavioural barriers. As a consequence, lack of gene flow between ant colonies should lead to genetic differentiation among them.

Nestmate recognition in ants usually relies on cuticular hydrocarbons (CHC’s) (Vander Meer and Morel 1998; Lahav et al. 1999; Akino et al. 2004; Torres et al. 2007; Brandstaetter et al. 2008) although other substance classes can also be involved, such as fatty acids (Franks et al. 1990). CHC’s are largely genetically determined (Adams 1991; Pennanec'h et al. 1997; Takahashi et al. 2001; van Zweden et al. 2009; van Zweden et al. 2010), which implies that genetic differentiation among sympatric ant colonies may entail the diversification of genetically based CHC profile compounds through genetic drift and the accumulation of supercolony-specific mutations. Thus, in ant species with strict intranidal mating, CHC-profiles may differ between colonies not only in terms of relative abundances of epicuticular compounds (quantitative differences, as is the case in most ant species with intercolonial mating), but also in the composition of the profile itself (qualitative differences).

As the origin and future of supercolonialism in ants is unclear (Helanterä et al. 2009), studying the degree and nature of differentiation within and between supercolonies may help in understanding the evolutionary paths that those species might take (Helanterä et al. 2009). We thus measured patterns of behavioural, genetic and chemical differentiation within and between six spatially separated supercolonies in north-eastern Borneo, and discuss the data with respect to potential evolutionary trajectories of *A. gracilipes*. As suggested by previous studies, NE-Borneo is inhabited by a mosaic of variably related, ecologically dominant *A. gracilipes* supercolonies (Drescher et al. 2007; Drescher et al. 2011) with unusually high intracolonial relatedness estimates compared to all other supercolonial ant species studied so far (Helanterä et al. 2009; Drescher et al. 2010). Thus, assuming that the scenario described above applies to *A. gracilipes*, we expect that the varying genetic differentiation between supercolonies corresponds to the degree of CHC-profile differentiation. As intranidal mating may be the dominant, if not exclusive, reproductive strategy in *A. gracilipes*, we furthermore expect that CHC-profiles differ both quantitatively as well as qualitatively between supercolonies, and that qualitative differences between the CHC-profiles of supercolonies are independent of substance classes as would be expected by an accumulation of random, supercolony-specific mutations.

METHODS

Selection and maintenance of colonies

We localized six *Anoplolepis gracilipes* supercolonies within the study area around Poring Hot Springs, Sabah, Malaysia (6°04' N, 116°70' E, colonies are from here on referred to as supercolonies P1-P6, Figure 9). All supercolonies included in this study were at least 200m in diameter, except for one slightly smaller supercolony (P1), which spanned only 100m (Fig. 9). The supercolonies were between 150 m (P1-P2) to ca. 15 km (P6-P5) apart. Workers, brood and queens from a single nest per supercolony were collected and transferred into plastic containers (45 × 25 × 25 cm) treated with Fluon™ to prevent escape (henceforth termed subcolony). All subcolonies were offered newspaper as nesting material and were fed water, honey and tuna every third day. Each of the subcolonies contained at least 1500 workers, 200 pupae/larvae and 3-12 queens (except for P1, which contained about 500 workers, 100 pupae and two queens).

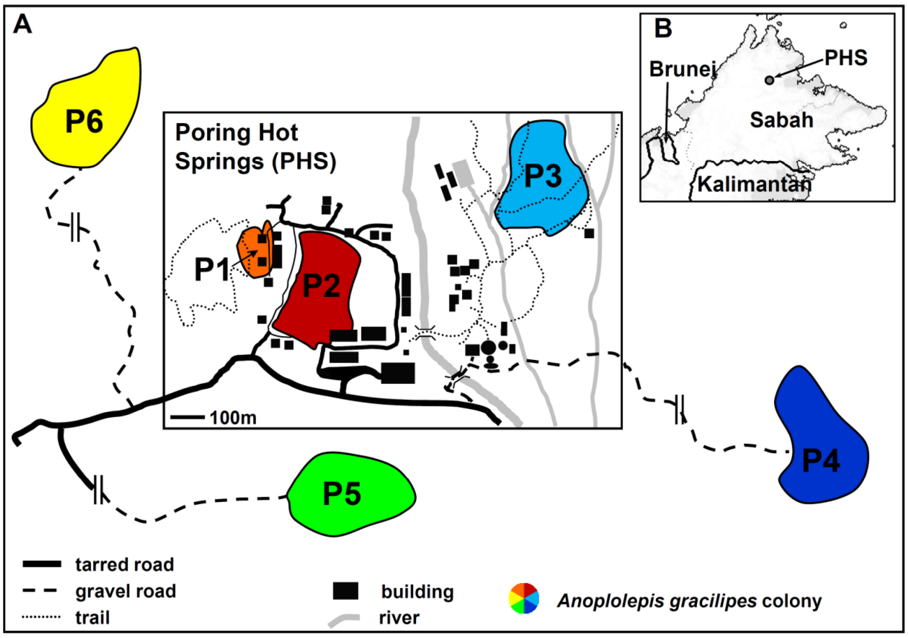


Figure 9. Location of six *Anoplolepis gracilipes* supercolonies in Sabah, Malaysia. A. Location of the six *Anoplolepis gracilipes* colonies in the study area. B. Location of Poring Hot Springs in NE-Borneo. Colour codes correspond to colony affiliation and to the results of a Bayesian clustering algorithm under the assumption of *K*=4 genetic clusters (Fig. 12).

Behavioural assays

Behavioural assays were performed within and between each of the six subcolonies (15 intercolonial combinations) using two different indices (Aggression Index *AI* and Mortality Index *MI*). Both indices were measured by placing 5 individuals of each colony (10 ants per trial per pairwise colony combination) inside a Fluon™ coated plastic cylinder (diameter=10 cm, height=5 cm) on a sheet of paper which was replaced after each trial. The average maximum level of aggression (henceforth termed aggression index *AI*) was obtained as the average of the most aggressive interaction within 5 minutes across ten replicates according to the following categories: 1 – ants displayed no reaction towards each other/no physical contact, 2 – reciprocal antennation, 3 – ants biting and spread-eagling one another, 4 – ants protruding gaster/ spraying formic acid while biting opponent. Aggression levels 1 and 2 were considered nonaggressive while aggression levels 3 and 4 were considered antagonistic/aggressive. In order to measure the Mortality Index *MI* (as described in (Drescher et al. 2007)), encounters between five workers of each subcolony were observed for a period of 60 minutes. Every minute, the number of dead individuals was counted. For each trial, the mortality index *MI* was obtained as *MI* = (y/2)/t50, with y being the total number of individuals killed at the end of the experiment (60 min) and t50 the time (in steps of 1 min) when half of this number (y/2) was already killed. Thus, this index allowed us to describe aggression by combining both the number of dead individuals as well as the speed at which ants kill each other.

Additionally, we measured aggression of workers towards allocolonial sexuals, i.e. virgin alate queens and males. As not enough virgin queens and males could be collected from the supercolonies in Poring Hot Springs, these tests were performed within and between three supercolonies from the Sepilok Forest Reserve (supercolonies S6, S7 and S10, 5°52’ N, 117°57’ E) and one from the Lower Kinabatangan Nature Reserve (supercolony K1, 5°29’ N, 118°16’ E), Sabah, Malaysia. Aggression towards allocolonial sexuals in a combination of supercolonies was measured by placing five workers from one supercolony and either one alate queen or one male from another supercolony in an arena and vice versa. We then scored presence/absence of aggression (biting, spread-eagling of the queen/male, protruding gaster and spraying acid) in 10 trials of 60 min each. Overall aggression towards allocolonial virgin queens/males was obtained by pooling the data from the 20 replicates per reproductive caste per supercolony combination. Aggression between workers of the same supercolonies was measured similarly, only that five individuals of each supercolony were placed in the arena (N=10 replicates per supercolony combination).

Relatedness and population structure

Workers from each supercolony were sampled in 98.8% EtOH p.A. 20 workers from each of the six subcolonies were genotyped using six polymorphic microsatellite loci (Ano1, Ano3, Ano4, Ano6, Ano8, Ano10) according to the protocol in Feldhaar *et al.* (2006). Genetic diversity within supercolonies was measured by genotyping five workers of two to four additional nests per supercolony (two additional nests for P1 and P5, four nests for P2, P3, P4 and P6). This resulted in a total number of 220 genotyped individuals.

Relatedness within and between the six supercolonies was calculated using Relatedness 5.0.8 (Queller and Goodnight 1989), including all individuals as reference population. All *R* values presented in this study arise from the half-matrix resulting from 220 × 220 pairwise comparisons of individual relatedness excluding comparisons of each individual with itself. Secondly, we conducted a three-level hierarchical analysis of molecular variance (AMOVA) over the entire data set to determine how genetic variability was distributed across three levels (between individuals within nests, among nests within a supercolony, among supercolonies) in the population using ARLEQUIN 3.01 (Excoffier et al. 2005). Thirdly, we performed assignment tests using STRUCTURE 2.2 (Pritchard et al. 2000; Falush et al. 2003, 2007) to determine to what extent the patterns obtained with aggression tests correlated with genetic differentiation between supercolonies. This software infers the number of clusters (*K*) that best fits a data set by maximizing Hardy-Weinberg equilibrium and calculates the assignment probability for each individual under any assumed *K*. We performed ten independent runs for *K* from one to six. All genotyped individuals of a supercolony were included in this analysis.

Cuticular Hydrocarbon (CHC) Profiles

Cuticular hydrocarbons were extracted from 20 pooled workers from the same nests as those sampled for bioassays (subcolonies) and genetic analysis plus two additional nests for supercolony P2. Single workers did no yield sufficient amounts of substances to allow analysis of the CHC profiles. The individuals were frozen at -20°C for 30 min prior to 8 min extraction in hexane. Extracts were reduced under a gentle stream of nitrogen to 10 to 15 µl and immediately used for analysis or stored at -20°C. 1 µl of the extract was analysed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett-Packard HP 6890 gas chromatograph (GC, equipped with a J & W DB-5 fused silica capillary column: 30 m x 0.25 mm ID; film thickness: 0.25 µm) coupled with a Hewlett-Packard HP 5973 mass selective detector (Hewlett-Packard, Waldbronn, Germany). We used a temperature program starting from 60°C with an increase of 5°C/min until a final temperature of 300°C, which was kept for 10 min. A split/splitless injector was set to splitless mode for 60 sec at a temperature of 250°C. Helium was used as carrier gas with a constant flow of 1 ml/min. Electron ionization mass spectra (EI-MS) were recorded at 70 eV with a source temperature of 230°C.

Only hydrocarbons, which were identified by their typical mass spectra, were included in our analysis. Furthermore, all molecules smaller than C19-bodies were discarded, as they only occurred in traces and were absent in most ant CHC profiles (Martin and Drijfhout 2009). We compared differences in cuticular hydrocarbon profiles between supercolonies by performing permutation tests (adonis, R-package vegan 1.15, 10000 runs) based on relative peak areas (proportion of each peak's integrated peak area to total integrated peak area). To visualize differences in CHC profiles, we performed non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities (d_{ij}) of relative peak areas of CHC profiles.

Correlations between behaviour, genetic and chemical properties of workers from different supercolonies

We constructed a matrix of the proportion of alleles that were not shared by supercolony pairs and, likewise, a matrix of the proportion of qualitatively differing cuticular hydrocarbon substances between supercolony pairs. We then tested for correlation between these two matrices and the matrices of pairwise aggression (*AI*, *MI*), relatedness (*R*) and Bray-Curtis-distances of CHC profiles (d_{ij}) by performing mantel tests (10000 permutations) between matrix pairs. If not stated otherwise, all statistical analyses were performed with *Statistica* 7.1 (StatSoft, 2005) or R 2.9.2 (Ithaka and Gentleman 1996).

RESULTS

Behavioural assays

Both types of behavioural assays (*AI*, *MI*) yielded consistent results (R=0.65, p=0.002, Mantel test). Aggression differed among supercolonies: While workers from P1-P2 and P3-P4 tolerated each other, workers from the remaining pairwise bioassays were aggressive towards each other (Kruskal-Wallis ANOVA, *AI*: H = 177.5, p < 0.0001; *MI*: H = 144.4, p < 0.0001; Figure 10). Assuming that P1-P2 and P3-P4 belonged to the same supercolony, despite being separated up to ca. 1.5 km (P3-P4), we performed the statistical analysis again without colony pairs P1-P2 and P3-P4. Still, aggression differed among the remaining pairs (*AI*: H = 36.1, p = 0.0003; *MI*: H = 60.4, p < 0.0001; Fig. 10). Furthermore, we observed high levels of aggression of workers towards allocolonial sexuals (virgin queens/males) in at least 50% of all replicates in each pairwise supercolony combination (Table 2).

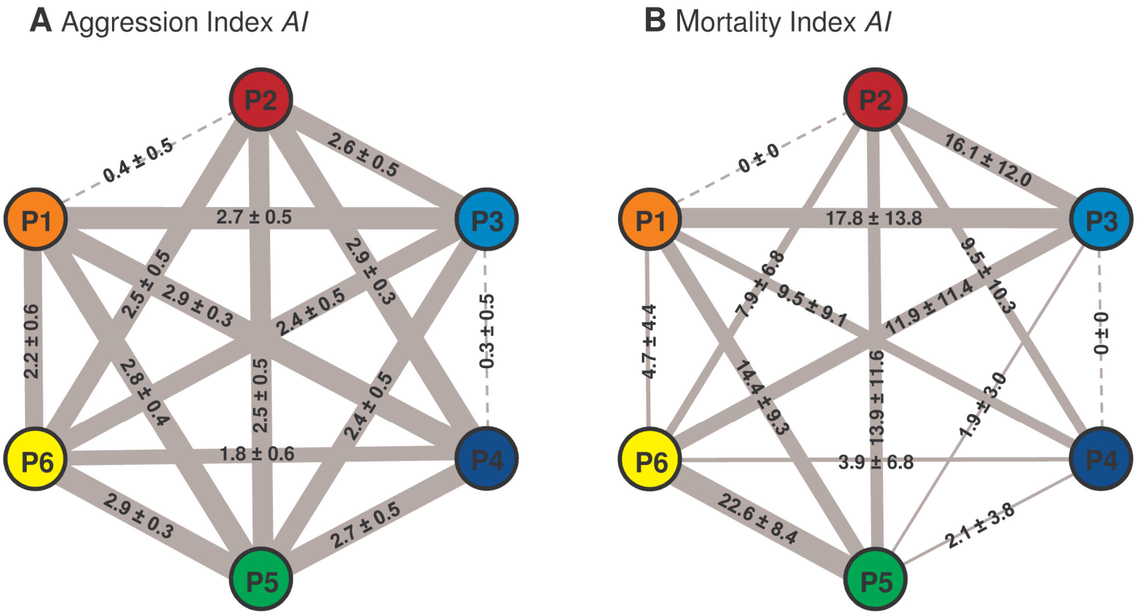


Figure 10. Aggression between six *Anoplolepis gracilipes* supercolonies from Sabah, Malaysia. Aggression was measured as (A) Aggression Index *AI* (mean ± SD, range: 1 - 4) and (B) Mortality Index *MI* (mean ± SD). The line widths are equivalent to the degree of aggression and dotted lines represent absent aggression. Colour codes correspond to colony affiliation and to the results of a Bayesian clustering algorithm under the assumption of *K*=4 genetic clusters (Fig. 12).

Table 2. Aggression of workers towards allocolonial workers, males and queens. The number of crosses represent the frequency of aggression of workers towards allocolonial queens (red crosses), males (blue crosses) and workers (black crosses) from four different *Anoplolepis gracilipes* supercolonies (supercolonies K1, S6, S7and S10; ++: aggression was observed in more than 50% of all replicates; +++: aggression observed in more than 75% of all replicates).

	K1	**S6**	**S7**	**S10**
K1	---	+++/+++	++/++	++/++
S6	+++	---	++/++	+++/++
S7	++	++	---	++/++
S10	++	+++	+++	---

Relatedness and population structure

We found 29 alleles in the entire population (215 individuals from six spatially separated colonies genotyped at six microsatellite loci). The number of alleles per locus ranged from three to 11, while the number of alleles per locus per supercolony ranged between two and four (Table 3). Laboratory subcolonies contained the same alleles as the other genotyped nests from their respective supercolony. Mutually aggressive subcolony pairs shared less than half of their alleles (33.1 ± 17.5%, mean ± SD), while mutually tolerant subcolonies P1-P2 and P3-P4 shared either all alleles (P1-P2) or 85.7% (P3-P4, 16 of 18 alleles in common) of the pairwise allele pool (Table 4). Workers within subcolonies were closely related (R = 0.85 ± 0.09, mean ± SE, range: 0.74 — 0.98, Fig. 11), while relatedness between reciprocally aggressive subcolony pairs was low (R = -0.31 ± 0.47, mean ± SE, range: -0.69 — 0.51). The mean relatedness between the two tolerant supercolony pairs P1-P3 and P3-P4 was as high as intracolonial relatedness (Fig. 11). Accordingly, the genetic diversity in the population sample originated from differences between supercolonies (AMOVA, $F_{SCOLONY\text{-}TOTAL}$ = 0.247, Table 5) rather than differences between individuals in nests ($F_{IND\text{-}NEST}$ = -0.925) or nests within supercolonies ($F_{NEST\text{-}SCOLONY}$ = -0.002). Without *a priori* information on the origin of the sampled individuals, the Bayesian clustering algorithm implemented in STRUCTURE 2.2 revealed the highest likelihood of the data (Ln P(D)) between K=4 and K=6, with K=4 showing the smallest standard deviation (Fig. 12 A). When using *a priori* information on assumed numbers of K, the assignment of all genotyped individuals to K=4 genetic clusters was clearest, especially for individuals from colonies P3 and P4 (Fig. 12 B).

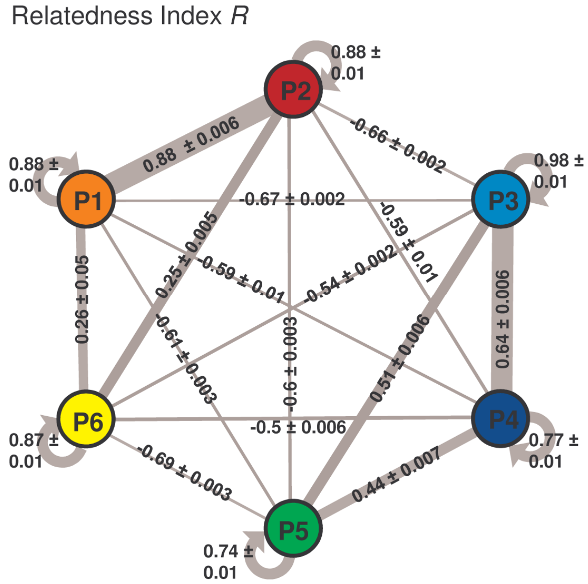


Figure 11. Relatedness within and between six *Anoplolepis gracilipes* supercolonies from Sabah, Malaysia. Relatedness was calculated as R (mean ± SE, range: -1 – 1). The line widths are equivalent to the degree of relatedness. Colour codes correspond to colony affiliation and to the results of a Bayesian clustering algorithm under the assumption of K=4 genetic clusters (Fig. 12).

Table 3 Alleles of six microsatellite loci in six *Anoplolepis gracilipes* supercolonies. The alleles of six microsatellite loci (Ano1, Ano3, Ano4, Ano6, Ano8, Ano10) are given in rows, the six *Anoplolepis gracilipes* supercolonies (P1-P6) and the entire study area (Total) are given in columns. N^n indicates the number of nests and N^w the number of workers genotyped per supercolony.

	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**Total**
Ano1	99 103	99 103	99 103	99 103	99 103	99 105	99 103 105
Ano3	164 166	164 166	144 156	144 156	144 156	164 166 168	144 156 164 166 168
Ano4	159 175	157 175	157 165	157 165	157 165	159 175	157 159 165 175
Ano6	116 130	116 130	116 130	116 130	116 118 130	116 130	116 118 130
Ano8	214 224 294	214 224 294	220 274 278	212 220 278	208 212 260 274	220 286 292	208 212 214 220 224 260 274 278 286 292 294
Ano10	234 242	234 242	234 238	234 238	234 238	234 242	234 238 242
N^n	3	5	5	5	3	5	26
N^w	30	40	40	40	30	40	220

Table 4. Percentage of alleles differing between *Anoplolepis gracilipes* supercolonies in relation to the pairwise allele pool.

	P1	**P2**	**P3**	**P4**	**P5**
P2	0				
P3	76.2	76.2			
P4	76.2	76.2	14.3		
P5	78.3	78.3	35.3	35.3	
P6	50	50	77.3	77.3	84

Table 5. Multi-level genetic analysis of molecular variance (AMOVA) of six *Anoplolepis gracilipes* supercolonies. *: p < 0.05; **: p<0.01; ***: p < 0.001. Significance values were obtained by bootstrapping 10000 times over loci.

	$F_{IND\text{-}NEST}$	$F_{NEST\text{-}SCOLONY}$	$F_{SCOLONY\text{-}TOTAL}$
Ano1	-1	0	**0.141*** **
Ano3	-0.923	-0.003	**0.361*** **
Ano4	-1	0	**0.376*** **
Ano6	-0.945	-0.001	**0.009* **
Ano8	-0.712	-0.009	**0.373*** **
Ano10	-1	0	**0.231*** **
All	-0.925	-0.002	**0.247*** **

Cuticular Hydrocarbon (CHC) Profiles

In total, we scored 154 peaks from GC-MS chromatograms of 26 *A. gracilipes* nest sites in Poring Hot Springs. The chemical profiles of pooled workers of reciprocally tolerant subcolonies were much more similar (Bray-Curtis distances $d_{P1\text{-}P2} = 0.12$ and $d_{P3\text{-}P4} = 0.14$, respectively) than those of mutually aggressive subcolonies ($d_{ij} = 0.32 \pm 0.06$, mean ± SD, range: 0.22 – 0.42, Figure 13). Likewise, the cuticular hydrocarbon profiles from additional nests of the six *Anoplolepis gracilipes* supercolonies in the field clustered according to supercolony affiliation (ADONIS, F = 10.78, p< 0.0001, Fig. 14) and were highly similar between the nests of supercolonies of which subcolonies were mutually tolerant (ADONIS, P1-P2: F = 2.84, p = 0.18; P3-P4: F = 1.80, p = 0.23).

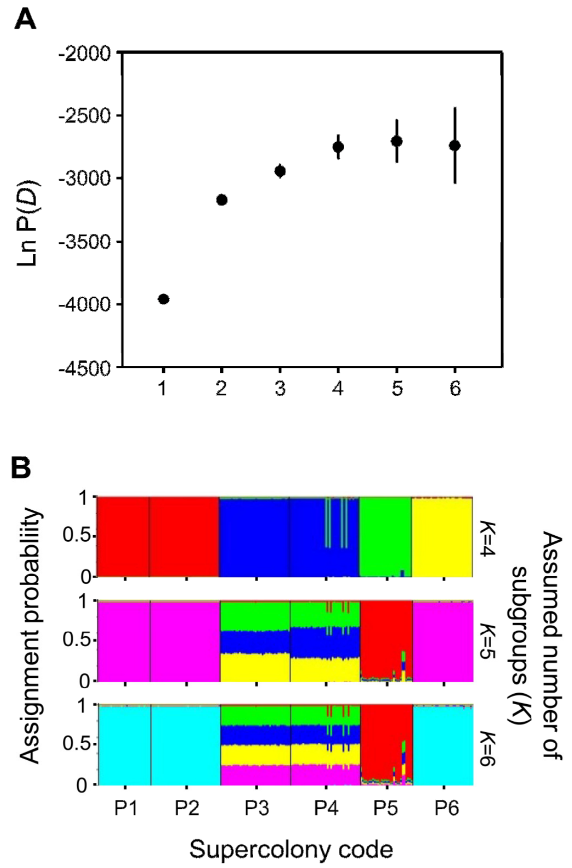


Figure 12. Estimated number of genetic clusters and assignment probability of six *A. gracilipes* supercolonies. (A) Average of the logarithm of the likelihood (Ln P(*D*)) of the data to be assigned to *K* genetic clusters as calculated with STRUCTURE 2.2. (B) Assignment probability of N=220 *Anoplolepis gracilipes* workers from colonies P1 — P6 to *K*=4, *K*=5 and *K*=6 genetic clusters. Identical colours indicate that subgroups belong to the same genetic cluster. At *K*=5 and *K*=6, individuals from colonies P3 and P4 can not be assigned to a specific cluster. At *K*=4, individuals from colonies P1 and P2 are assigned to the red cluster, individuals from colonies P3 and P4 are assigned to the blue cluster and individuals from colonies P5 and P6 are each assigned an individual cluster.

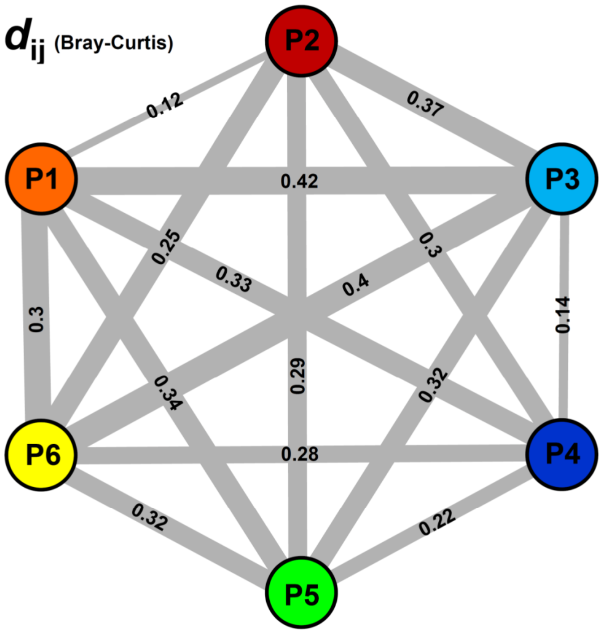


Figure 13. Bray-Curtis dissimilarities of relative peak areas of cuticular hydrocarbon profiles between six *Anoplolepis gracilipes* supercolonies. Colour codes correspond to colony affiliation and to the results of a Bayesian clustering algorithm under the assumption of *K*=4 genetic clusters (Fig. 12).

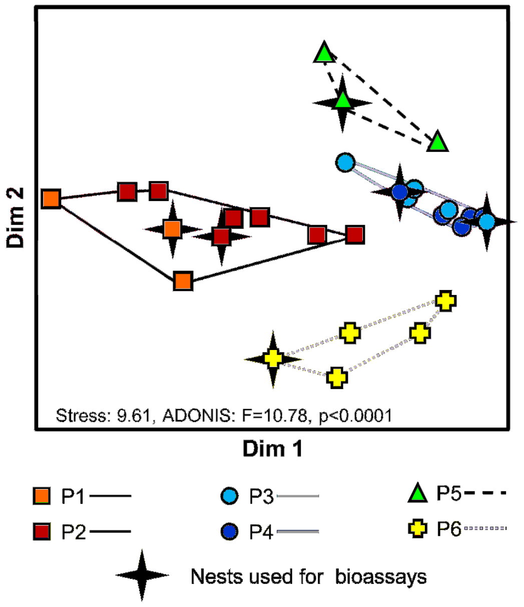


Figure 14. NMDS plot of Bray-Curtis dissimilarities of CHC profiles between six *A. gracilipes* colonies. All symbols are coloured according to colony affiliation and the assignment to common genetic clusters (Fig. 12). Colony profiles differ between the four clusters (ADONIS, F=10.78, p=0.0001), but not between colonies P1-P2 (F = 2.84, p = 0.11) and P3-P4 (F = 1.80, p = 0.20).

We then limited the array of analyzed peaks to only those, which constituted at least 1% of relative peak area in at least one sample, thus reducing the array of cuticular compounds from 154 (all substances) to 30. Nevertheless, ADONIS results and NMDS arrangements remained largely the same (Fig. 15). The remaining 30 substances comprised a mix of unbranched and methyl-branched alkanes and alkenes, 16 of which differed qualitatively between supercolonies (i.e. compounds that were present in CHC profiles of one or several supercolonies while being absent in others, Fig. 16).

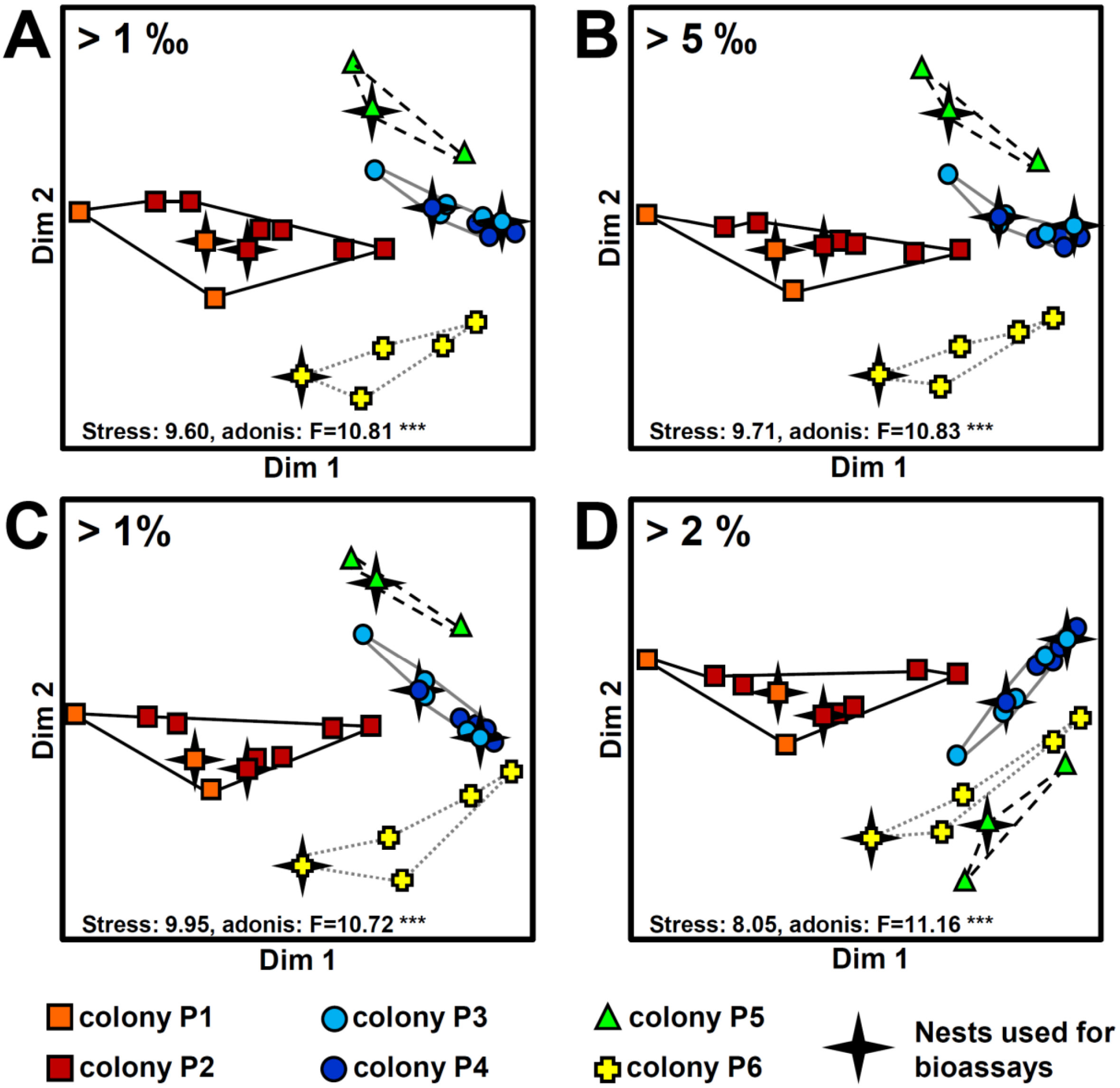


Figure 15. NMDS plots of Bray-Curtis dissimilarities of CHC profiles of six *A. gracilipes* supercolonies. We calculated Bray-Curtis dissimilarities of the CHC profiles using four different scoring thresholds (A-D). (A) Relative peak areas (RPA) were larger than 1‰ in at least one sample. (B) RPA's larger than 5‰ in at least one sample. (C) RPA's larger than 1% in at least one sample. (D) RPA's larger than 2% in at least one sample. Colour codes correspond to colony affiliation and to the results of a Bayesian clustering algorithm under the assumption of *K*=4 genetic clusters (Fig. 12). While adonis results and stress values of the NMDS plots remained similar regardless of the predefined thresholds, the arrangements of NMDS plots were similar to the original arrangement at thresholds >1‰, >5‰ and >1% (A-C), but not at >2% (D).

Qualitative differences between CHC profiles involved all identified compound-classes, but were overrepresented in dimethyl-branched alkanes (Fig. 16). Qualitatively differing compounds constituted between 1.01% and 8.21% per compound of the respective CHC profiles (average: 2.59 % ± 0.5 %, mean ± SE). Moreover mutually aggressive colonies shared only 73.8% of the compounds in their CHC profiles on average, while mutually tolerant colonies (P1-P2, P3-P4) shared 95.8% of the compounds (presence/absence of compounds, Table 6).

Correlations between behaviour, genetic and chemical properties of workers from different supercolonies

The behavioural patterns among subcolonies were in perfect agreement with intercolonial relatedness and Bray-Curtis similarities of CHC profiles. Intercolonial aggression (in both *AI* and *MI*) was negatively correlated to relatedness (AI~R: $r = -0.68$, $p = 0.023$; MI~R: $r = -0.84$, $p = 0.002$, Mantel test, Table 7) and positively correlated to Bray-Curtis distances of the CHC profiles (AI~d_{ij}: $r = 0.72$, $p = 0.015$; MI~R: $r = 0.62$, $p = 0.023$). Thus, high aggression

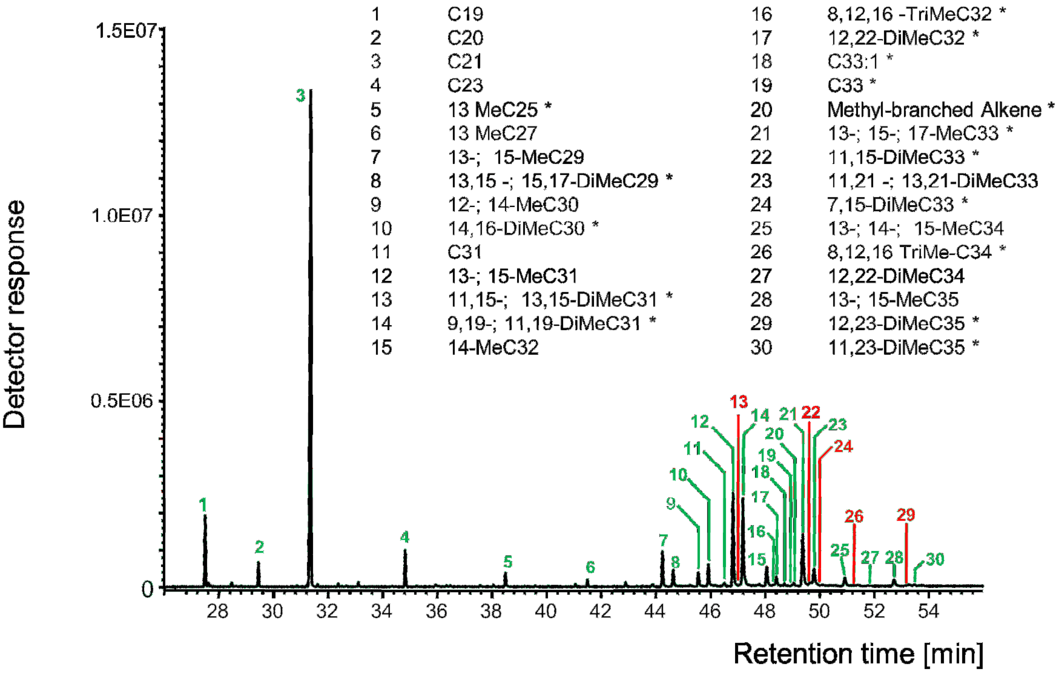


Figure 16. GC-MS chromatogram of a pooled sample of 20 *Anoplolepis gracilipes* workers from supercolony P3. 30 cuticular substances remained after applying a 1% scoring threshold. Green labels indicate cuticular compounds that are present in this sample, whereas red labels indicate substances that are absent from this sample, but present in cuticular extracts from other supercolonies. Asterisks in the compound list indicate the 16 substances that differ qualitatively between supercolony CHC-profiles.

Table 6. Percentage of Cuticular hydrocarbon compounds (CHC's) differing between six *Anoplolepis gracilipes* supercolonies in relation to the pairwise compound pool.

	P1	P2	P3	P4	P5
P2	4.2				
P3	12	8			
P4	8.3	12	4.2		
P5	37.9	34.5	34.5	37.9	
P6	33.3	29.6	29.6	33.3	29.6

occurred at low relatedness and high CHC profile dissimilarity, while low aggression matched high relatedness and low dissimilarity of CHC profiles. Accordingly, relatedness and CHC profile dissimilarity were negatively correlated (r = -0.77, p = 0.007). Moreover, the proportion of alleles that differed between supercolonies pairs was positively correlated to the proportion of CHC compounds that differed between supercolony profiles (r = 0.26, p = 0.044).

Table 7 Correlation coefficients between aggression, relatedness and chemical and genetic differentiation between six *Anoplolepis gracilipes* supercolonies. Abbreviations represent aggression (*AI*, *MI*), relatedness (*R*), Bray-Curtis distances of cuticular hydrocarbon profiles (d_{ij} CHC), percentage of distinct alleles (% Alleles) and percentage of distinct CHC compounds (% CHC) between six *Anoplolepis gracilipes* supercolonies. Asterisks indicate p-levels: $^{+}$p<0.1, *p<0.05, **p<0.01.

	AI	*MI*	*R*	d_{ij} **CHC**	**% Alleles**
MI	0.65**				
R	-0.68*	-0.84**			
d_{ij} **CHC**	0.72*	0.62*	-0.77**		
% Alleles	0.85**	0.45^{+}	-0.36^{+}	0.50^{+}	
% CHC's	0.38*	0.05	0.09	0.25	0.26*

DISCUSSION

The present study revealed a significant positive correlation of aggression with differentiation of the cuticular hydrocarbon profile as well as genetic differentiation among sympatric supercolonies of the Yellow Crazy Ant *Anoplolepis gracilipes* in a study site in NE-Borneo. Intercolonial aggression varied among supercolony pairs, ranging from tolerance to mortal encounters in the bioassays. Mutually aggressive supercolonies were distantly related and showed highly differentiated CHC profiles, while two non-aggressive though spatially separated supercolonies were found to have highly similar CHC profiles and relatedness values that were comparable to those found within supercolonies. Similar patterns have been observed in the Argentine Ant *Linepithema humile*, where mutually tolerant supercolonies from different locations (even from different continents) can be highly similar in genetic and chemical terms, and are thus thought to have been introduced from the same source population (Brandt et al. 2009b; Sunamura et al. 2009a; van Wilgenburg et al. 2010). Likewise, supercolonies that are highly differentiated in genetic and chemical terms (even if they are close to each other geographically) are thought to have been introduced from different source populations (Sunamura et al. 2009b; Vogel et al. 2010). Hence, the varying degrees of behaviour as well as genetic and chemical differentiation that we observed in *A. gracilipes* in this study may potentially be explained by the same mechanisms, i.e. supercolony pairs P1-P2 and P3-P4 may have been introduced from the same source population whereas supercolonies P5 and P6 may have been introduced from yet another population, respectively. Alternatively, two tolerant supercolonies may belong to the same supercolony separated by uninhabitable terrain (very likely for the pair P1-P2 which was separated by only 150m) or one supercolony in a tolerant supercolony pair may have originated from the other through via human-mediated jump-dispersal within the study site (possibly true for P3-P4).

Cuticular hydrocarbon (CHC) profiles have repeatedly been shown to enable ants to discriminate between nestmates and non-nestmates (Vander Meer and Morel 1998; Lahav et al. 1999; Akino et al. 2004). Typically, differing CHC profiles lead to aggression while similar CHC profiles do not. Aggressive responses towards individuals bearing allocolonial CHC profiles may be evoked according to a threshold rule (e.g. *Cataglyphis iberica*) or may be gradual (e.g. *Myrmica rubra*) (reviewed in (Lenoir et al. 1999)). The positive correlation between CHC profile dissimilarity and aggression that we observed between individuals from different supercolonies in *A. gracilipes* may suggest the existence of a graded response mechanism, which has also been found in other invasive ant species such as *Lasius neglectus* (Ugelvig et al. 2008), *Wasmannia auropunctata* (Errard et al. 2005) and *Linepithema humile* (Suarez et al. 2002; Torres et al. 2007; Blight et al. 2010).

Supercolonies of *A. gracilipes* showed both quantitative and qualitative differences in their CHC profiles across various types of hydrocarbons (Fig. 4, Table 6). This is remarkable since in most ant species, CHC profiles tend to differ quantitatively between conspecific colonies and qualitatively between species (Vander Meer and Morel 1998; Elmes et al. 2002; Hefetz 2007). For instance, 14 out of 41 epicuticular compounds differed qualitatively between the CHC profiles of six *Formica* species (*Formica* sensu stricto) in a study by Martin *et al.* (2008b), if the same threshold was applied as in our study. In comparison, 16 out of 30 substances differed qualitatively between the profiles of six different supercolonies of *A. gracilipes* in our study. On occasion, high degrees of qualitative differences between CHC profiles of colonies from non-invasive ant species have been reported, but analyses either include all substances (including trace substances, e.g. (Elmes et al. 2002)) or qualitative differences are only detected in one substance class, (e.g. homologous series of (Z)-9-alkenes or positional isomers of dimethyl-branched C25-bodies, (Martin et al. 2008a)). In invasive ant species, qualitative differences between CHC profiles of different supercolonies have repeatedly been found, e.g. between invasive and native populations of *Linepithema humile* (Brandt et al. 2009b) and *Wasmannia auropunctata* (Errard et al. 2005). CHC profiles were found to be less complex in introduced areas, with substances having been lost from the profiles in comparison to colonies in the native range (Errard et al. 2005). However, supercolonies of *Linepithema humile* also differ in their CHC profiles in the invasive range (Blight et al. 2010).

Here, we found unusually high degrees of qualitative differences between CHC profiles of different supercolonies of *A. gracilipes*. We identified 30 major CHC compounds that accounted for at least 1% of the entire CHC profile and found qualitative differences in compounds that accounted for up to 8.21% of the profiles of different *A. gracilipes* supercolonies. The qualitatively differing substances comprised different compound classes such as alkanes, alkenes, methyl-branched alkenes as well as mono-, di- and trimethyl-branched alkanes of varying chain length (Fig. 4), as would be expected if supercolonies diverge in a drift-like process of neutral evolution.

In our study, genetic distances corresponded to chemical distances between colonies, as has been observed in several other studies on various social insect species (ants (Dronnet et al. 2006), termites (Dapporto et al. 2009) or paper wasps (Ugelvig et al. 2008; Vogel et al. 2009)). The correlation between differentiation in CHC profile and differentiation in microsatellite loci in *A. gracilipes* suggests that cuticular hydrocarbons are to a large extent genetically determined in this species (as is in many ant species (Adams 1991; Pennanec'h et al. 1997; Takahashi et al. 2001; van Zweden et al. 2009; van Zweden et al. 2010)), albeit environmental cues may also be involved (Liang and Silverman 2000; Florane et al. 2004).

Helanterä *et al.* suggested that unicoloniality (the ability of ant species to form populations consisting of one or more supercolonies (Pedersen et al. 2006; Helanterä et al. 2009)), might be an evolutionary dead-end (Helanterä et al. 2009), since selfish behaviour of distantly related nestmates should lead to supercolony instability as predicted by kin-selection theory. This view is supported by the scattered distribution of unicoloniality in the ant phylogeny as well as the fact that no unicolonial ant species has a sister species that is also unicolonial (Helanterä et al. 2009). In contrast to other supercolonial ant species such as *L. humile* and *S. invicta*, however, workers in *A. gracilipes* supercolonies are closely related (Drescher et al. 2007; Helanterä et al. 2009), and thus selfish behaviour is less likely to evolve.

Despite this potential evolutionary dead-end, the limited or even absent gene flow between supercolonies of unicolonial ant species (Jaquiery et al. 2005; Pedersen et al. 2006; Thomas et al. 2006; Thomas et al. 2010b) may be conducive to speciation (Helanterä et al. 2009). As speciation occurs on timescales beyond reach for current research, indirect approaches are the only way to find clues regarding that question, e.g. by measuring how much supercolonies have diverged in neutral or selected traits (Helanterä et al. 2009). As we have shown, *A. gracilipes* supercolonies are indeed differentiated in neutral traits, i.e. alleles of microsatellite loci and several cuticular compounds. Regardless of the origin of this initial differentiation that we see today, one should expect both the genetic and chemical differentiation to increase in the prolonged absence of gene flow between supercolonies. Although we cannot infer absence of gene flow directly, the sociogenetic structure of the supercolonies in NE-Borneo resembles that of the supercolonies found on Christmas Island, where gene flow among the two supercolonies can be excluded based on mitochondrial and microsatellite markers (Thomas et al. 2010b). In addition, behavioural characteristics of *A. gracilipes*, such as intranidal mating soon after eclosion of female reproductives (pers. obs.) and aggression towards allocolonial sexuals by workers (Table 2), suggest that mating is non-random, resulting in strongly reduced or absent gene flow between supercolonies. Similar patterns (intranidal mating, limited or absent intercolonial gene flow) have been reported for the likewise invasive ant *Linepithema humile* both in its native (Pedersen et al. 2006; Vogel et al. 2009) and invasive range (Giraud et al. 2002; Jaquiery et al. 2005; Thomas et al. 2006).

The initial differences that would be necessary in order for subsequent differentiation between neighbouring supercolonies to occur are likely to accumulate in allopatry, e.g. if supercolonies stem from different source populations or if supercolonies are divided into two or more fragments through human-mediated jump dispersal. In the latter scenario, lack of contact zones (and thus mating) between the resulting allopatric fragments is likely to lead to accumulation of colony (or fragment-) specific mutations and CHC compounds. If both fragments come into secondary contact again, genetic and chemical differentiation may have advanced enough to result in mutual aggression and lack of gene flow. This pattern was very recently described for *Linepithema humile* supercolonies from Corsica and the European mainland, where the introduction of one or several *L. humile* propagules from the mainland to Corsica likely entailed an interruption of gene flow between Corsican and European supercolonies, resulting in the pronounced chemical and behavioural differentiation observable today (Blight et al. 2010). This scenario may also explain parts of our data, as supercolonies P3 and P4 are likely to be established fragments of the same supercolony which have already accumulated supercolony-specific alleles and cuticular compounds despite still tolerating each other (Tables 4 and 6). Over time, the spatial separation (allopatry) of the two supercolonies P3 and P4 should facilitate increasing genetic and chemical differentiation, which in turn should lead to mutual aggression as soon as CHC profiles are sufficiently different. Furthermore, genetic and chemical differentiation should also entail behavioural suppression of intercolonial gene flow through worker aggression against allocolonial sexuals even if they came into contact again (e.g. as a result of range expansion).

Thus, we suggest that the combination of exclusive intranidal mating and budding on the one hand and positive feedback between genetic, chemical and behavioural traits on the other hand may drive supercolonies towards ever increasing differentiation, possibly even involving reproductive isolation and thus, speciation. In contrast to the majority of ant species, *A. gracilipes* supercolonies are characterized by a combination of traits (polygyny, intranidal mating, and lack of active dispersal other than budding) that ensure the continuous production of generation upon generation of reproductives that all stay within the supercolony. Coupled with an unusual reproductive system that may potentially avoid the negative effects of inbreeding depression (Drescher et al. 2007), the extreme polygyny and polydomy of *A. gracilipes* immensely reduces the risk of a breakdown of the entire supercolony, resulting in virtual immortality of the supercolony superorganism. Furthermore, in eusocial species with strict intranidal mating, a colony-specific signal should be sufficient for recognition between the sexes and thus, selection towards a species-specific signal that allows recognition between sexes from different supercolonies should be relaxed (as suggested in Martin et al. 2009). In concert with the virtual immortality of *A. gracilipes* supercolonies, relaxed selection towards a species-specific signal may even lead to a complete breakdown of intraspecific recognition between sexuals from different colonies, potentially leading to prezygotic, reproductive isolation. This, in turn, implies that different *A. gracilipes* supercolonies would no longer belong to the same species according to the biological concept of species, which states that species are groups of interbreeding natural populations that are reproductively isolated from other such groups (Mayr 1942). Currently it is unclear whether different *A. gracilipes* supercolonies should be perceived as belonging to the same species, as apparent lack of random mating between sexuals from different supercolonies, intranidal mating and aggression of workers towards allocolonial sexuals may already pose a sufficient barrier preventing intercolonial gene flow. Thus, we argue that intercolonial differentiation may potentially result in reproductive barriers between colonies, ultimately leading to independent units on different evolutionary trajectories, and thus possibly speciation between neighbouring *A. gracilipes* supercolonies.

ACKNOWLEDGEMENTS

We thank the Economic Planning Unit (EPU) for permission to conduct research in Malaysia, Prof. Dr. Datin Maryati Mohamed for collaboration and Dr. Maklarin bin Lakim (Sabah Parks) for permission to conduct our work at Poring Hot Springs, as well as H. Helanterä and three anonymous reviewers for valuable comments on this manuscript. All experiments conducted comply with the current laws of Malaysia.

VII. WORKER AGGRESSION TOWARDS ALLOCOLONIAL MALES AND QUEENS MAY FACILITATE SPECIATION BETWEEN *ANOPLOLEPIS GRACILIPES* SUPERCOLONIES

ABSTRACT

Some ant species form large polygynous, polydomous supercolonies in which mating takes place within the nest (intranidal mating) and which grow via the occupation of suitable nesting sites by fertilized queens along the supercolony boundary (colony budding). Supercolonial ant species rarely, if ever, perform nuptial mating flights and as a consequence, gene flow may be largely confined within supercolonies whereas gene flow between supercolonies may be limited or even absent. In the absence of mating flights, the migration of reproductive individuals from one supercolony to another may represent the only way by which gene flow between different supercolonies may be maintained. This, however, would require workers to tolerate males or queens from different supercolonies. We tested the potential of reproductive individuals to migrate between supercolonies using 14 supercolonies of the Yellow Crazy Ant *Anoplolepis gracilipes* from North-East Borneo. A previous study suggested that mutually aggressive supercolonies of this ant species may be highly differentiated both genetically (in nuclear microsatellite loci) and chemically (in cuticular hydrocarbons, CHC's) suggesting limited or absent gene flow between them (Chapter VI, Drescher et al. 2010). The present study revealed highly congruent patterns of genetic, chemical and behavioural differentiation between *A. gracilipes* supercolonies, thus supporting the findings of the previous study. Intercolonial differentiation in mitochondrial DNA also corresponded to nuclear genetic, chemical and behavioural differentiation, implying that maternal ancestry may be an important factor in shaping intercolonial differentiation. Arena experiments showed that workers were highly aggressive towards males and queens from different supercolonies, suggesting that they may act as behavioural boundary to a potential migration of sexuals between supercolonies, thus possibly impairing gene flow between supercolonies. A series of preliminary breeding experiments revealed that despite males and queens from different supercolonies may be able to successfully mate in the absence of workers, queens fertilized by allocolonial males may potentially not be able to produce viable worker offspring. Thus, gene flow between different *A. gracilipes* supercolonies may not only

be impaired by worker aggression towards allocolonial reproductives, but may further be the result of reproductive isolation between sexuals from different supercolonies. Both potential mechanisms may enhance genetic and chemical differentiation between different *A. gracilipes* supercolonies by impeding intercolonial gene flow, and might ultimately contribute to the diversification of different *A. gracilipes* supercolonies into different species.

INTRODUCTION

Biological invasions are considered as one of the main threats to global biodiversity, as invaders often negatively influence native taxa (e.g. Sanders et al. 2003; Kenis et al. 2009), potentially leading to severe economic and environmental damage (Mack et al. 2000b; Mooney and Cleland 2001; Perrings et al. 2002). Social insects, especially ants, belong to the most devastating groups of invaders (Moller 1996) and five ant species are found among the 100 invasive species declared most destructive worldwide (Lowe et al. 2000): the Yellow Crazy Ant (*Anoplolepis gracilipes*), the Argentine Ant (*Linepithema humile*), the Big-headed Ant (*Pheidole megacephala*), the Little Fire Ant (*Wasmannia auropunctata*) and the Red Imported Fire Ant (*Solenopsis invicta*). The ecological success of introduced ant species in new habitats seems to be tightly linked to their unusual ability to form high-density supercolonies, which are often polygynous (contain more than one queen) and in which workers roam freely between neighboring nests (Holway et al. 2002; Pedersen et al. 2006). As opposed to most ants, supercolonial ant species show a tendency to mate within the nest (intranidal mating) instead of mating in periodic nuptial flights. Queens often stay within the maternal nest after fertilization, enter neighboring nests within the supercolony or establish nests at the supercolony boundary (known as supercolony budding). While the main dispersal mode over short distances seems to be colony budding, the predominant long-range dispersal mode of supercolonial ant species is human-mediated jump-dispersal of propagules (Holway et al. 2002). Recent studies on *L. humile* (Jaquiery et al. 2005; Pedersen et al. 2006; Thomas et al. 2006) and *A. gracilipes* (Drescher et al. 2010; Thomas et al. 2010a) suggest that as a consequence of this combination of mating and dispersal strategies, gene flow may be largely confined within supercolonies and thus extremely limited or even absent between them, thus possibly resulting in ant supercolonies following independent evolutionary trajectories (Drescher et al. 2010).

A vital prerequisite for supercolonies to diverge through absence of intercolonial gene flow is that males and queens from different supercolonies do not mate. In most ant species, males and queens from different colonies mate in synchronized nuptial mating flights. The absence of nuptial mating flights, however, is a characteristic that is shared by several invasive ant species such as *Linepithema humile* (Passera and Aron 1993; Aron 2001) or *Lasius neglectus* (Espadaler and Rey 2001; Espadaler et al. 2004). Even though mating flights were suggested for *Anoplolepis gracilipes* in a survey on Christmas Island (Abbott 2006), a later study demonstrated that should these mating flights occur, they do not result in random mating among sexuals from the different supercolonies on Christmas Island (Thomas et al. 2010a).

An alternative way by which males and queens could find allocolonial mates would be to immigrate into foreign supercolonies. However, in order to facilitate such allocolonial mating by migration of sexuals and hence gene flow between supercolonies, resident workers would have to tolerate foreign males or queens entering their supercolony. Otherwise, mating between males and queens from different colonies may be unlikely. Consequently, rejection of allocolonial sexuals might represent an effective behavioural barrier to gene flow between supercolonies, facilitating an independent co-existence and evolution of supercolonies.

In order to study whether the potential rejection of foreign sexuals by workers may act as mechanism facilitating differentiation between supercolonies of *Anoplolepis gracilipes*, we studied 14 *A. gracilipes* supercolonies in NE-Borneo using four different approaches (behavioural assays, genetic analyses of mtDNA and microsatellites as well as chemical analyses of cuticular hydrocarbon profiles). An earlier study suggested that aggression between *A. gracilipes* supercolonies is positively correlated to their genetic distance, which is reflected by a higher dissimilarity in their composition of cuticular hydrocarbons (Drescher et al. 2010). This study further revealed that mutually aggressive *A. gracilipes* supercolonies shared only a third of their alleles across six microsatellite loci and only three quarters of the compounds in their cuticular hydrocarbon profiles (Drescher et al. 2010). Thus, we expect aggression between workers from the 14 different supercolonies to be reflected by both the genetic and chemical differentiation between them, and that mutually aggressive supercolonies share only a fraction of their alleles and CHC compounds. Furthermore, supercolonies of the same matriline (inferred by an identical mtDNA haplotype) might be more closely related than supercolonies from different matrilines, since they may have been derived from the same founding queen. Similar cuticular hydrocarbon profiles between such closely related supercolonies may lead to lower levels of aggression, as opposed to encounters between different haplotypes. Finally, high degrees of genetic and chemical differentiation between mutually aggressive invasive ant supercolonies may suggest a lack of gene flow between them (Drescher et al. 2010; Thomas et al. 2010a). We thus investigate whether the immigration of allocolonial sexuals may be hampered by aggression of resident workers towards them. We expect workers from mutually aggressive supercolonies to show a corresponding aggression towards males and queens as well.

METHODS

Selection and maintenance of colonies

Anoplolepis gracilipes subcolonies were collected and maintained from 14 supercolonies in three focal regions in NE-Borneo (Fig. 17): Three from Poring Hot Springs (PHS, supercolonies P3, P5, and P6, Drescher et al. 2010; Drescher et al. 2011), ten from the Sepilok Forest Reserve (SEP, supercolonies S1-S10) and one from the Lower Kinabatangan Nature Reserve (KIN, supercolony K1). Queens, workers and brood were collected from each supercolony and transferred into plastic buckets (Vol. = 25 l) treated with Fluon™ to prevent escape. All colonies were offered newspaper and cardboard as nesting material and were fed water, honey and crayfish scraps twice a week. Initially, each of the subcolonies contained at least 10.000 workers, 500 pupae, 50 males and 20 queens.

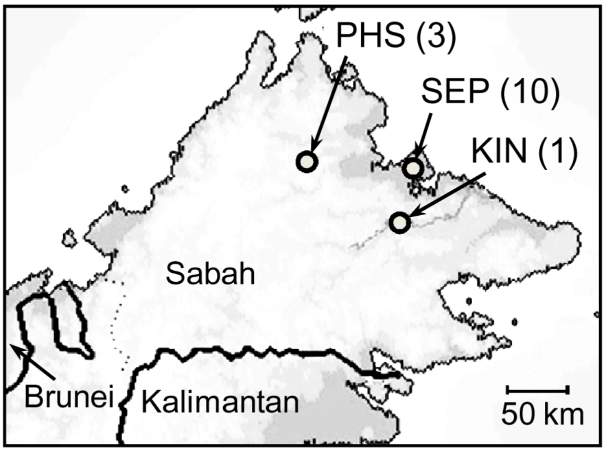


Figure 17. Location of the 14 Anoplolepis gracilipes supercolonies used in this study. Ants were sampled from supercolonies in three regions in NE-Borneo: Poring Hot Springs (POR, three supercolonies), Sepilok Nature Reserve (SEP, ten Supercolonies) and Kinabatangan Nature Reserve (KIN, one colony).

Behavioural assays

Aggression between workers of the 14 subcolony pairs was obtained using two indices, the Mortality Index *MI* (Drescher et al. 2007; Drescher et al. 2010) and the Aggression Latency T_{bite}. To measure the Mortality Index *MI*, five workers of each subcolony (10 ants per trial per pairwise colony combination) were placed within a Fluon coated plastic cylinder (diameter = 10 cm, height = 5 cm) on a sheet of paper which was replaced after each trial (n=10 trials per colony combination). Every minute, the number of dead individuals was counted over a period of 60 min. For each trial, the mortality index *MI* was obtained as MI = $(y/2)/t_{50}$, with y being the total number of individuals killed at the end of the experiment (60 min) and t_{50} the time (in steps of 1 min) when half of this number ($y/2$) was already killed.

To measure the Aggression Latency T_{bite}, five 'resident' workers were confronted with one allocolonial worker in an arena as described above. Within ten minutes, the Aggression Latency T_{bite} was recorded as the time lapse from first antennal contact between 'resident' workers and the single allocolonial worker until the singe allocolonial worker was bitten for the first time. We performed 20 trials for each subcolony combination, ten of which were performed using 'resident' workers from one colony and the other ten using 'resident' workers from the other subcolony. Overall aggression towards allocolonial workers in a subcolony combination was obtained by pooling the Aggression Latency T_{bite} across the two sets of reciprocal bioassays. Likewise, aggression of workers towards allocolonial reproductives was measured as the Aggression Latency T_{bite} among workers, males and dealate queens from four different subcolonies (K1, S6, S7, S10). The Mortality Index *MI* was not obtained for bioassays involving allocolonial workers and reproductives, as the lowered aggression of workers towards allocolonial sexuals did not meet the requirements of the Mortality Index *MI*.

Genetic analyses – mitochondrial DNA

As preliminary results yielded no more than one haplotypes per supercolonies (unpub.), one individual each of the 14 supercolonies was used for DNA extraction using the Puregene® DNA Purification Kit (Gentra Systems) according to the manufacturer's recommendations. Mitochondrial DNA was amplified using the primer pairs Horst (5'-AC(TC)ATACTTTTAACTGATCG-3') designed by D. Kronauer (unpubl.) / Ben (5'-GC(AT)AC(AT)AC(AG)TAATA(GT)GTATCATG-3') (Moreau et al. 2006) for partial Cytochromeoxidase I (*COI*) (Genebank accession numbers: DQ888821-DQ888825) corresponding to positions 2407-2427 (Horst) and 2891-2914 (BEN) relative to the mitochondrial genome of *Apis mellifera* (Crozier and Crozier 1993) and CBI/CBII for partial Cytochrome B (*Cytb*) (Genebank accession numbers: DQ888817-DQ888820) (Crozier et al. 1991). PCR reactions were performed as described in Drescher et al. (2007). The purified mtDNA fragments were sequenced by SEQLAB, analyzed using Chromas Lite 2.01 (Technelysium Pty Ltd) and aligned using ClustalW (Thompson et al. 1994) implemented in BioEdit 7.0.5.3 (Hall 1999). We then constructed a haplotype network based on the concatenated sequence of 904bp (consisting of a 460bp fragment of the *COI* gene and a 444bp fragment of the *Cytb*) using the 95% parsimony algorithm implemented in TCS 1.21 (Clement et al. 2000a).

Genetic analyses – nuclear DNA / microsatellites

DNA was extracted from 8 individuals per subcolony as described above and genotyped using six polymorphic microsatellite loci (Ano1, Ano3, Ano4, Ano6, Ano8, Ano10) according to the protocol in Feldhaar et al. (2006). Relatedness within and between the 14 supercolonies was calculated using Relatedness 5.0.8 (Queller and Goodnight 1989), including all individuals as reference population. All R values in this study result from the half-matrix of 112 × 112 pairwise comparisons of individual relatedness, excluding comparisons of each individual with itself.

Cuticular hydrocarbon profiles

Cuticular hydrocarbons (CHCs) were extracted from 20 pooled workers from each of the 14 supercolonies and analyzed according to the protocol in Drescher et al. (2010). Only hydrocarbons, which were identified by their typical mass spectra, were included in our analysis. Furthermore, all molecules smaller that C19-bodies were discarded since they only occurred in traces and were absent in most of the investigated cuticular hydrocarbons (Martin and Drijfhout 2009). To visualize differences in CHC profiles, we performed non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities (d_{ij}) of relative peak areas of CHC profiles.

Correlations between behaviour, genetic and chemical properties of workers from different supercolonies

We constructed a matrix of the percentage of alleles that were not shared by supercolony pairs as well as a matrix of the percentage of qualitatively differing cuticular hydrocarbon compounds between supercolony pairs. We then tested for correlation between these two matrices, the matrices of both aggression indices (Mortality Index *MI* and Aggression Latency T_{bite}), relatedness R, Bray-Curtis-distances of CHC profiles and the genetic distance between mtDNA haplotypes (i.e. the number of mutational steps needed to transform one haplotype into another by substitution of single nucleotides) by performing mantel tests (10000 permutations) between matrix pairs.

Mating experiments between A. gracilipes supercolonies (preliminary)

Based on observations of worker aggression towards foreign sexuals, we examined whether males and queens from different supercolonies were generally able to mate and start new colonies, both in the presence and absence of workers from the queen supercolony. Virgin queens were obtained by removing queen pupae from lab colonies and letting them hatch and be cared for by their fellow workers in small subcolonies. In each of eight replicates, one virgin alate queen was confronted with five males from a different lab colony in a plastic container (10cm x 10cm, height = 5cm) which contained a small burrow made of plaster as well as water, honey and bits of cockroaches. In four of the eight replicates, virgin queens were accompanied by 150 workers from their own colony before five foreign males were added. In the other four replicates, virgin queens and males were left alone for 72h after which the five males were removed and 150 workers from the queen's colony were added. Every month, the presence/absence of all stages of brood was noted. After six months, experiments were aborted and the queen's spermathecae were dissected to verify whether the queens had been inseminated. To compare colony development after intercolonial and intracolonial mating, we removed 35 dealate queens from various supercolonies (thus already mated queens), placed them in plastic containers as described above and added 150 workers. The presence/absence of brood was noted as above. After three to six months, queens were removed and their spermathecae dissected. As *A. gracilipes* supercolonies are highly genetically differentiated (Drescher et al. 2010), the thorax and spermatheca content of each queen was genotyped as described above to confirm that queens had been fertilized by males from their own supercolony.

RESULTS

Behavioural, genetic and chemical differentiation between supercolonies

The intensity of aggression between allocolonial workers varied between supercolony pairs, confirmed by both aggression indices (Kruskal-Wallis-ANOVA, *MI*: $H = 559.8$, $p < 0.0001$;

T_{bite}: H = 589.3, $p < 0.0001$; Mantel test between *MI* and T_{bite}: R = -0.26, p = 0.0009, Table 8), ranging from trials with no mortality to encounters in which 9 out of 10 ants died. Overall, 2.3 ± 1.5 (mean ± SD) individuals died in bioassays between supercolonies, while not a single individual was killed in intracolonial control tests. Workers of all supercolonies were aggressive towards each other, and in 5:1 live assays, single workers were bitten after T_{bite} = 7.3 ± 5.5 sec (mean ± SD) after first contact with the 'resident' workers.

We found 32 alleles in the entire population. Relatedness between supercolonies was lower (*R* = -0.04 ± 0.37, mean ± SD, range: -0.6 – 0.65) than within supercolonies (*R* = 0.81 ± 0.12, range: 0.58 – 1; U = 62.0, $p < 0.0001$, Mann-Whitney U-test), and on average, supercolonies shared 46.4% ± 16.4% of their alleles (mean ± SD). The GC-MS analyses of cuticular hydrocarbons revealed a total of 129 cuticular compounds. Supercolonies shared 86.7% ± 5.3% of the compounds in their CHC profiles. Furthermore, intercolonial aggression, genetic distance and qualitative chemical differentiation were significantly correlated between the colony pairs, but quantitative distances of CHC profiles were only significantly correlated with relatedness *R* (Table 8).

Table 8. Correlation coefficients between aggression and chemical and genetic differentiation among 14 *Anoplolepis gracilipes* supercolonies. Abbreviations stand for pairwise Mortality Index ('*MI*'), Aggression Latency ('T_{bite}'), Relatedness ('*R*'), percentage of differing alleles ('% Alleles'), Bray-Curtis distance of cuticular hydrocarbon profiles ('d_{ij} CHC'), percentage of differing CHC compounds ('% CHC's') and number of mtDNA mutational steps ('mtDNA mutations') between workers of 14 *A. gracilipes* supercolony pairs in Sabah, Malaysia. P-levels are: $^{+}p<0.1$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}<0.001$, mantel tests.

	MI	**T_{bite}**	***R***	**% Alleles**	**d_{ij} CHC**	**% CHC's**
T_{bite}	-0.26**					
R	-0.54***	0.37***				
% Alleles	0.55***	-0.30**	-0.80***			
d_{ij} CHC	0.28^{+}	-0.08	-0.22*	0.10		
% CHC's	0.44***	-0.25*	-0.61***	0.51***	0.15	
mtDNA mutations	0.19	-0.06	-0.15^{+}	0.23^{+}	-0.18	0.29*

Behavioural, genetic and chemical differentiation between mtDNA haplotypes

We identified seven different mitochondrial haplotypes across the 14 *A. gracilipes* supercolonies which were between 2 and 9 mutational steps apart (Fig. 18). The most common haplotype A was found in five supercolonies from Sepilok whereas the other six haplotypes were found in either one or two colonies. While six of the seven haplotypes were exclusive to a single sampling region, haplotype C was found in Poring as well as in Sepilok. The number of mutational steps that separated supercolony haplotypes was not correlated to their aggression (MI, T_{bite}), genetic differentiation (R, percentage of differing alleles) or quantitative dissimilarities between CHC profiles, but were positively correlated to the percentage of distinct CHC compounds (R = 0.29, p = 0.017, Table 8, Mantel test). Workers of all supercolonies displayed aggression towards each other. However, workers from the same mtDNA haplotype were slightly less aggressive towards each other than workers from supercolonies carrying a different haplotype ($MI_{\text{within}} = 3.8 \pm 7.0$, $MI_{\text{between}} = 9.2 \pm 7.9$, Fig. 19 A; $T_{bite\text{-within}} = 8.6 \pm 6.2$ sec, $T_{bite\text{-between}} = 10.1 \pm 28.3$, Fig. 19 B, mean ± SD). Likewise, relatedness R was higher within a haplotype than between haplotypes ($R_{within} = 0.28 \pm 0.35$; $R_{between} = -0.05 \pm 0.38$, Fig. 20 A) as was allelic differentiation (percentage of

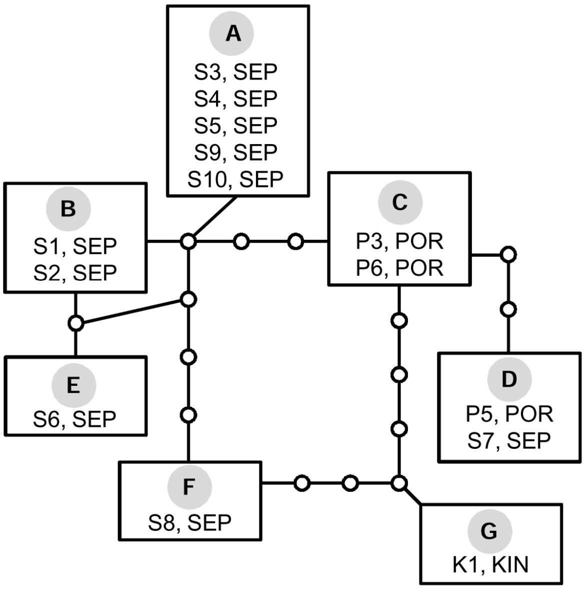


Figure 18. 95%-parsimony haplotype network based on a 904bp fragment of *COI* (460bp) and Cyt*b* (444bp). Abbreviations denominate colony identity and sampling region (supercolonies P3, P5 and P6 from Poring/POR, supercolonies S1-S10 from Sepilok/SEP and supercolony K1 from Kinabatangan/KIN) that are distributed among 7 haplotypes (A-G). Nodes between haplotypes indicate the number of parsimonious mutational differences.

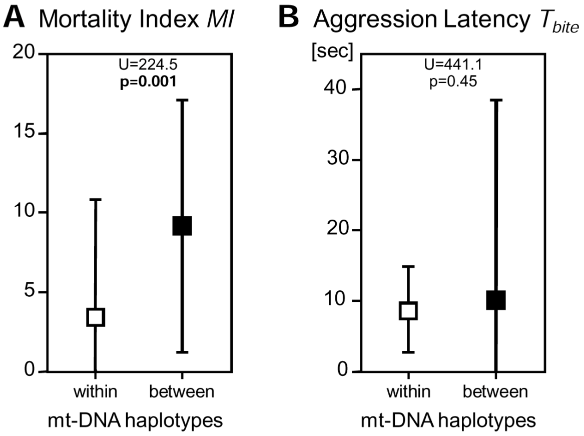


Figure 19. Aggression between *Anoplolepis gracilipes* supercolonies of the same ('within') or differing ('between') mt-DNA haplotypes. Aggressoin was measured as (A) Mortality Index MI and (B) Aggression Latency T_{bite} (mean ± SD, including Mann-Whitney U-test statistics).

different alleles within haplotypes: 44.1% ± 17.3%, between haplotypes: 55.1% ± 15.8%, Fig. 20 B). Whereas the quantitative chemical differentiation within and between haplotypes did not differ significantly (Fig. 21 A), the qualitative chemical composition did (percentage of different CHC compounds within haplotypes: 7.9% ± 4.3%; between haplotypes: 14.2% ± 4.9%, Fig. 21 B). The genetic distance between mtDNA haplotypes was positively correlated to the quantitative chemical differentiation and marginally significantly correlated to nuclear genetic differentiation (both *R* and proportion of differing alleles), but not to aggression or quantitative distances between CHC-profiles (Table 8).

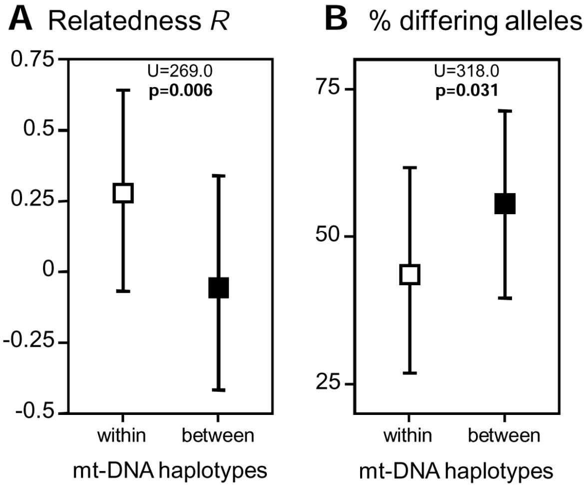


Figure 20. Genetic differentiation between *Anoplolepis gracilipes* supercolonies of the same ('within') or differing ('between') mt-DNA haplotypes. Genetic differentiation was measures as (A) Relatedness R and (B) percentage of differing alleles between supercolony pairs (mean ± SD, including Mann-Whitney U-test statistics).

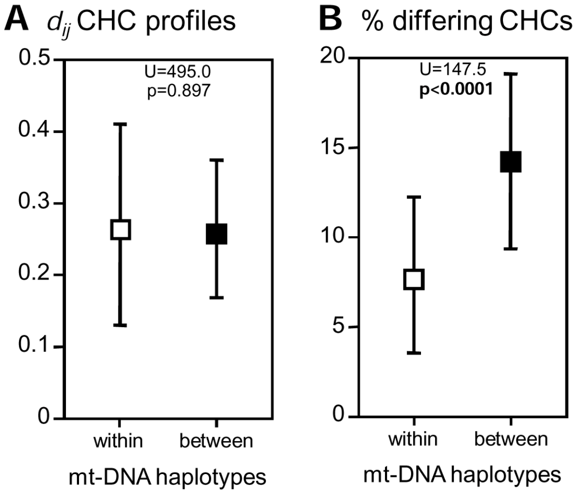


Figure 21. Chemical differentiation between *Anoplolepis gracilipes* supercolonies of the same ('within') or differing ('between') mt-DNA haplotypes. Chemical differentiation was measured as (A) Bray-Curtis dissimilarities of CHC profiles and (B) percentage of compounds differing between CHC-profiles (mean ± SD, including Mann-Whitney U-test statistics).

Worker aggression towards allocolonial males and queens

Workers were aggressive towards allocolonial workers as well as towards allocolonial males and dealate queens (Fig. 22). However, workers attacked allocolonial workers much more rapidly (T_{bite} = 9.2 ± 5.8 sec, mean ± 95% CI, Fig. 22 A) than allocolonial males (T_{bite} = 150.5 ± 62.5 sec) or allocolonial queens (T_{bite} = 234.3 ± 50.0 sec). Furthermore, workers killed a higher number of allocolonial workers and males than allocolonial queens (Fig. 22 B). Worker aggression against allocolonial workers and males differed significantly among supercolonies (aggression towards allocolonial workers: T_{bite} , H = 29.6, $p < 0.0001$;

aggression towards allocolonial males: T_{bite}, H = 15.8, p = 0.007, Kruskal-Wallis ANOVA, Fig. 23 A-B), unlike aggression against allocolonial queens (Fig. 23 C). Nonetheless, worker aggression towards allocolonial males and queens was positively correlated (T_{bite}, R = 0.75, p = 0.042), but not between workers and males or workers and queens, respectively.

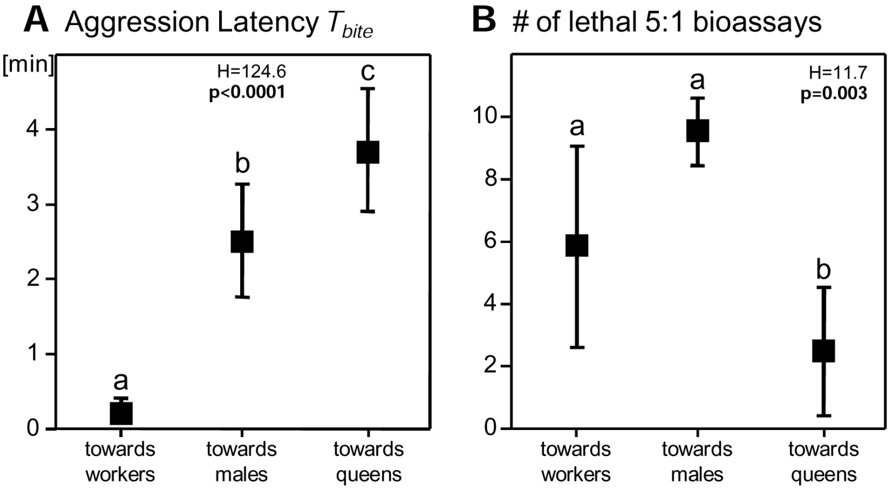


Figure 22. Aggression of *Anoplolepis gracilipes* workers towards allocolonial workers, males and queens. Aggression was measured as (A) Aggression Latency T_{bite} and (B) number of mortal encounters in 5:1 live bioassays (mean ± 95% CI, including Kruskal-Wallis ANOVA test statistics).

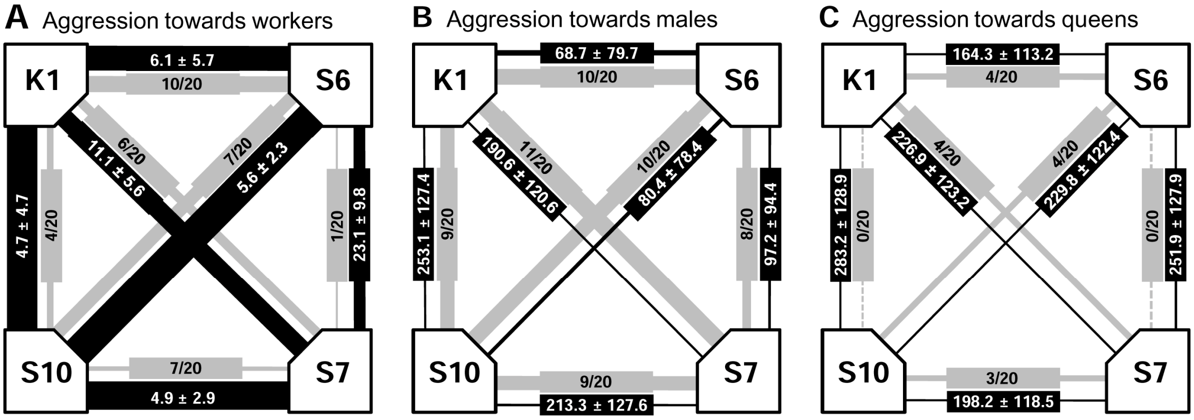


Figure 23. Aggression of workers from four *Anoplolepis gracilipes* supercolonies (K1, S6, S7, S10) towards (A) allocolonial workers, (B) allocolonial males and (C) allocolonial queens. Two aggression measures are displayed: Aggression Latency T_{bite} (black bars, white letters) and the number of mortal encounters in N=20 trials (grey bars, black letters; each trial consisted of 5 workers vs. 1 allocolonial worker/male/queen, mean ± 95% CI).

Mating experiments between A. gracilipes supercolonies (preliminary results)

In intercolonial breeding experiments in which males were added to a subcolony containing 150 workers and one virgin queen from a different colony, all males were instantly attacked and killed within 30 minutes. Consequently, queens and males could not mate and no brood was produced (see Table 9). In breeding experiments in which workers from the queen's colony were added 72 hours later than allocolonial males, all queens mated with one or several of the allocolonial males, which was both observed as well as verified by the filled spermathecae of all four queens. In two of the four replicates, no brood was produced despite the fact that the queens were fertilized, and queens died after four months. In the remaining two replicates, queens produced eggs and larvae, but no pupae were produced within six months (Table 9). In contrast, all of the 35 dealate queens taken directly from within supercolonies were inseminated (by males from their own supercolony) and started producing pupae after a maximum of three months.

Table S1 Development of *Anoplolepis gracilipes* subcolonies in allocolonial mating experiments under presence/absence of 150 workers from the queens' colony within the first 72 hours of the experiment.

queens/ worker colony	Males colony	Workers present?	Queens fertilized?	Colony development
S9	P5	YES	NO	all males died within 10min
S9	P5	YES	NO	all males died within 10min
S9	P5	YES	NO	all males died within 30min
S9	P5	YES	NO	all males died within 20min
S9	S1	NO	YES	4th month: queen died, no brood during that time
S9	S1	NO	YES	4th month: queen died, no brood during that time
S9	P5	NO	YES	1st month: eggs, 3rd month: larvae, no pupae/callows until abortion of the experiment
S9	P5	NO	YES	1st month: eggs, 3rd month: larvae, no pupae/callows until abortion of the experiment

DISCUSSION

In an earlier study, we showed that supercolonies of the Yellow Crazy Ant *Anoplolepis gracilipes* can be substantially differentiated both genetically (using nuclear DNA microsatellites) and chemically to an extent that suggests lack of gene flow between them (Drescher et al. 2010). In the present study, we (A) confirm the previous data with an increased sample size of 14 supercolonies, (B) show that differentiation in mitochondrial DNA corresponds to an increased aggression, nuclear genetic and chemical differentiation between supercolonies and (C) suggest a mechanism by which gene flow may be suppressed between supercolonies, i.e. the aggression of workers towards foreign sexuals. Moreover, we present preliminary data indicating that – in contrast to males and virgin queens from the same supercolony – allocolonial reproductives may be able to mate but not necessarily capable of producing viable worker offspring.

Behavioural, genetic and chemical differentiation between supercolonies

Recently, several studies have suggested limited or even absent gene flow between supercolonies of supercolonial ant species such as *Linepithema humile* (Jaquiery et al. 2005; Pedersen et al. 2006; Thomas et al. 2006) or *Anoplolepis gracilipes* (Drescher et al. 2010; Thomas et al. 2010a), using behavioural and genetic or chemical properties (or both) as indicators of differentiation between supercolonies. In this paper, we used two aggression indices (Mortality Index *MI* and Aggression Latency T_{bite}), two measures of genetic (Relatedness *R* and proportion of differing alleles) and two measures of chemical properties (quantitative and qualitative distances of CHC profiles), all of which were correlated among each other with the exception of quantitative CHC distance. By confirming previous data (Drescher et al. 2010) in a second region containing a larger set of supercolonies, this study is consistent with the hypothesis that the majority of *A. gracilipes* supercolonies existing today may not exchange genetic material. Instead, they seem to represent different independent units, i.e. potentially separated evolutionary trajectories.

Behavioural, genetic and chemical differentiation between mtDNA haplotypes

The 14 supercolonies in this study contained seven different mtDNA haplotypes which were between two and nine mutational steps apart. Workers from all 14 supercolonies were aggressive towards each other, which suggests that different haplotypes may virtually always represent different supercolonies, while the same haplotype may define groups either from the same or from different supercolonies (Drescher et al. 2010). Moreover, supercolonies of the same haplotype may have been either introduced from the same source population or derived from each other by dispersing queens (most likely as part of an anthropogenically dispersed propagule). In contrast to data on the Argentine Ant *Linepithema humile* (Vogel et al. 2009), *A. gracilipes* supercolonies with identical haplotype were genetically more similar that those with different haplotypes, both in terms of pairwise relatedness as well as the percentage of different alleles. Additionally, we observed less mortal aggression and a lower qualitative chemical differentiation between supercolonies with identical haplotype. Thus, our data suggest that ancestral maternal lineages may represent the first stage of differentiation between *A. gracilipes* supercolonies followed by a second stage of differentiation within maternal lineages, e.g. through accumulation of supercolony-specific nuclear genetic mutations in allopatry (Drescher et al. 2010).

Aggression towards foreign sexuals and breeding experiments

As opposed to most social insects, supercolonial ant species such as *Anoplolepis gracilipes* or *Linepithema humile* are characterized by a strong tendency to mate within the nest rather than in synchronized nuptial mating flights. As a result, the immigration of reproductives into foreign supercolonies may represent the only alternative possibility by which gene flow between supercolonies may be achieved. A vital prerequisite for immigration of foreign queens and males would thus be the tolerance by resident workers towards them. Our results, however, indicate that a potential immigration of reproductives into foreign supercolonies may consistently fail due to worker aggression towards allocolonial males and queens. Both measures, the aggression latency T_{bite} and the number of lethal bioassays, yielded conclusive evidence suggesting that whenever workers of different supercolonies are aggressive towards each other, they are also likely to be aggressive towards each others reproductive castes. Aggression differed to some extent between castes. While the workers' aggression towards foreign workers and males was both more rapid and more intense, it was significantly reduced towards foreign queens. A higher tolerance towards dealate, fertilized queens may be due to a fertility signal which has been shown to reduce worker aggression towards egg-layers in several ponerine ant species such as *Pachycondyla sublaevis* (Ito and Higashi 1991), *Dinoponera quadriceps* (Monnin and Peeters 1999), *Diacamma ceylonense* (Cuvillier-Hot et al. 2002), and *Streblognathus peetersi* (Cuvillier-Hot et al. 2004).

Nevertheless, workers were aggressive towards both males and queens that did not belong to the same supercolony, suggesting that a potential immigration of foreign reproductives may likely be prevented by resident workers. This would effectively prohibit gene flow between supercolonies. This view is further supported by preliminary data on mating experiments between queens and males from different supercolonies (Table 9), which revealed that workers fight off and kill foreign males even if they were in the presence of an unmated alate queen. Moreover, our preliminary results indicate that the reproductive potential of *A. gracilipes* queens fertilized by allocolonial males may be severely lowered, even though egg-policing by the workers accompanying the queen can not be entirely excluded.

Overall, the present study is in concert with previous findings by Drescher et al. (2010), demonstrating that neighboring *Anoplolepis gracilipes* supercolonies are strongly differentiated both genetically and chemically, which translates into increased aggression between supercolonies. In addition, the present study revealed that the affiliation to mtDNA haplotypes corresponds to intercolonial aggression as well as nuclear genetic and chemical differentiation, i.e. supercolonies that may stem from the same ancestral queen are slightly less aggressive and genetically and chemically more similar than supercolonies from different maternal lineages. Finally, the present study revealed a potential mechanism by which gene flow between *A. gracilipes* supercolonies may be restricted or even entirely prohibited, i.e. the aggression of workers towards allocolonial sexuals. As *A. gracilipes* supercolonies are virtually immortal due to their capability to continuously produce generation after generation of reproductives, a prolonged absence of gene flow may further enhance genetic and chemical differentiation between supercolonies through neutral drift over long time spans. Positive feedback between behavioural, genetic and chemical differentiation should further enhance intercolonial segregation, possibly even until reproductive isolation, and thus speciation, between different *A. gracilipes* supercolonies. In fact, the preliminary allocolonial mating experiments may already have detected an intermediate form of reproductive isolation between *A. gracilipes* supercolonies, as colony development was impeded in all four replicates despite the fact that the queens were fertilized.

ACKNOWLEDGEMENTS

We thank the Economic Planning Unit (EPU) for permission to conduct research in Malaysia, and Prof. Dr. Datin Maryati Mohamed for collaboration. Dr. Maklarin bin Lakim (Sabah Parks) and Dr. Arthur Chung (Sabah Forestry Department) are thanked for permission to conduct our study in Poring Hot Springs and Sepilok Forest Reserve, respectively. All experiments conducted comply with the current laws of Malaysia.

VII. Worker aggression towards foreign sexuals

VIII. ECOLOGICAL DOMINANCE OF *ANOPLOLEPIS GRACILIPES* IN AN ANT COMMUNITY IN NORTH-EAST BORNEO

This Chapter has been published as:

Drescher J, Feldhaar H, Blüthgen N (2011) Interspecific aggression and resource monopolization of the invasive ant *Anoplolepis gracilipes* in Malaysian Borneo. *Biotropica* 43: 93-99

ABSTRACT

Invasions by introduced ant species can be ecologically destructive and affect a wide range of taxa, particularly native ants. Invasive ant species often numerically dominate ant communities and outperform native ant species in effective resource acquisition. Here we describe interactions between the invasive ant *Anoplolepis gracilipes* (Smith) and resident ant species in disturbed habitats in north-eastern Borneo. We measured interference competition abilities of *A. gracilipes* by performing arena bioassays between two *A. gracilipes* colonies and seven local ant species, and measured its effective resource competition at baits within supercolonies and at supercolony boundaries. Furthermore, we compared ant species diversity and composition at baits among (A) core areas of *A. gracilipes* supercolonies, (B) supercolony boundaries and (C) outside supercolonies.

A. gracilipes was behaviourally dominant over most ant species except *Oecophylla smaragdina*. Within supercolonies, *A. gracilipes* discovered all food baits first, and monopolized the vast majority throughout the course of the experiment. At supercolony boundaries, *A. gracilipes* discovered baits later than resident ant species, but subsequently monopolized half of the baits. Furthermore, the activity and diversity of the ant community within *A. gracilipes* supercolonies was lower than at its boundaries and outside supercolonies, and the ant communities differed significantly between infested and non-infested areas. Our study supports the hypothesis that successful establishment of *A. gracilipes* in anthropogenically disturbed habitats may negatively affect resident ant communities through

high levels of direct interspecific aggression and almost complete monopolization of resources within high-density supercolonies.

INTRODUCTION

Biological invasions rank among the most important drivers of environmental degradation (Mack et al. 2000b). Successful invasions by introduced ant species have been shown to cause decreases in diversity and abundance of both vertebrate and invertebrate populations (Suarez et al. 2005; Wetterer and Moore 2005; Davis et al. 2008) and may even lead to alteration of whole ecosystems, e.g. through predation on key-stone species (O'Dowd *et al.* 2003), competitive advantages over native invertebrates (McNatty *et al.* 2009), disruption of essential mutualisms such as seed dispersal (Christian 2001; Davis et al. 2010), pollination (Blancafort and Gomez 2005) or disturbance of native ant communities (Holway 1999).

Ants within a community compete with each other either directly (interference competition, predation) or indirectly (via exploitation of mutually required space or food resources). Invasive ant species often share characteristics that render them superior in both interference and exploitation competition (reviewed by Holway *et al.* 2002). Among these features are (1) pronounced interspecific aggression (Holway 1999; Human and Gordon 1999), (2) superiority at resource discovery and monopolization (Davidson 1998; Holway 1999) and (3) numerical dominance within the invaded habitat (Walters and Mackay 2005). Introduced ant species often form large polygynous and polydomous supercolonies in which large numbers of workers move freely among nests (Buczkowski et al. 2004; Espadaler et al. 2004; Le Breton et al. 2004; Abbott 2005). The continuous epigaeic activity of worker presence may allow introduced ant species to outperform other ant species locally in terms of resource discovery and monopolization (Holway 1999), as they are likely to discover resources before other ants do in areas of high activity and have the worker capacity to monopolize it. Thus, the size of an ant colony (in terms of worker quantity) and its competitive abilities in resource discovery and monopolization are often linked, posing a ‘chicken and egg’ dilemma (Oliveras et al. 2005; Oliver et al. 2008). Likewise, the worker density in the foraging territory of an ant colony and its ability to prevail in aggressive inter- and intraspecific encounters may be intertwined (e.g. Palmer 2004). Thus, it may be important to compare outcomes of direct competitive interactions among workers of different species in different ratios, and compare resource discovery and monopolization patterns between areas of high worker density and low worker density (e.g. in the centre of a colony and at its boundaries).

Most of our knowledge on invasive ants is derived from studies on two focal species, the Argentine Ant *Linepithema humile* and the Red Imported Fire Ant (RIFA) *Solenopsis invicta*. To elucidate whether the mechanisms at work in invasions by the Argentine Ant or RIFA are of the same importance in other invasive ant species, we studied behavioural and ecological characteristics of a potentially invasive population of supercolonies the Yellow Crazy Ant *Anoplolepis gracilipes* in NE-Borneo. Despite its current distribution in tropical regions of SE Asia and the Indopacific is well mapped, its origin (and thus its status as invasive/native in several regions including SE-Asia) remains unclear (Wetterer 2005). Several introduced populations have been studied on Hawaii (Kirschenbaum and Grace 2007), the Seychelles (Gerlach 2004), Tokelau (Lester and Tavite 2004; Abbott et al. 2007) or Christmas Island (O'Dowd et al. 2003; Abbott 2004; Thomas et al. 2010b), and potentially introduced populations were found in Central Sulawesi (Bos et al. 2008) and NE Borneo (Drescher et al. 2007; Pfeiffer et al. 2008; Brühl and Eltz 2009). Few studies, however, aimed to identify the mechanisms facilitating the ecological success of *A. gracilipes*. Those that did were limited to Pacific islands (e.g. Lester and Tavite 2004; Abbott et al. 2007), on which native ant communities are largely absent, or focused on the interaction of *A. gracilipes* with other invading ant species (Lester and Tavite 2004; Kirschenbaum and Grace 2008).

In this study, we describe the ecological dominance of *A. gracilipes* supercolonies within a resident ant community in anthropogenically altered habitats in north-eastern Borneo and explore the underlying mechanisms by testing interference and exploitative abilities of *A. gracilipes*. We measured: (1) the aggression of two different *A. gracilipes* laboratory colonies in bioassays against seven resident ant species (six native species and one introduced species); (2) the resource discovery and monopolization patterns of ants in the field by installing artificial baits (honey, tuna) within, outside and across *A. gracilipes* supercolony boundaries; and (3) the ant community composition along bait transects within, outside and across boundaries of *A. gracilipes* supercolonies.

METHODS

All experiments were performed between October 2006 and January 2007 in Poring Hot Springs, Sabah, Malaysia, except for bioassays using colony D1 (see below) which were performed in March 2005 in Danum Valley, Sabah. The area in and around Poring Hot Springs is comprised of recreational parkways (referred to as park, consisting mostly of lawn, shrubs and bamboo) and private orchards (e.g. durian, mango, banana, rambutan) along the edge of mildly disturbed submontane rainforest dominated by dipterocarps. Locations and labels of *Anoplolepis gracilipes* supercolonies overlap with those of a previous study focusing on population genetics (Drescher et al. 2007).

A. gracilipes was found in almost all anthropogenically altered habitats (e.g. residential area, park, orchard and secondary forest edge) except within secondary forest. However, *A. gracilipes* scouts of supercolonies located at the forest edge were observed to venture as far as ca. 40 m into the forest. Consequently, we categorized four types of habitats, in which experiments were performed: (1) secondary forest, (2) secondary forest edge, (3) plantation/orchard, (4) park. Core regions of supercolonies were characterized by a continuous above-ground activity of *A. gracilipes* workers foraging, carrying brood, males, callow workers and food items from one nest or bivouac to the other. The density of *A. gracilipes* workers decreased from the core region of supercolonies to its boundaries, where workers were occasionally present as scouts or small groups of workers carrying food items. We thus categorized three different presence/absence categories: (A) within supercolonies, (B) at supercolony boundaries and (C) outside supercolonies. *A. gracilipes* was sighted in categories (A) and (B), but not in (C).

Since our study was located in anthropogenically altered habitats, in which at least one other introduced ant species, *Tapinoma melanocephalum*, was also found, we refer to the local ant community as ‘resident’ as opposed to ‘native’.

Interspecific Aggression and numerical dominance

On two occasions, we collected subsets of two *A. gracilipes* supercolonies (colony D1, Danum Valley; colony P3, Poring Hot Springs, see Drescher et. al. 2007) and transferred the ants into plastic containers (P3: 28.2 liter, D1: 6.75 liter) with Fluon™-coated walls. The laboratory colonies were given soil and leaves (colony D1) or cardboard and newspaper (colony P3) as nesting material. Upon setup of the laboratory colonies, colony P3 contained at least 2500 workers, 500 pupae/larvae and 13 adult queens. Colony D1 contained ca. 1500 workers, 300 pupae/larvae and 5 queens. The colonies were fed water, honey water and tuna every three days.

In each region (Danum Valley, Poring Hot Springs), we selected colonies of several local ant species (*Oecophylla smaragdina* Fabricius, *Dolichoderus thoracicus* Smith, *Crematogaster coriaria* Mayr, *C. inflata* Smith, *C. rogenhoferi* Mayr, *Technomyrmex* sp. and *Paratrechina longicornis* Latreille) which occurred close to supercolony boundaries (100 – 200m) in similarly disturbed habitats and which could be sampled continuously without destroying the colony. Only, *O. smaragdina* and *D. thoracicus* were found in both regions in sufficient abundance to be used in the bioassays. Ant species within supercolonies were not selected, because of their general low abundance and various sampling difficulties (e.g. arboreal nests in *Polyrachis* spp., too small colony sizes in *Diacamma rugosum, Odontomachus* spp. or *Odontoponera* spp., frequent colony movements in *Leptogenys* spp.).

Aggression tests were performed in PVC cylinders (height = 5 cm, diameter = 10 cm, Fluon™-coated walls, referred to as ‘arena’) based on a sheet of paper which was replaced after each trial. For each species combination, varying numbers of workers from colonies D1/P3 (*A. gracilipes*) and from one of the seven local ant species were allowed to enter the arena by crossing a bridge between their laboratory nests and the arena. We tested five different ratios between workers of colony P3 and the resident ant species (1:10, 1:5, 5:5, 5:1 and 10:1) and three ratios (5:1, 5:5, 1:5) in tests of colony D1 against resident ant species, plus 10:1 in tests against *O. smaragdina*. In uneven ratios, the more numerous species was allowed into the arena first, whereas in the 5:5 ratio, each species was allowed into the arena first in half of the replicates. After 2 min, individuals of the second ant species were transferred into the arena in a similar fashion. When both ant species were in the arena, the number of dead individuals of each species was counted in 5-min-intervals over a period of 60 min. In total, we performed N=432 replicates (12 replicates per ratio and species for each colony). We calculated the average survival time for each species in every trial and determined the difference in mean survival time for each ratio and species combination.

Composition of ant communities at bait transects

We installed three types of transects of varying length (90m-120m, see Tables 10-13) in each of six supercolonies (resulting in N=18 transects): (A) within supercolonies, (B) across supercolony boundaries and (C) outside supercolonies. Transects across supercolony boundaries (B) were installed perpendicular to the supercolony boundary, with one half situated within the supercolony and the other half outside the supercolony. However, since supercolony boundaries of *A. gracilipes* are diffuse instead of sharply defined borders (Abbott 2006), boundaries were not always positioned centrally in each transect. Each transect consisted of 12 evenly distributed measuring points in a straight line, and each measuring point contained one honey bait and one tuna bait. Baits consisted of ca. 1 cm^3 of honey or tuna placed on a circular polyethylene disc (diameter = 10 cm, with a 1 cm deep central depression). During the course of the experiment, honey and tuna were replenished wherever necessary. The baits were observed for five hours (between 0900 h and 1600 h during dry weather conditions) and the incidence of ant species was recorded on an hourly basis. Wherever possible, ants were identified in the field to the species level or assigned morphospecies. In cases where species/morphospecies affiliation was unclear, individuals were photographed and identified by collecting up three specimen from the area surrounding the bait disc, so that recruitment was disturbed to the least possible extent.

We compared the activity density, species richness and diversity of ant species other than *A. gracilipes* among transects (A) within supercolonies, (B) across boundaries and (C) outside supercolonies and the four habitat types (1 – secondary forest, 2 – secondary forest edge, 3 – plantation/orchard, 4 – park). Activity density *A* of the entire ant community in a transect was defined as $A = \sum_{i=1}^{S} a_i$, with a_i representing the number of baits at which species *i* occurs. Species richness *S* was the sum of all species observed per transect (excluding *A. gracilipes*). For species diversity, we used the Simpson's reciprocal diversity index $1/D = 1/\sum_{i=1}^{S} p_i^2$, based on relative activity density (p_i) of each species (except *A. gracilipes*) at transects, with $p_i = \frac{a_i}{A}$. Furthermore, we compared the ant community composition in response to *A. gracilipes* (A – C) and habitat type (1 – 4) by performing permutation tests (adonis, R-package vegan 1.15, 10000 runs) based on relative activity densities (p_i). To visualize the differences in community composition among transects, we performed non-metric multidimensional scaling (NMDS) based on Bray-Curtis-distances of relative activity densities (p_i) among transects.

Resource discovery and monopolization

To obtain a finer temporal resolution of species succession at resources than in the above ant surveys, we observed baits for two hour periods in the field between 0900 h and 1600 h during dry weather conditions. Baits were prepared and treated as above. A pair of one honey and one tuna bait was placed 1 m apart (A) within supercolonies (three replicates), (B) at supercolony boundaries (six replicates) and (C) outside supercolonies (five replicates). For each bait type, we recorded the time until bait discovery for each ant species and counted the foragers in five minute intervals. During the observation period, honey and tuna were replenished if necessary.

RESULTS

Interspecific Aggression and numerical dominance

Generally, interspecific aggression was high and at least one individual was killed in each trial. *Anoplolepis gracilipes* survived longer (*i.e.* killed more workers than was killed) than many of the tested ant species in most ratios except *O. smaragdina* (Fig. 24 A, B), and the patterns of competitive superiority were similar between colonies P3 and D1. In all nine pair-wise species combinations, an increased proportion of *A. gracilipes* in the arena correlated positively with superior competitive performance (Spearman rank correlation, all $P < 0.01$, Fig. 24 A, B). In some cases, the superiority of *A. gracilipes* was already pronounced even when ratios favored the other ant species (i.e. when outnumbered by 5:1 or 10:1 in combinations against P3 — *Technomyrmex* sp., P3 — *C. coriaria*, D1 — *C. inflata*). In these combinations, *A. gracilipes* workers killed more than one opponent (1.0±1.1, mean±SD) while rarely being killed, resulting in a significantly longer survival.

Composition of ant communities at bait transects

In total, 46 ant species (excluding *A. gracilipes*) from 22 genera and four subfamilies were found across all bait transects (see Supporting Tables 10 - 13 at the end of this Chapter). The activity density (A) of resident ant species inside supercolonies was lower than at transects across supercolony boundaries or transects outside supercolonies (ANOVA, $F_{2, 15} = 22.5$, $P < 0.0001$, Tukey's post-hoc $P < 0.05$, Fig. 25 A), and the same trend was found for Simpson's diversity $1/D$ ($F_{2, 15} = 13.6$, $P < 0.001$, Tukey's $P < 0.01$, Fig. 25 B) and species

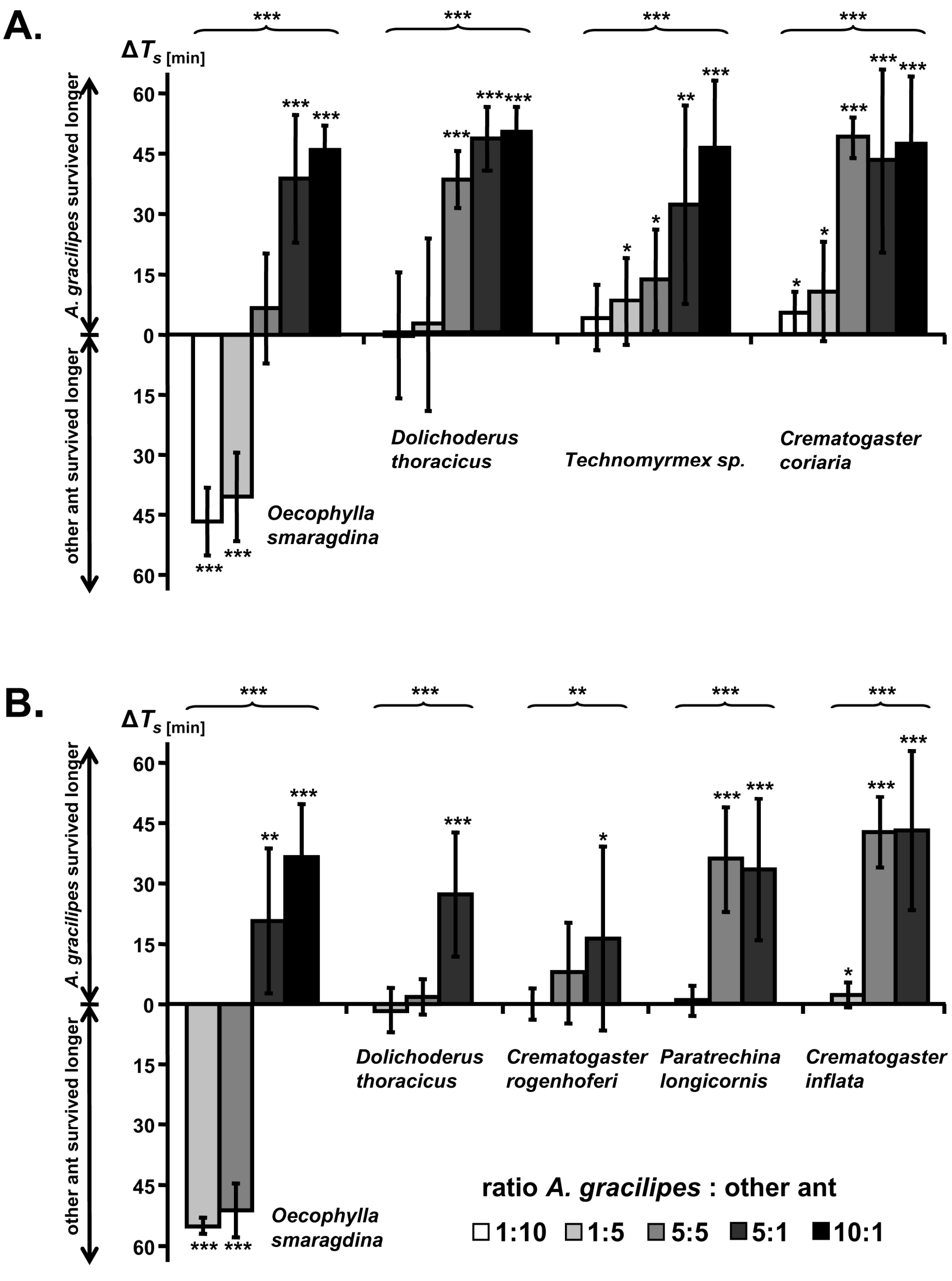


Figure 24. Difference in survival time in arena bioassays between *Anoplolepis gracilipes* and resident ant species. The difference in survival time was calculated as $\Delta T_S = T_{Anoplolepis} - T_{\text{other ant}}$ (mean ± SEM) and arena bioassays were performed with (A) *A. gracilipes* supercolony P3, Poring Hot Springs, and (B) *A. gracilipes* supercolony D1, Danum Valley. Worker ratios of *A. gracilipes* to the other ant species vary from 1:10 to 10:1. Asterisks above the brackets indicate a significant positive correlation between worker ratio and difference in survival (Spearman rank correlation). Asterisks above or below columns indicate significant deviations from zero (one-sample *t*-test): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

richness S ($F_{2,15} = 27.0$, $P < 0.0001$, Tukey's $P < 0.001$). Neither the activity density A, ant species diversity 1/*D*, nor richness *S* differed significantly among the four habitat types (1 – secondary forest, 2 – secondary forest edge, 3 – plantation/orchard, 4 – park) ($F_{3,\,14} \leq 2.7$, $P \geq 0.19$) across all sample areas.

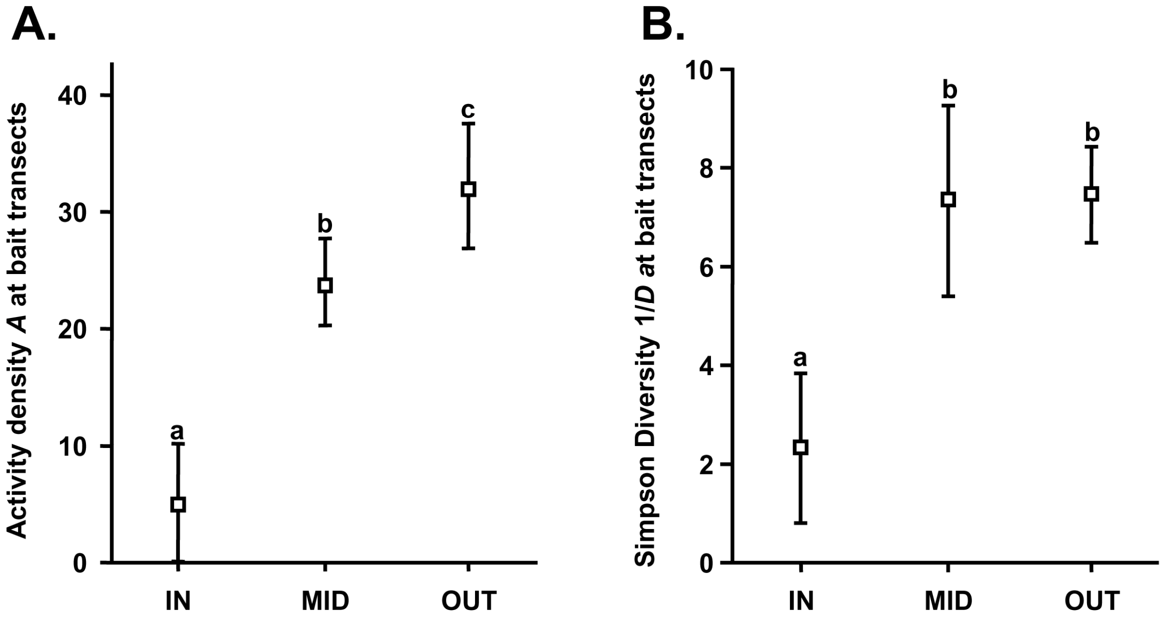


Figure 25. (A) Activity density *A* (number of sightings at baits) and (B) reciprocal Simpson diversity (1/*D*) of the resident ant community. Values are shown for transects within *A. gracilipes* supercolonies (IN), across supercolony boundaries (MID) and outside supercolonies (OUT) (mean ± SD). Letters indicate significant differences among groups (ANOVA, Tukey-post-hoc).

In transects across supercolony boundaries, we observed a gradual increase of *A. gracilipes* worker abundance at baits when moving towards the supercolony core region (Fig. 26). The increase of *A. gracilipes* forager abundance from the margins of a supercolony to its center corresponded to a decrease in the species richness of resident ants (baits outside supercolony boundaries were excluded from the analysis, Spearman rank correlation, $R = -0.79$, $P = 0.033$, Fig. 26). *Anoplolepis gracilipes* often monopolized baits and was only observed at baits together with other species in low numbers (e.g. when its scouts and those of other species discovered the bait at the same time in early phases of recruitment, or when *A. gracilipes* scouts discovered baits that were already being exploited by other ant species).

Ant community composition significantly differed among areas of *A. gracilipes* presence/absence (adonis, $F = 1.6$, $P = 0.019$), whereas the four habitat categories did not differ significantly ($F = 0.89$, $P = 0.67$). In the NMDS ordination (Fig. 27), this pattern is reflected by the fact that ant assemblages cluster in response to the presence/absence of *A. gracilipes* (categories A-C), but not in response to habitat categories.

Resource discovery and monopolization

Inside supercolonies, *A. gracilipes* was first to discover all baits, and did so within the first minute (50.6 ± 30.6 sec, mean ± SD). Both types of baits were exploited to a similar extent (average number of individuals at bait within two hours: 50.4 ± 14.9 for honey, 48.5 ± 12.2 for tuna). In only one case, *Acanthomyrmex* sp2 at a tuna bait, was any other ant species observed to feed on any of the baits (Fig. 5 A, B). Occasionally, other ant species were observed to approach the baits, but were aggressively chased away by *A. gracilipes*. At supercolony boundaries, resident ant species discovered both honey and tuna baits significantly earlier than *A. gracilipes* (resident ants: 2.4 ± 1.8 min for honey and 5.6 ± 3.1min for tuna baits; *A. gracilipes*: 26.5 ± 9.1 min for honey and 16.6 ± 3.1 min for tuna baits, *t*-test, all $P < 0.01$). Despite discovering honey baits later than resident ants, *A. gracilipes* subsequently monopolized five out of six honey baits (Fig. 5 C). Tuna baits, however, were largely monopolized by resident ants (Fig. 5 D). Outside supercolonies, the baits were fully discovered and recruited to by the resident ant species.

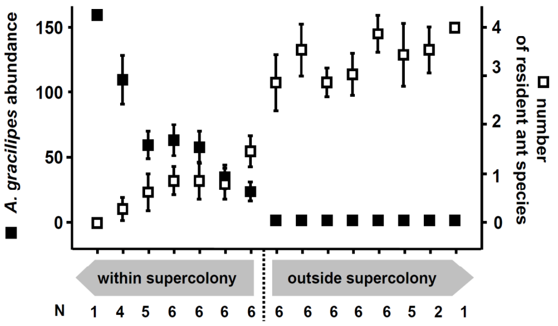


Fig. 26. Abundance of *Anoplolepis gracilipes* (black squares) and number of resident ant species (white squares) at bait transects (mean ± SEM). Transects comprised 12 measuring points (10 m apart from each other), but were readjusted (*post hoc*) to superpose the supercolony boundaries for this analysis.

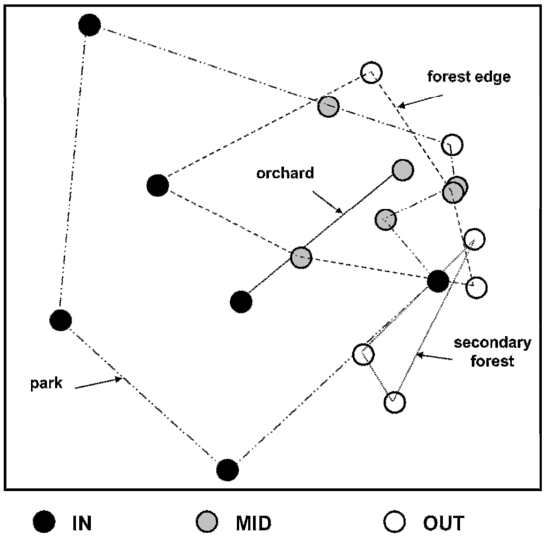


Figure 27. Multidimensional scaling (NMDS) plot of the ant assemblages at bait transects. Transects were within supercolonies (IN), across supercolony boundaries (MID) and outside *Anoplolepis gracilipes* supercolonies (OUT, and across four habitat types (park, orchard, forest edge, secondary forest) (Stress = 19.98, non-metric fit $R^2 = 0.96$).

DISCUSSION

Anoplolepis gracilipes was highly aggressive towards other ant species and readily engaged in lethal fights. Its workers survived much longer than workers of most other species – when a single *A. gracilipes* worker was confronted with up to ten competitors, it sometimes survived until the end of the trial and killed some competitors. Furthermore, *A. gracilipes'* competitive performance increased as its ratio in aggression tests increased. *Oecophylla smaragdina* was the only ant species that matched the competitive superiority of *A. gracilipes*, which may in part be due to its larger size compared to the other tested ant species including *A. gracilipes*, but may also reflect its increased aggressive behaviour as a dominant ant species (Basu 1997). This, however, would imply that the other tested ant species are less dominant, which is not the case, e.g. in *Dolichoderus thoracicus*.

Under natural circumstances, ants adjust their behaviour towards potential opponents depending on the context such as next proximity or group size (e.g. Buczkowski and

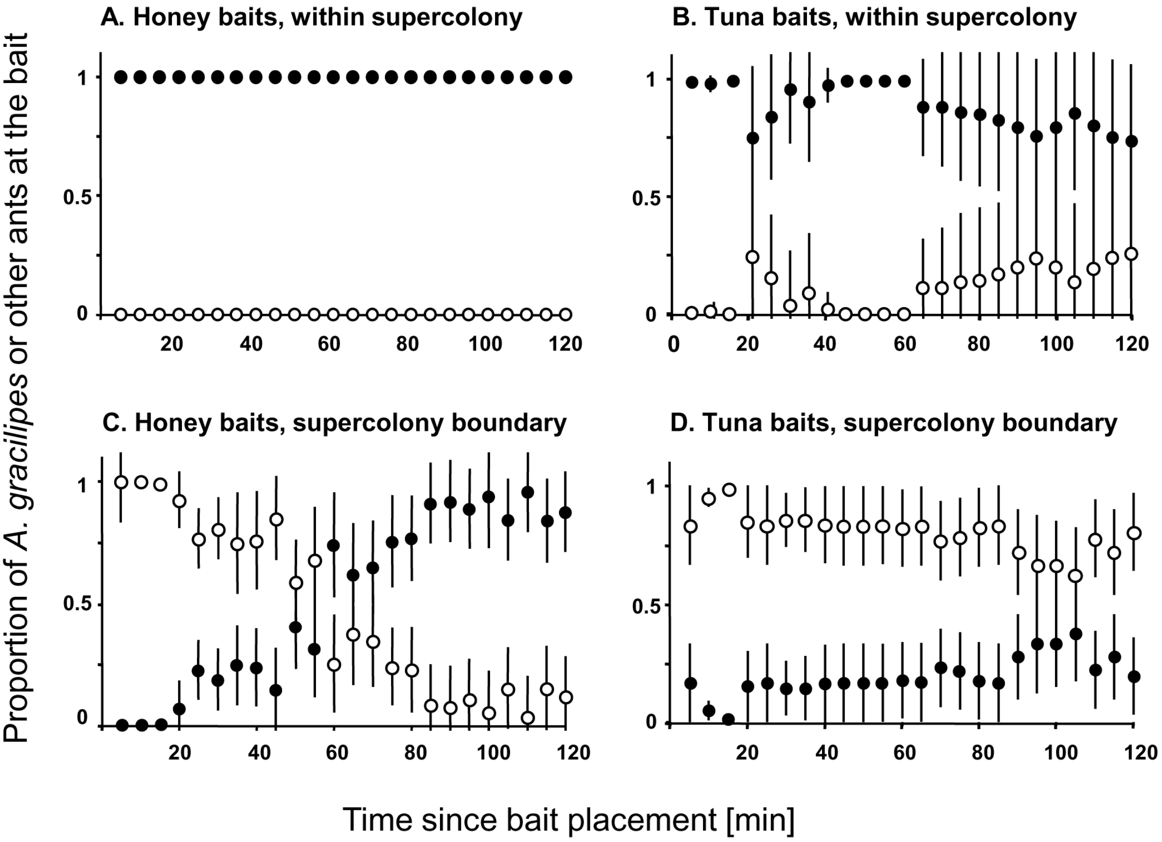


Figure 28. Relative abundances of Anoplolepis gracilipes and resident ants at baits. *Anoplolepis gracilipes* (black circles) and members of the resident ant community (white circles, pooled data) were observed on two types of baits in two types of colonization states within 120 min (mean ± SD). In Fig 5 B, *Acanthomyrmex* sp 2 is the only ant species other than *A. gracilipes*.

Silverman 2005; Sagata and Lester 2009), and may often avoid a confrontation (Human and Gordon 1999). In arena experiments, individuals are largely deprived of the social context as well as the chance to escape confrontation, and may thus display behaviour which differs from natural conditions. However, arena experiments demonstrate the competitive potential of each participant in the field, which is what was aimed for in this particular setup. The results characterize *A. gracilipes* as a highly aggressive ant which may dominate other ant species in both superior and inferior numbers, which may point towards highly efficient fighting capabilities. In fact, within their colony territories, *A. gracilipes* workers were repeatedly observed to attack and pursue foragers of other species such as *Odontoponera* sp. or *Diacamma* sp., and dead individuals of those two and other species were frequently found within nests of *A. gracilipes* (JD, pers. obs.). None of the seven ant species used for bioassays were observed to nest within supercolony territories. This pattern may be the result of the unilateral competitive superiority of *A. gracilipes* towards many ant species, while it may reflect reciprocal competitive exclusion in the case of the likewise dominant ant species *O. smaragdina* (e.g. Basu 1997; Bluthgen and Fiedler 2004).

Within supercolonies, *A. gracilipes* outperformed most other ant species in terms of resource discovery and monopolization. This ability to dominate resource acquisition within territorial boundaries is known from other invasive ant species such as *Linepithema humile* (Human and Gordon 1999) and *Solenopsis invicta* (Calcaterra *et al.* 2008) and may be the cause or consequence of local displacement of competing ant species in invaded habitats. At supercolony boundaries, the abundance of *A. gracilipes* workers was lower than inside core areas. Here, *A. gracilipes* discovered resources later than other ant species, but displaced many of them after discovery. This, in turn, may give other ant species time to exploit a resource before recruitment of *A. gracilipes* starts (dominance-discovery trade-off, see Fellers 1987; Davidson 1998; Holway 1999) and might facilitate species coexistence with *A. gracilipes* in areas of low abundance. This *status quo*, however, might only be temporary, as boundaries of *A. gracilipes* supercolonies are highly dynamic and may spread as much as 0.5 m per day (Abbott 2006). Furthermore, our study showed that the activity, diversity and composition of ant communities at bait transects were affected by the presence/absence of *A. gracilipes* and not by differences between the four disturbed habitat types, suggesting that *A. gracilipes* may also dominate natural food resources within the study area. As competitive exclusion from food resources by dominant ants may control ant species richness at the assemblage level (Parr 2008), the numerical dominance of *A. gracilipes* at baits reliably reflects its ecological dominance within the resident ant community. *Acanthomyrmex* sp2 was one of the few ant morphospecies that were observed at baits within supercolonies, and the only one that successfully monopolized a bait within *A. gracilipes* territory. As this species could not be assigned to any presently known *Acanthomyrmex* species from Borneo, it is unclear whether *Acanthomyrmex* sp2 represents a species that has the potential to may commonly prevail in the presence of *A. gracilipes* or whether that observation was an exception to the rule.

We conclude that within disturbed habitats, *A. gracilipes* has the potential to displace many species of local ant communities. Its spread is facilitated by a combination of direct interspecific aggression, resource monopolization and numerical dominance. *A. gracilipes* has strong individual competitive abilities, which may aid in prevailing in new habitats as a propagule. This study once again emphasizes the capability of invasive ants to influence entire ant communities by dominating both interspecific encounters and exploitation of food resources.

ACKNOWLEDGMENTS

We thank the Economic Planning Unit (EPU) for permission to conduct research in Malaysia, Prof. Dr. Datin Maryati Mohamed and Dr. Arthur Chung for collaboration and support. Albinus Ongkudon (DVMC) and Dr. Maklarin bin Laklim (Sabah Parks) facilitated our work at Danum Valley and Poring Hot Springs. Janina Eulenburg is thanked for help with fieldwork and data collection. This work was part of the Royal Society's Southeast Asia Rainforest Research Programme (SEARRP) and was funded by the Deutsche Forschungsgemeinschaft DFG (SFB 554 'Evolution and mechanisms of arthropod behaviour', project E2). All experiments conducted comply with the current laws of Malaysia.

SUPPORTING TABLES

Table 10. Species composition of six transects inside *Anoplolepis gracilipes* supercolonies (IN) within five hours. Numbers indicate at how many of 12 measuring points a species was found. Each measuring point consisted of one honey and one tuna bait.

Subfamily	Genus / species	IN 1	IN 2	IN 3	IN 4	IN 5	IN 6
Formicinae	*Anoplolepis gracilipes*	12	12	12	9	12	12
Formicinae	*Euprenolepis* sp1				1		
	Paratrechina sp1					1	
	Paratrechina sp3				1		
	Paratrechina sp5			2			
	Paratrechina sp6				4		
	Paratrechina sp7				1	4	
Myrmicinae	*Acanthomyrmex* sp4				4		
	Cardiocondyla sp1			2			
	Crematogaster sp3						2
	Lophomyrmex sp1						1
	Lordomyrma sp1					1	
	Pheidologeton sp2		1			1	
Ponerinae	*Odontomachus* sp1	1			1	1	
	Odontoponera sp2				2		
Transect length		90	120	100	90	120	90
Habitat type		park	park	park	park	orchard	forest edge

Table 11. Species composition of six transects across *Anoplolepis gracilipes* supercolony boundaries (EDGE) within five hours. Numbers indicate at how many of 12 measuring points a species was found. Each measuring point consisted of one honey and one tuna bait.

Subfamily	**Genus / species**	**EDGE 1**	**EDGE 2**	**EDGE 3**	**EDGE 4**	**EDGE 5**	**EDGE 6**
Formicinae	*Anoplolepis gracilipes*	5	4	6	6	5	7
Dolichoderinae	*Dolichoderus* sp1					1	
Formicinae	*Camponotus* sp1						1
	Camponotus sp2				1		1
	Camponotus sp3			1			
	Euprenolepis sp1	1					
	Paratrechina sp1	1		2		2	5
	Paratrechina sp2			3	4		
	Paratrechina sp3	1			2		
	Paratrechina sp4			2	2		
	Polyrhachis sp2	1					
Myrmicinae	*Acanthomyrmex* sp2		3			3	
	Acanthomyrmex sp3	1					
	Acanthomyrmex sp4	3	8		4	10	
	Cardiocondyla sp1		1		2		
	Crematogaster sp2	4					
	Crematogaster sp3	2					5
	Lordomyrma sp1						1
	Monomorium sp1	3				1	3
	Pheidologeton sp1		1			1	2
	Pheidologeton sp2	1		2			
	Tetramorium sp1			2			1
	Tetramorium sp2				1		
Ponerinae	*Diacamma rugosum*	5	3	3	4	3	3
	Leptogenys sp1						1
	Odontomachus sp1	2			1		3
	Odontomachus sp2		4	1		4	
	Odontoponera sp2	2	2	1	1	3	
	Odontoponera sp3			1	1		
Transect length		120	120	120	120	120	120
Habitat type		park	park	park	orchard	forest edge	forest edge

Table 12. Species composition of six transects outside *Anoplolepis gracilipes* supercolony boundaries (OUT) within five hours. Numbers indicate at how many of 12 measuring points a species was found. Each measuring point consisted of one honey and one tuna bait.

Subfamily	**Genus / species**	**OUT 1**	**OUT 2**	**OUT 3**	**OUT 4**	**OUT 5**	**OUT 6**
Dolichoderinae	*Dolichoderus* sp1					1	
	Tapinoma melanocephalum			4			
Formicinae	*Camponotus gigas*				3		1
	Camponotus sp2				4		
	Camponotus sp3				1		
	Camponotus sp4						1
	Camponotus sp5					2	
	Camponotus sp6					1	
	Oecophylla smaragdina		1				
	Paratrechina sp1		3		1		1
	Paratrechina sp2		3	2			
	Paratrechina sp3					1	
	Paratrechina sp6				10		8
	Paratrechina sp7		2	6			
	Polyrhachis sp1					2	3
	Polyrhachis sp2						1
Myrmicinae	*Acanthomyrmex* sp1				2		4
	Acanthomyrmex sp2	3				1	
	Acanthomyrmex sp3	3					
	Acanthomyrmex sp4	7		3		5	
	Aphaenogaster sp1					1	
	Cardiocondyla sp1						1
	Crematogaster sp1			1	1		
	Crematogaster sp2	5				6	3
	Crematogaster sp3				3		
	Lophomyrmex sp1			1			
	Lordomyrma sp1					1	6
	Monomorium sp1	3					
	Myrmicaria sp1		5				
	Myrmicaria sp2	2					
	Pheidole sp1		1	1	2		2
	Pheidologeton sp1		1		1		
	Pheidologeton sp2	1					
	Tetramorium sp1	1					
Ponerinae	*Diacamma rugosum*	3	4	1	3	2	
	Odontomachus sp1			2	6	1	1
	Odontomachus sp2						6
	Odontoponera sp1				2		
	Odontoponera sp2	3		9		4	
	Odontoponera sp3	1	6				
Transect length		90	120	120	100	100	100
Habitat type		park	forest edge	forest edge	secondary forest	secondary forest	secondary forest

IX. AGILITY DETERMINES THE SURVIVAL OF THE CLEPTOPARASITIC CRICKET *MYRMECOPHILUS PALLIDITHORAX* IN SUPERCOLONIES OF *ANOPLOLEPIS GRACILIPES*

ABSTRACT

Myrmecophily is a taxonomically widespread phenomenon which includes mutualistic, commensalistic and parasitic associations between ants and other organisms. Organisms exploiting the resources offered by ant colonies, such as food and shelter, have evolved a variety of morphological, behavioural and chemical mechanisms to circumvent the colonies' defences. In this study, we examined the mechanisms employed by the myrmecophilous cricket *Myrmecophilus pallidithorax* (Orthoptera, Myrmecophilidae) that enable it to survive in supercolonies of the invasive Yellow Crazy Ant *Anoplolepis gracilipes*. By reducing *M. pallidithorax'* agility (through induced autotomy of the cricket's hind legs), we were able to show that the ability to avoid potentially lethal encounters with its hosts by evasive jumping significantly increases the survival of *M. pallidithorax* in standardized *A. gracilipes* subcolonies. Moreover, we observed significantly higher survival of handicapped *M. pallidithorax* individuals in subcolonies that consisted of crickets and ants from the same *A. gracilipes* supercolony than in subcolonies that consisted of ants and crickets from mutually aggressive *A. gracilipes* supercolonies. The apparently colony-specific discrimination suggests that the cuticular hydrocarbon (CHC) profile of *M. pallidithorax* may differ between populations from different host supercolonies. Preliminary data show a high similarity between CHC profiles of *M. pallidithorax* and *A. gracilipes* from the same supercolony. We discuss our findings with respect to the potential mechanisms facilitating the successful establishment of *M. pallidithorax* in supercolonies of its host.

INTRODUCTION

Social insects invest much of their time constructing shelters and gathering food, providing them with protection for their queen(s) and brood and a stock of resources as an insurance against variable conditions (Hölldobler and Wilson 1990; Blüthgen and Feldhaar 2010). While the almost perpetual availability of food and shelter in social insect societies provides the basis for many mutualistic associations that they engage in, it also represents valuable resources that are exploited by a vast array of commensals and parasites (Hölldobler and Wilson 1990). In ants, an estimated 80,000 – 100,000 morphologically distinct species of myrmecophilous insects exist worldwide, 5000 – 20000 of which are thought to be obligate social parasites (Elmes 1996). Once inside ant colonies, they prey upon colony members (including eggs and larvae), parasitize food resources, scrounge food via trophallaxis or enjoy social benefits such as protection from their own enemies (Hölldobler and Wilson 1990; Dettner and Liepert 1994). Ant colonies are well defended and workers are usually hostile towards intruders. Thus, in order to enter and live inside an ant colony, social parasites evolved strategies to overcome the colony defences, e.g. mimicking the ants' communication. Members of ant communities recognize each other by a mix of tactile (antennation) and chemical cues (mainly cuticular hydrocarbons, CHC's)(Vander Meer and Morel 1998; Lahav et al. 1999; Akino et al. 2004; Torres et al. 2007). Accordingly, many social parasites of ants show morphological, behavioural and/or chemical adaptations that allow them to enter and remain within their hosts colonies largely undetected, such as chemical mimicry or camouflage (Dettner and Liepert 1994; Lenoir et al. 2001; Akino 2008).

In this study, we explore the adaptations that allow the myrmecophilous cricket *Myrmecophilus pallidithorax* to enter and live inside nests of the invasive Yellow Crazy Ant *Anoplolepis gracilipes*. *M. pallidithorax* (Chopard 1930)[= *M. mayaealberti*, Hugel & Matyot (2006) syn. nov. and *M. leei*, Kistner & Chong (2007), syn. nov., according to Ingrisch (2010)] belongs to the ca. 40-60 species of myrmecophilous crickets described to date (Kistner 1982; Kistner et al. 2007), which belong almost exclusively to the genus *Myrmecophilus* (syn. *Myrmecophila* Latreille, 1927; exception: *Camponophilus*). Like all *Myrmecophilus* crickets, *M. pallidithorax* is characterized by its small size (<5mm), aptery and plump body form. Despite the fact that myrmecophilous crickets were studied as early as 1799 (Panzer 1799), little is known about their feeding behaviour in ant nests as well as the mechanisms by which aggression from the host is averted. Some *Myrmecophilus* crickets may intercept or even elicit trophallaxis of their host ants (Henderson and Akre 1986; Komatsu et al. 2009), thereby acquiring regurgitated food such as honeydew or prey haemolymph. Aggression from the respective host ant species seems to be avoided by escaping hostile workers and by chemical camouflage, in which *Myrmecophilus* crickets acquire the host's species-specific CHC-profile by direct contact with the ants (Akino et al. 1996).

A previous study showed that *A. gracilipes* supercolonies may be highly differentiated chemically up to the point that CHC profiles of workers from different supercolonies possess a high proportion of qualitatively different hydrocarbons (Drescher et al. 2010). Provided that *M. pallidithorax* uses chemical camouflage, the CHC profiles of *M. pallidithorax* specimen from different *A. gracilipes* supercolonies may thus also differ to a great extent, possibly resulting in higher aggression of ants towards crickets from different supercolonies than towards crickets from their own supercolony.

This study focused on the relative importance of two potential adaptations that appear to facilitate *M. pallidithorax'* survival in supercolonies of its host, i.e. escape from precarious situations by jumping and deception of the host by chemical camouflage. In particular, we introduced handicapped crickets (unable to jump) into *A. gracilipes* subcolonies, expecting them to suffer from a higher mortality than intact crickets that retained the ability to jump. Furthermore, we introduced both handicapped and intact crickets into *A. gracilipes* subcolonies consisting of ants sampled from the crickets' 'home' supercolony as well as 'foreign' supercolonies, expecting them to experience higher aggression (and thus mortality) in 'foreign colony' as opposed to 'home colony' assays.

METHODS

Selection and maintenance of colonies

Anoplolepis gracilipes and *Myrmecophilus pallidithorax* were sampled from four different supercolonies in NE-Borneo, two from Poring Hot Springs (6°04' N, 116°70' E; supercolonies P3 and P6, see Drescher et al. 2010) and two from Sepilok Forest Reserve (5°51' N, 117°57' E, supercolonies S6 and S7). Pieces of bamboo and drainage pipes were placed next to nest entrances within supercolonies and were readily accepted by *A. gracilipes* as alternative nesting sites. Within several days, *A. gracilipes* supercolony subsets containing queens, males and brood as well as *M. pallidithorax* and other ant guests (Stys et al. 2010) had moved in and were subsequently transferred into plastic buckets (Vol. = 25 l) treated with Fluon™ to prevent escape. All colonies were offered newspaper and cardboard as nesting material and were fed water, honey and tuna or crayfish scraps *ad libitum*.

Manipulation of crickets and experimental setups

Like many crickets (Fleming et al. 2007), *M. pallidithorax* has a pair of hind legs which can be shed off along a breakage plane at the femur-trochanter junction, a defensive mechanism termed autotomy. 'Handicapped' crickets were generated by inducing autotomy, i.e. by pinching the femurae of the hind legs with sharp forceps which caused the shedding of the leg. We could not detect haemolymph leaking out of the wounds upon autotomy of the hind legs. Nevertheless, 'handicapped' crickets were kept isolated for 24-48 h prior to the experiments, thus minimizing the risk of open wounds evoking aggression by *A. gracilipes* or introducing lethally wounded crickets into the experimental colonies, both of which would have biased the experimental setup. To reduce potential differences in CHC profiles between 'handicapped' and 'intact' crickets, 'intact' crickets were also kept isolated for 24-48h prior to the experiments.

Groups of ten crickets, both 'intact' and 'handicapped', were then introduced into *A. gracilipes* laboratory subcolonies consisting of ants from the crickets' 'home' supercolony or 'foreign' supercolony, or were kept without ants at all (see Table 1 for sample sizes). Due to limited cricket availability, experiments were performed in each region separately (Poring and Sepilok, respectively), i.e. no crickets from *A. gracilipes* supercolonies in Poring were introduced into laboratory subcolonies consisting of ants from Sepilok and vice versa. For each replicate, experimental *A. gracilipes* subcolonies consisted of 500 workers, three to five males, one queen, 50 pupae/large larvae and some egg packages. Subcolonies were kept in plastic containers (Vol. = 1100 ml) with Fluon™-coated walls. Cardboard and newspaper was added as nesting material and subcolonies were fed water, honey and tuna/crayfish *ad libitum*.

Prior to the introduction of crickets, ants were given one hour to familiarize themselves with the new environment. Every day for four consecutive days, subcolonies were screened for dead *M. pallidithorax* individuals. For data analysis, we pooled the number of dead crickets in 'home colony' assays and 'foreign colony' assays, respectively. Overall, we obtained data from 1370 crickets (137 trials containing ten crickets each, Tab. 14).

Table 14. Setup of experimental subcolonies containing *Anoplolepis gracilipes* and M*yrmecophilus. Pallidithorax* specimen.

	***M. pallidithorax* 'home colony'**	***A. gracilipes* supercolony**	**Ants from…**	**Cricket treatment**	**$N_{replicates}$**
Poring Hot Springs	P3	P3	home colony	intact	5
	P6	P6	home colony	intact	5
	P3	P6	foreign colony	intact	5
	P6	P3	foreign colony	intact	5
	P3	P3	home colony	handicapped	10
	P6	P6	home colony	handicapped	10
	P3	P6	foreign colony	handicapped	10
	P6	P3	foreign colony	handicapped	10
Sepilok Forest	S6	S6	home colony	intact	5
	S7	S7	home colony	intact	5
	S6	S7	foreign colony	intact	5
	S7	S6	foreign colony	intact	5
	S6	S6	home colony	handicapped	10
	S7	S7	home colony	handicapped	10
	S6	S7	foreign colony	handicapped	10
	S7	S6	foreign colony	handicapped	10
	various	-	crickets alone	handicapped	17

Cuticular Hydrocarbon (CHC) Profiles

To obtain preliminary data on the similarity of M. *pallidithorax* CHC profiles and the CHC profiles of their respective *A. gracilipes* host supercolonies, CHC's from 10 adult *M. pallidithorax* and 20 *A. gracilipes* workers from supercolony P3 were extracted with Hexane and analyzed according to the protocol in Drescher at al. (2010). Due to the lack of replicates from other supercolonies, the two resulting CHC profiles are simply presented and discussed as preliminary evidence for the potential capability of *M. pallidithorax* to adjust their CHC profile to the CHC profile of their host (Akino et al. 1996).

RESULTS

In all experimental setups, *Myrmecophilus pallidithorax* individuals were killed by the ants throughout the experiment, with the highest mortality occurring within the first 24 h after introduction of the crickets into *A. gracilipes* colonies (Fig. 29). After four days, handicapped crickets had experienced significantly higher mortality ($N_{dead} = 9.0 \pm 1.2$) than intact crickets

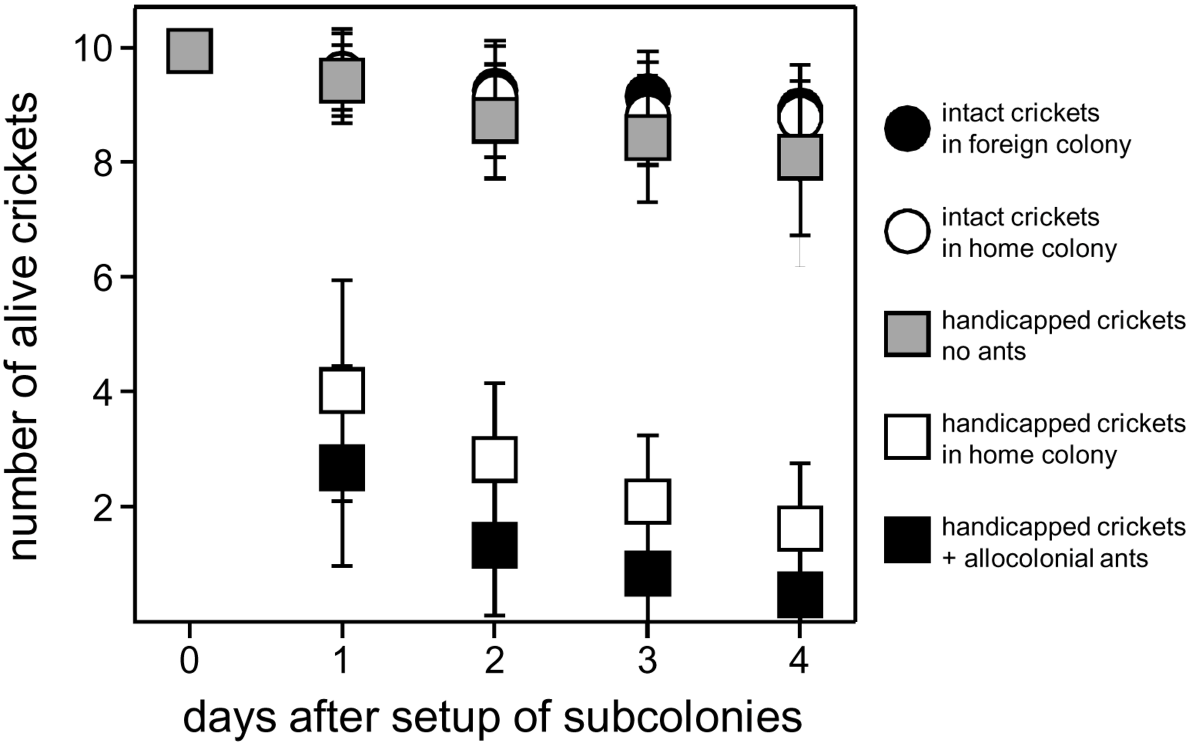


Fig. 1 Demise of *Myrmecophilus pallidithorax* individuals in Anoplolepis gracilipes subcolonies within four days (mean ± SD). Crickets were either untreated (‘intact’, circles) or had their hind legs removed by induced autotomy (‘handicapped’, squares). They were either kept without ants (grey squares), introduced into *A. gracilipes* subcolonies containing ants from the supercolony that the crickets themselves were sampled from (‘home colony’, white circles/squares) or into *A. gracilipes* subcolonies containing ants from different supercolonies (‘foreign colony’, black circles/squares).

in ant subcolonies ($N_{dead} = 1.2 \pm 0.9$, $p < 0.001$, ANOVA, Tukey-Kramer HSD post-hoc test for unequal sample sizes, Fig. 30 A) or handicapped crickets kept without ants ($N_{dead} = 1.9 \pm 0.8$, $p < 0.001$). Moreover, handicapped crickets ‘foreign colony’ assays suffered from higher mortality ($N_{dead} = 9.6 \pm 0.8$) than handicapped crickets in ‘home colony’ assays ($N_{dead} = 8.4 \pm 1.2$, $p < 0.001$, ANOVA, Tukey-Kramer HSD post-hoc for unequal N, Fig. 30 B). There was no significant difference between mortality of intact crickets in ‘home colony’ assays, intact crickets in ‘foreign colony’ assays and handicapped crickets kept without ants ($p > 0.3$ for all three pairwise comparisons, Fig. 30 B). The CHC profiles of *M. pallidithorax* and *A. gracilipes* sampled from the same supercolony showed considerable congruency (Fig. 31, preliminary data). At least 11 of the peaks were found in profiles of both species.

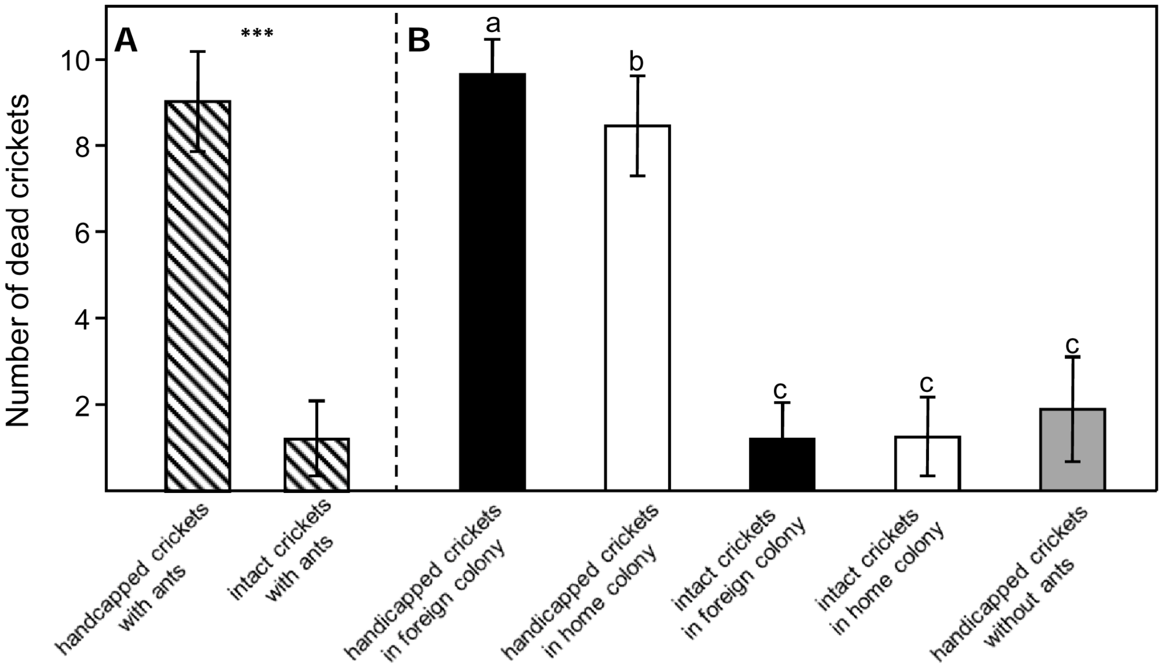


Fig. 30 Mortality of *M. pallidithorax* in subcolonies of *A. gracilipes* at day four (mean ± SD). A. Mortality of intact crickets vs. handicapped crickets in *A. gracilipes* subcolonies (pooled data for intracolonial and allocolonial setups; ***: p<0.001). B. Mortality of intact and handicapped crickets in different subcolonies. Color coding is equivalent to the description in Fig. 1. Letters indicate significant differences (ANOVA, Tukey-Kramer HSD post-hoc test for unequal sample size).

DISCUSSION

Our data suggest that *Myrmecophilus pallidithorax* may possess two strategies that facilitate its survival in supercolonies of *Anoplolepis gracilipes*: (1) avoidance of potentially precarious interactions with its host by evasive jumping /escape, and (2) a CHC profile that shows considerable congruency with the CHC profile of its host.

Autotomy is a defensive behavioural strategy employed by a wide variety of animals (Maginnis 2006; Fleming et al. 2007) and it is commonly viewed as a mechanism to escape predation, interspecific conflicts, infection or other injuries (Bateman and Fleming 2005). The loss of a limb, however, carries costs such as increased susceptibility to predation, reduced escape speed or lowered mating abilities (Bateman and Fleming 2005, 2006). It is commonly accepted that in order for autotomy to have evolved, net benefits of this strategy must outweigh net costs. Our assumption that *M. pallidithorax* uses autotomy as a strategy to escape predation is based on the ease by which the hind legs were shed upon seizure with forceps, the predominant shedding of the leg at the femur-trochanter junction (suggesting a predefined breakage plane) and the observation that roughly 10% of *M. pallidithorax* specimen caught under natural conditions were missing one or more limbs, including the hind legs. Autotomy of the hind legs may be an especially useful strategy when associated with ants, as ants tend to grasp competitors (other ants) and prey insects predominantly by the legs. Our data suggest that the injuries caused by autotomy alone do not account for the high mortality of handicapped crickets in *A. gracilipes* subcolonies (reaching 84-96%) as handicapped crickets kept without ants suffered from much lower mortality (~ 19%). Dead crickets that were removed from the subcolonies throughout the course of the experiment were often shrivelled or even torn apart, further suggesting that they did not die due to direct effects of the treatment but that they were preyed upon by *A. gracilipes*. In contrast, intact *M. pallidithorax* specimen kept together with *A. gracilipes* suffered from much lower mortality (12-13%), which signifies the importance of the hind legs for the avoidance of critical encounters with the host species *A. gracilipes*.

Ant social parasites, including myrmecophiles, have repeatedly been shown to employ chemical tactics that target ant nestmate recognition and alarm communication (Hölldobler and Wilson 1990). These tactics include chemical mimesis (including chemical phytomimesis, avoiding detection by ants by being ‘invisible’ via background-matching), chemical mimicry and camouflage (avoiding attacks by ants by pretending to be nestmates) and chemical propaganda (interfering with host nestmate recognition by creating alarm responses)(Akino 2008). Myrmecophilous crickets from the genus *Myrmecophilus* spp. are thought to employ chemical camouflage (Akino et al. 1996; Akino 2008; Komatsu et al. 2009), which differs from chemical mimicry in that CHC profiles are acquired by contact with the host *during* infestation as opposed to the biosynthesis of host species-specific CHC profiles *before* infestation (Dettner and Liepert 1994; Akino 2008). This difference has important implications as chemical mimicry would suggest a coevolutionary history of parasite and host, which in the case of *M. pallidithorax* is an invasive ant species of unknown origin (Wetterer 2005). Our data imply that *M. pallidithorax’* CHC-profiles may differ between supercolonies, as significantly less crickets died in setups where ants and crickets were from the same supercolony than when they were from different supercolonies.

Assuming that *M. pallidithorax* utilized chemical mimicry to reduce detection by *A. gracilipes*, the severe chemical differentiation between different *A. gracilipes* supercolonies (Drescher et al. 2010) would require that *M. pallidithorax* populations inhabiting different *A.*

gracilipes supercolonies would have to biosynthesize different cuticular compounds. This, in turn, would suggest that coevolution between *A. gracilipes* and *M. pallidithorax* occurred on the supercolony level rather than on the species level, i.e. different *M. pallidithorax* populations from different supercolonies might have to differentiate genetically and chemically in parallel to the supercolonies they inhabited. Chemical camouflage, on the other hand, may represent a more parsimonious explanation for the potentially different CHC profiles of *M. pallidithorax* specimen from different supercolonies, as CHC profiles could be passively acquired rather than actively biosynthesized, thus not requiring chemical coevolution between *M. pallidithorax* and *A. gracilipes*. Adjustment of the own CHC profile to the profile of the host could be achieved either by direct contact with the host ants or by contact with nesting material or the refuse pile. Given the considerable aggression by *A.*

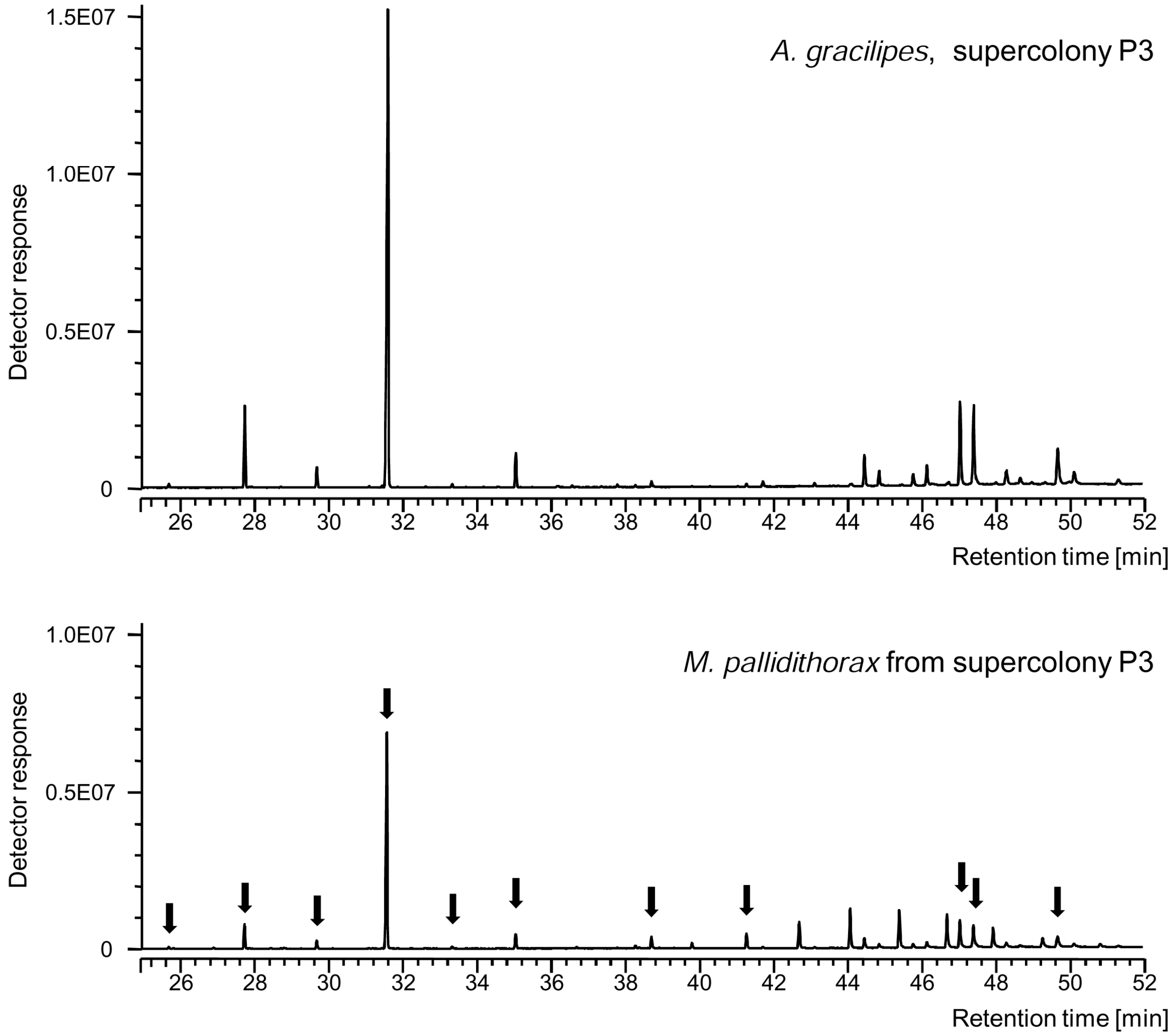


Fig. 31 CHC profiles of *A. gracilipes* workers and *M. pallidithorax* in from the same supercolony. Arrows indicate the peaks/ cuticular compounds that were found in CHC profiles of both species

gracilipes towards *M. pallidithorax*, acquisition of the supercolony odour by direct contact with the host may seem unlikely. In turn, *M. pallidithorax* was repeatedly observed in piles of shed pupal skins, which were often found in areas of lower *A. gracilipes* densities at the fringe of individual nests. Thus, *M. pallidithorax* may likely utilize chemical mimicry to deceive its host and may possibly acquire the supercolony-specific CHC profile from shed pupal skins.

Further evidence regarding the potential utilization of chemical mimicry by *M. pallidithorax* comes from its taxonomical history. According to Ingrisch (2010), *M. pallidithorax* (Chopard, 1930) is the original synonym of two subsequently described species (*M. mayaealberti*, Hugel & Matyot, 2006 and *M. leei*, Kistner & Chong, 2007), one of which has been reported from nests of the likewise invasive ant *Paratrechina longicornis* (Hugel and Matyot 2006). Thus, *M. pallidithorax* may potentially be able to immigrate into colonies of more than just one host species, rendering chemical mimicry (which requires coevolution with a host species) an unlikely strategy. However, further research is needed in order to clearly identify *M. pallidithorax*' chemical strategy. In particular, analyzing the CHC profiles of *M. pallidithorax* from both *A. gracilipes* and *P. longicornis* supercolonies as well as following the CHC profile development of *M. pallidithorax* specimen that were freshly introduced into colonies of either species will surely provide further evidence concerning the question whether the crickets use chemical mimicry or chemical camouflage to reduce aggression by its host.

Conclusion

Our data suggest that avoidance of direct interactions with its host seems to be the main strategy facilitating survival of *Myrmecophilus pallidithorax* in *Anoplolepis gracilipes* supercolonies. Our data further suggest that CHC profiles of *M. pallidithorax* may differ between *A. gracilipes* supercolonies and that aggression may be reduced by a CHC profile similar to that of the host ant. The crickets' CHC profile, however, was insufficient to completely avoid hostility from *A. gracilipes*, as nearly 90% of crickets were killed by ants from their host colony once movement was impaired by autotomy of the hind legs.

ACKNOWLEDGEMENTS

We thank the Economic Planning Unit (EPU) for permission to conduct research in Malaysia, Prof. Dr. Datin Maryati Mohamed and Dr. Arthur Chung for collaboration and support.

Dr. Maklarin bin Lakim (Sabah Parks) and Justinah Parantis facilitated our work at Poring Hot Springs, Syvlia Alsistu and Eugene Tan in Sepilok. Prof. Dr. K.-Eduard Linsenmair, Dr. Brigitte Fiala and Deborah Schweinfest are thanked for important observations and helpful comments on the experimental design. This work was part of the Royal Society's Southeast Asia Rainforest Research Programme (SEARRP) and was funded by the Deutsche Forschungsgemeinschaft DFG (SFB 554 'Evolution and mechanisms of arthropod behaviour', project E2).

X. REFERENCES

Abbott, K. L. 2004. Alien ant invasion on Christmas Island, Indian Ocean: the role of ant-scale associations in the dynamics of supercolonies of the yellow crazy ant, *Anoplolepis gracilipes*. PhD Thesis, Biological Sciences, Monash University, Melbourne.227pp.

Abbott, K. L. 2005. Supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on an oceanic island: Forager activity patterns, density and biomass. *Insectes Sociaux* 52: 266–273.

Abbott, K. L. 2006. Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. *Diversity and Distributions* 12: 101-110.

Abbott, K. L., S. N. J. Greaves, P. A. Ritchie, P. J. Lester. 2007. Behaviourally and genetically distinct populations of an invasive ant provide insight into invasion history and impacts on a tropical ant community. *Biological Invasions* 9: 453-463.

Adams, E. S. 1991. Nest-mate recognition based on heritable odors in the termite *Microcerotermes arboreus*. *Proceedings of the National Academy of Sciences of the United States of America* 88: 2031-2034.

Adams, J. M., W. Fang, R. M. Callaway, D. Cipollini, E. Newell. 2009. A cross-continental test of the Enemy Release Hypothesis: leaf herbivory on *Acer platanoides* (L.) is three times lower in North America than in its native Europe. *Biological Invasions* 11: 1005-1016.

Akino, T. 2008. Chemical strategies to deal with ants: a review of mimicry, camouflage, propaganda, and phytomimesis by ants (Hymenoptera: Formicidae) and other arthropods. *Myrmecological News* 11: 173-181.

Akino, T., R. Mochizuki, M. Morimoto, R. Yamaoka. 1996. Chemical camouflage of myrmecophilous cricket *Myrmecophilus sp.* to be integrated with several ant species. *Japanese Journal of Applied Entomology and Zoology* 40: 39-46.

Akino, T., K. Yamamura, S. Wakamura, R. Yamaoka. 2004. Direct behavioral evidence for hydrocarbons as nestmate recognition cues in *Formica japonica* (Hymenoptera: Formicidae). *Applied Entomology and Zoology* 39: 381-387.

Aloo, P. A. 2003. Biological diversity of the Yala Swamp lakes, with special emphasis on fish species composition, in relation to changes in the Lake Victoria Basin (Kenya): threats and conservation measures. *Biodiversity and Conservation* 12: 905-920.

Aron, S. 2001. Reproductive strategy: an essential component in the success of incipient colonies of the invasive Argentine ant. *Insectes Sociaux* 48: 25-27.

Atkinson, I. A. E., E. K. Cameron. 1993. Human influence on the terrestrial biota and biotic communities of New Zealand. *Trends in Ecology & Evolution* 8: 447-451.

Baier, R. E., R. L. Forsberg, A. E. Meyer, J. J. Zambon, B. Galil, N. Hulsmann, L. A. Drake, F. C. Dobbs. 2003. Ballast water biofilms as "benchmarks" for bioinvasion risk management. *Abstracts of Papers of the American Chemical Society* 226: U363-U363.

Balas, M. T., E. S. Adams. 1996. Nestmate discrimination and competition in incipient colonies of fire ants. *Animal Behaviour* 51: 49-59.

Basu, P. 1997. Competition hierarchy in the ground foraging ant community in a wet evergreen forest (Western Ghats, India): Role of interference behaviour. *Current Science* 73: 173-179.

Bateman, P. W., P. A. Fleming. 2005. Direct and indirect costs of limb autotomy in field crickets, *Gryllus bimaculatus*. *Animal Behaviour* 69: 151-159.

Bateman, P. W., P. A. Fleming. 2006. Increased susceptibility to predation for autotomized house crickets (*Acheta domestica*). *Ethology* 112: 670-677.

Bernasconi, G., J. E. Strassmann. 1999. Cooperation among unrelated individuals: the ant foundress case. *Trends in Ecology & Evolution* 14: 477-482.

Blancafort, X., C. Gomez. 2005. Consequences of the Argentine ant, *Linepithema humile* (Mayr), invasion on pollination of *Euphorbia characias* (L.) (Euphorbiaceae). *Acta Oecologica* 28: 49-55.

Blight, O., M. Renucci, A. Tirard, J. Orgeas, E. Provost. 2010. A new colony structure of the invasive Argentine ant (*Linepithema humile*) in Southern Europe. *Biological Invasions* 12: 1491-1497.

Blüthgen, N., H. Feldhaar. 2010. 'Food and shelter: how resources influence ant ecology' in '*Ant Ecology*', pp. 115-136, edited by L. Lach, C. L. Parr, and K. Abbott. Oxford University Press, New York.

Bluthgen, N., K. Fiedler. 2004. Competition for composition: Lessons from nectar-feeding ant communities. *Ecology* 85: 1479-1485.

Bolton, B. 1995. *A New General Catalogue of the Ants of the World*. Harvard University Press, Cambridge.

Born, W., F. Rauschmayer, I. Brauer. 2005. Economic evaluation of biological invasions - a survey. *Ecological Economics* 55: 321-336.

Bos, M. M., J. M. Tylianakis, I. Steffan-Dewenter, T. Tscharntke. 2008. The invasive Yellow Crazy Ant and the decline of forest ant diversity in Indonesian cacao agroforests. *Biological Invasions* 10: 1399-1409.

Boudouresque, C. F., M. Verlaque. 2002. Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. *Marine Pollution Bulletin* 44: 32-38.

Brandstaetter, A. S., A. Endler, C. J. Kleineidam. 2008. Nestmate recognition in ants is possible without tactile interaction. *Naturwissenschaften* 95: 601-608.

Brandt, M., E. van Wilgenburg, R. Sulc, K. J. Shea, N. D. Tsutsui. 2009a. The scent of supercolonies: the discovery, synthesis and behavioural verification of ant colony recognition cues. *Bmc Biology* 7: -.

Brandt, M., E. Van Wilgenburg, N. D. Tsutsui. 2009b. Global-scale analyses of chemical ecology and population genetics in the invasive Argentine ant. *Molecular Ecology* 18: 997-1005.

Brühl, C. A., T. Eltz. 2009. Fuelling the biodiversity crisis: species loss of ground-dwelling forest ants in oil palm plantations in Sabah, Malaysia (Borneo). *Biodiversity and Conservation* 19: 519-529.

Buczkowski, G., J. Silverman. 2005. Context-dependent nestmate discrimination and the effect of action thresholds on exogenous cue recognition in the Argentine ant. *Animal Behaviour* 69: 741-749.

Buczkowski, G., E. L. Vargo, J. Silverman. 2004. The diminutive supercolony: the Argentine ants of the southeastern United States. *Molecular Ecology* 13: 2235-2242.

Calcaterra, L. A., J. P. Livore, A. Delgado, J. A. Briano. 2008. Ecological dominance of the red imported fire ant, *Solenopsis invicta*, in its native range. *Oecologia* 156: 411-421.

Caldera, E. J., K. G. Ross, C. J. DeHeer, D. D. Shoemaker. 2008. Putative native source of the invasive fire ant *Solenopsis invicta* in the USA. *Biological Invasions* 10: 1457-1479.

Carlton, J. T. 1996. Biological invasions and cryptogenic species. *Ecology* 77: 1653-1655.

Carlton, J. T., M. R. Ruiz. 2005. 'Vector Science and Integrated Vector Management in Bioinvasion Ecology: Conceptual Frameworks' in '*Invasive Alien Species - A New Synthesis*', pp. 36-58, edited by H. A. Mooney, R. N. Mack, J. A. McNeely, L. E. Neville, P. J. Schei, and J. K. Waage. Island Press, Washington, D.C.

Case, T. J. 1996. Global patterns in the establishment and distribution of exotic birds. *Biological Conservation* 78: 69-96.

Chopard, L. 1930. The Gryllidae of Sarawak. *Sarawak Museum Journal Kuching* 4: 1-42.

Christian, C. E. 2001. Consequences of a biological invasion reveal the importance of mutualism for plant communities. *Nature* 413: 635-639.

Cincotta, C. L., J. M. Adams, C. Holzapfel. 2009. Testing the enemy release hypothesis: a comparison of foliar insect herbivory of the exotic Norway maple (*Acer platanoides* L.) and the native sugar maple (*A. saccharum* L.). *Biological Invasions* 11: 379-388.

Clausen, R., R. York. 2008. Economic growth and marine biodiversity: Influence of human social structure on decline of marine trophic levels. *Conservation Biology* 22: 458-466.

Clement, M., D. Posada, K. A. Crandall. 2000a. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1659.

Clement, M., D. Posada, K. A. Crandall. 2000b. TCS: A computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1659.

Colautti, R. I., A. Ricciardi, I. A. Grigorovich, H. J. MacIsaac. 2004. Is invasion success explained by the enemy release hypothesis? *Ecology Letters* 7: 721-733.

Corin, S. E., P. J. Lester, K. L. Abbott, P. A. Ritchie. 2007. Inferring historical introduction pathways with mitochondrial DNA: the case of introduced Argentine ants (*Linepithema humile*) into New Zealand. *Diversity and Distributions* 13: 510-518.

Crawley, M. J., ed. 1997. *Plant Ecology*. University Press, Cambridge.

Cremer, S., L. V. Ugelvig, F. P. Drijfhout, B. C. Schlick-Steiner, F. M. Steiner, B. Seifert, D. P. Hughes, A. Schulz, K. S. Petersen, H. Konrad, C. Stauffer, K. Kiran, X. Espadaler, P. d'Ettorre, N. Aktac, J. Eilenberg, G. R. Jones, D. R. Nash, J. S. Pedersen, J. J. Boomsma. 2008. The evolution of invasiveness in Garden Ants. *Plos One* 3: -.

Crooks, J. A. 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos* 97: 153-166.

Crozier, R. H., Y. C. Crozier. 1993. The mitochondrial genome of the honeybee *Apis mellifera*: Complete sequence and genome organization. *Genetics* 133: 97-117.

Crozier, Y. C., S. Koulianos, R. H. Crozier. 1991. An improved test for africanized honeybee mitochondrial-DNA. *Experientia* 47: 968-969.

Cuvillier-Hot, V., R. Gadagkar, C. Peeters, M. Cobb. 2002. Regulation of reproduction in a queenless ant: aggression, pheromones and reduction in conflict. *Proceedings of the Royal Society of London Series B-Biological Sciences* 269: 1295-1300.

Cuvillier-Hot, V., A. Lenoir, R. Crewe, C. Malosse, C. Peeters. 2004. Fertility signalling and reproductive skew in queenless ants. *Animal Behaviour* 68: 1209-1219.

Dapporto, L., A. E. Liebert, P. T. Starks, S. Turillazzi. 2009. The relationships between cuticular hydrocarbon composition, faunal assemblages, inter-island distance, and population genetic variation in Tuscan Archipelago wasps. *Biochemical Systematics and Ecology* 37: 341-348.

Davidson, D. W. 1998. Resource discovery versus resource domination in ants: a functional mechanism for breaking the trade-off. *Ecological Entomology* 23: 484-490.

Davis, N. E., D. J. O'Dowd, P. T. Green, R. Mac Nally. 2008. Effects of an Alien Ant Invasion on Abundance, Behavior, and Reproductive Success of Endemic Island Birds. *Conservation Biology* 22: 1165-1176.

Davis, N. E., D. J. O'Dowd, R. Mac Nally, P. T. Green. 2010. Invasive ants disrupt frugivory by endemic island birds. *Biology Letters* 6: 85-88.

Dettner, K., C. Liepert. 1994. 'Chemical mimicry and camouflage' in '*Annual Review of Entomology*', pp. 129-154, edited by T. E. Mittler, F. J. Radovsky, and V. H. Resh. Annual Reviews Inc., Palo Alto, USA.

Drake, J. M., D. M. Lodge. 2004. Global hot spots of biological invasions: evaluating options for ballast-water management. *Proceedings of the Royal Society of London Series B-Biological Sciences* 271: 575-580.

Drake, L. A., M. A. Doblin, F. C. Dobbs. 2007. Potential microbial bioinvasions via ships' ballast water, sediment, and biofilm. *Marine Pollution Bulletin* 55: 333-341.

Drescher, J., N. Blüthgen, H. Feldhaar. 2007. Population structure and intraspecific aggression in the invasive ant species *Anoplolepis gracilipes* in Malaysian Borneo. *Molecular Ecology* 16: 1453-1465.

Drescher, J., N. Blüthgen, T. Schmitt, J. Bühler, H. Feldhaar. 2010. Societies drifting apart? Behavioural, genetic and chemical differentiation between supercolonies in the Yellow Crazy Ant *Anoplolepis gracilipes*. *Plos One* 5: e13581.

Drescher, J., H. Feldhaar, N. Blüthgen. 2011. Interspecific aggression and resource monopolization of the invasive ant *Anoplolepis gracilipes* in Malaysian Borneo. *Biotropica* 43: 93-99.

Dronnet, S., C. Lohou, J. P. Christides, A. G. Bagneres. 2006. Cuticular hydrocarbon composition reflects genetic relationship among colonies of the introduced termite *Reticulitermes santonensis* Feytaud. *Journal of Chemical Ecology* 32: 1027-1042.

Durka, W., O. Bossdorf, D. Prati, H. Auge. 2005. Molecular evidence for multiple introductions of garlic mustard (Alliaria petiolata, Brassicaceae) to North America. *Molecular Ecology* 14: 1697-1706.

Ehrenfeld, J. G. 2010. Ecosystem Consequences of Biological Invasions. *Annual Review of Ecology, Evolution, and Systematics* 41: 59-80.

Eldredge, L. G., S. E. Miller. 1997. Numbers of Hawaiian Species: Supplement2, including a review of freshwater invertebrates. *Musseum Occasional Papers* 48: 3-22.

Elmes, G. W. 1996. 'Biological diversity of ants and their role in ecosystem function' in '*Biodiversity research and its perspectives in East Asia*', pp. 33-48, edited by B. H. Lee, T. H. Kim, and B. Y. Sun. Chonbuk National University, Korea.

Elmes, G. W., T. Akino, J. A. Thomas, R. T. Clarke, J. J. Knapp. 2002. Interspecific differences in cuticular hydrocarbon profiles of Myrmica ants are sufficiently consistent to explain host specificity by Maculinea (large blue) butterflies. *Oecologia* 130: 525-535.

Errard, C., J. Delabie, H. Jourdan, A. Hefetz. 2005. Intercontinental chemical variation in the invasive ant *Wasmannia auropunctata* (Roger) (Hymenoptera, Formicidae): A key to the invasive success of a tramp species. *Naturwissenschaften* 92: 319-323.

Espadaler, X., S. Rey. 2001. Biological constraints and colony founding in the polygynous invasive ant Lasius neglectus (Hymenoptera, Formicidae). *Insectes Sociaux* 48: 159-164.

Espadaler, X., S. Rey, V. Bernal. 2004. Queen number in a supercolony of the invasive garden ant, *Lasius neglectus*. *Insectes Sociaux* 51: 232-238.

Excoffier, L., G. Laval, S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47-50.

Falush, D., M. Stephens, J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* 164: 1567-1587.

Falush, D., M. Stephens, J. K. Pritchard. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes* 7: 574-578.

Feare, C. 1999. Ants take over from rats on Birs Island, Seychelles. *Bird Conservation International* 9: 95-96.

Feldhaar, H., J. Drescher, N. Blüthgen. 2006. Characterization of microsatellite markers for the invasive ant species *Anoplolepis gracilipes*. *Molecular Ecology Notes* 6: 912-914.

Fellers, J. H. 1987. Interference and exploitation in a guild of woodland Ants. *Ecology* 68: 1466-1478.

Fleming, P. A., D. Muller, P. W. Bateman. 2007. Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biological Reviews (Cambridge)* 82: 481-510.

Florane, C. B., J. M. Bland, C. Husseneder, A. K. Raina. 2004. Diet-mediated inter-colonial aggression in the Formosan subterranean termite *Coptotermes formosanus*. *Journal of Chemical Ecology* 30: 2559-2574.

Foucaud, J., J. Orivel, D. Fournier, J. H. C. Delabie, A. Loiseau, J. Le Breton, P. Cerdan, A. Estoup. 2009. Reproductive system, social organization, human disturbance and ecological dominance in native populations of the little fire ant, *Wasmannia auropunctata*. *Molecular Ecology* 18: 5059-5073.

Foucaud, J., J. Orivel, A. Loiseau, J. H. C. Delabie, H. Jourdan, D. Konghouleux, M. Vonshak, M. Tindo, J. L. Mercier, D. Fresneau, J. B. Mikissa, T. McGlynn, A. S. Mikheyev, J. Oettler, A. Estoup. 2010. Worldwide invasion by the little fire ant: routes of introduction and eco-evolutionary pathways. *Evolutionary Applications* 3: 363-374.

Fournier, D., J. C. Biseau, S. Aron. 2009. Genetics, behaviour and chemical recognition of the invading ant *Pheidole megacephala*. *Molecular Ecology* 18: 186-199.

Fournier, D., A. Estoup, R. M. Orivel, J. Foucaud, H. Jourdan, J. Le Breton, L. Keller. 2005. Clonal reproduction by males and females in the little fire ant. *Nature* 435: 1230-1234.

Franks, N., M. Blum, R. K. Smith, A. B. Allies. 1990. Behavior and chemical disguise of cuckoo ant *Leptothorax kutteri* in relation to its host *Leptothorax acervorum*. *Journal of Chemical Ecology* 16: 1431-1444.

Geller, J. B., J. A. Darling, J. T. Carlton. 2010. Genetic Perspectives on Marine Biological Invasions. *Annual Review of Marine Science* 2: 367-393.

Gerlach, J. 2004. Impact of the invasive crazy ant *Anoplolepis gracilipes* on Bird Island, Seychelles. *Journal of Insect Conservation* 8: 15–25.

Giraud, T., J. S. Pedersen, L. Keller. 2002. Evolution of supercolonies: The Argentine ants of southern Europe. *Proceedings of the National Academy of Sciences of the United States of America* 99: 6075-6079.

Glancey, B. M., J. C. E. Nickerson, D. Wojcik, J. Trager, W. A. Banks, C. T. Adams. 1987. The increasing incidence of the polygynous form of the red imported fire ant, *Solenopsis invicta* (Hymenoptera, Formicidae), in Florida. *Florida Entomologist* 70: 400-402.

Grant, W. S., B. W. Bowen. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity* 89: 415-426.

Grapputo, A., S. Boman, L. Lindstrom, A. Lyytinen, J. Mappes. 2005. The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations. *Molecular Ecology* 14: 4207-4219.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series* 41: 95-98.

Harris, R., K. Abbott, K. Barton, J. Berry, W. Don, D. Gunawardana, P. Lester, J. Rees, M. Stanley, A. Sutherland, R. Toft. 2005. Invasive ant pest risk assessment project for Biosecurity New Zealand. Series of unpublished Landcare Research contract reports to Biosecurity New Zealand. (BAH/35/2004-1.

Hefetz, A. 2007. The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera: Formicidae) - interplay of colony odor uniformity and odor idiosynchrasy. A review. *Myrmecological News* 10: 59-68.

Helanterä, H., J. E. Strassmann, J. Carrillo, D. C. Queller. 2009. Unicolonial ants: where do they come from, what are they and where are they going? *Trends in Ecology & Evolution* 24: 341-349.

Henderson, G., R. D. Akre. 1986. Biology of the myrmecophilous cricket, *Myrmecophila manni* (Orthoptera: Gryllidae). *Journal of the Kansas Entomological Society* 59: 454-467.

Hewitt, C. L., M. L. Campbell, R. E. Thresher, R. B. Martin, S. Boyd, B. F. Cohen, D. R. Currie, M. F. Gomon, M. J. Keough, J. A. Lewis, M. M. Lockett, N. Mays, M. A. McArthur, T. D. O'Hara, G. C. B. Poore, D. J. Ross, M. J. Storey, J. E. Watson, R. S. Wilson. 2004. Introduced and cryptogenic species in Port Phillip Bay, Victoria, Australia. *Marine Biology* 144: 183-202.

Hill, M., K. Holm, T. Vel, N. J. Shah, P. Matyot. 2003. Impact of the introduced yellow crazy ant *Anoplolepis gracilipes* on Bird Island, Seychelles. *Biodiversity and Conservation* 12: 1969–1984.

Hoffmann, B. D., C. L. Parr. 2008. An invasion revisited: the African big-headed ant (*Pheidole megacephala*) in northern Australia. *Biological Invasions* 10: 1171-1181.

Hoffmann, B. D., W. C. Saul. 2010. Yellow crazy ant (*Anoplolepis gracilipes*) invasions within undisturbed mainland Australian habitats: no support for biotic resistance hypothesis. *Biological Invasions* 12: 3093-3108.

Hölldobler, B., E. O. Wilson. 1977. The number of queens: An important trait in ant evolution. *Naturwissenschaften* 64: 8-15.

Hölldobler, B., E. O. Wilson. 1990. *The Ants*. The Belknap Press of Harvard University, Cambridge, Massachusetts.

Hölldobler, B., E. O. Wilson. 2009. *The superorganism: the beauty, elegance, and strangeness of insect societies*. W. W. Norton & Company, Inc., New York.

Holway, D. A. 1998. Effect of Argentina ant invasions on ground-dwelling arthropods in northern California riparian woodlands. *Oecologia* 116: 252-258.

Holway, D. A. 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. *Ecology* 80: 238-251.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, T. J. Case. 2002. The causes and consequences of ant invasions. *Annual Reviews of Ecology and Systematics* 33: 181-233.

Holway, D. A., A. V. Suarez, T. J. Case. 1998. Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science* 282: 949-952.

Hugel, S., P. Matyot. 2006. A new *Myrmecophilus* from Seychelles (Orthoptera, Grylloidea). *Bulletin de la Societe Entomologique de France* 111: 469-473.

Human, K. G., D. M. Gordon. 1999. Behavioral interactions of the invasive Argentine ant with native ant species. *Insectes Sociaux* 46: 159-163.

Ignacio, B. L., L. M. Julio, A. O. R. Junqueira, M. A. G. Ferreira-Silva. 2010. Bioinvasion in a Brazilian Bay: Filling Gaps in the Knowledge of Southwestern Atlantic Biota. *Plos One* 5: e13065.

Ingrisch, S. 2010. Review of the ant loving crickets from South-East Asia (Orthoptera: Gryllidae, Myrmecophilinae). *Entomologische Zeitschrift* 120: 3-12.

Iriarte, P. J. F., J. Balanya, M. Pascual, F. Mestres, E. R. Hasson, A. Fontdevila, L. Serra. 2009. Tracking the origin of an invasive species: *Drosophila subobscura* in Argentina. *Journal of Evolutionary Biology* 22: 650-658.

Ithaka, R., R. Gentleman. 1996. R: A language for data analysis and graphics. *Journal of Computational and Graphical Statistics* 5: 299-314.

Ito, F., S. Higashi. 1991. A linear dominance hierarchy regulating reproduction and polyethism of the queenless ant *Pachycondyla sublaevis*. *Naturwissenschaften* 78: 80-82.

Jaquiery, J., V. Vogel, L. Keller. 2005. Multilevel genetic analyses of two European supercolonies of the Argentine ant, *Linepithema humile*. *Molecular Ecology* 14: 589-598.

Jerdon, T. C. 1851. A catalogue of the species of ants found in Southern India. *Madras Journal of Literature and Science* 17.

Jeschke, J. M., D. L. Strayer. 2005. Invasion success of vertebrates in Europe and North America. *Proceedings of the National Academy of Sciences of the United States of America* 102: 7198-7202.

Jones, G. P., M. I. McCormick, M. Srinivasan, J. V. Eagle. 2004. Coral decline threatens fish biodiversity in marine reserves. *Proceedings of the National Academy of Sciences of the United States of America* 101: 8251-8253.

Jousson, O., J. Pawlowski, L. Zaninetti, F. W. Zechman, F. Dini, G. Di Guiseppe, R. Woodfield, A. Millar, A. Meinesz. 2000. Invasive alga reaches California. *Nature* 408: 157-158.

Keane, R. M., M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17: 164-170.

Keller, L., K. G. Ross. 1999. Major gene effects on phenotype and fitness: The relative roles of Pgm-3 and Gp-9 in introduced populations of the fire ant *Solenopsis invicta*. *Journal of Evolutionary Biology* 12: 672-680.

Kenis, M., M. A. Auger-Rozenberg, A. Roques, L. Timms, C. Pere, M. Cock, J. Settele, S. Augustin, C. Lopez-Vaamonde. 2009. Ecological effects of invasive alien insects. *Biological Invasions* 11: 21-45.

Kiritani, K., K. Yamamura. 2003. 'Exotic Insects and Their Pathways for Invasion' in '*Invasive Species - Vectors and Managment Strategies*', pp. 44-67, edited by G. M. Ruiz, and J. T. Carlton. Island Press, Washington, D.C.

Kirschenbaum, R., J. K. Grace. 2007. Dominant ant species in four habitats in Hawaii (Hymenoptera : Formicidae). *Sociobiology* 50: 1069-1073.

Kirschenbaum, R., J. K. Grace. 2008. Agonistic interactions among invasive ant species (Hymenoptera : Formicidae) from two habitats on Oahu, Hawaii. *Sociobiology* 51: 543-553.

Kistner, D. H. 1982. 'The social insects' bestiary' in '*Social Insects vol. III*', pp. 1-244, edited by H. R. Hermann. Academic Press, New York.

Kistner, D. H., K. F. Chong, C. Y. Lee. 2007. A new Malaysian myrmecophilous cricket (Orthoptera : Myrmecophilidae). *Sociobiology* 50: 173-182.

Klimes, P., M. Janda, S. Ibalim, J. Kua, V. Novotny. 2011. Experimental suppression of ants foraging on rainforest vegetation in New Guinea: testing methods for a whole-forest manipulation of insect communities. *Ecological Entomology* 36: 94-103.

Komatsu, T., M. Maruyama, T. Itino. 2009. Behavioral differences between two ant cricket species in Nansei Islands: host-specialist versus host-generalist. *Insectes Sociaux* 56: 389-396.

Krieger, M. J. B. 2005. To b or not to b: A pheromone-binding protein regulates colony social organization in fire ants. *Bioessays* 27: 91-99.

Krushelnycky, P. D., L. L. Loope, N. J. Reimer. 2005. The Ecology, Policy, and Management of Ants in Hawaii. *Proceedings of the Hawaiian Entomological Society* 37: 1-25.

Lach, L., L. M. Hooper-Bùi. 2010. 'Consequences of Ant Invasions' in '*Ant Ecology*', pp. 261-286, edited by L. Lach, C. L. Parr, and K. Abbott. Oxford University Press Inc., New York.

Lach, L., C. V. Tillberg, A. V. Suarez. 2010. Contrasting effects of an invasive ant on a native and an invasive plant. *Biological Invasions* 12: 3123-3133.

Lahav, S., V. Soroker, A. Hefetz, R. K. Vander Meer. 1999. Direct behavioral evidence for hydrocarbons as ant recognition discriminators. *Naturwissenschaften* 86: 246-249.

Le Breton, J., J. H. C. Delabie, J. Chazeau, A. Dejean, H. Jourdan. 2004. Experimental evidence of large-scale unicoloniality in the tramp ant *Wasmannia auropunctata* (Roger). *Journal of Insect Behavior* 17: 263-271.

Leniaud, L., A. Pichon, P. Uva, A. G. Bagneres. 2009. Unicoloniality in *Reticulitermes urbis*: a novel feature in a potentially invasive termite species. *Bulletin of Entomological Research* 99: 1-10.

Lenoir, A., P. D'Ettorre, C. Errard, A. Hefetz. 2001. Chemical ecology and social parasitism in ants. *Annual Review of Entomology* 46: 573-599.

Lenoir, A., D. Fresneau, C. Errard, A. Hefetz. 1999. 'Individuality and colonial identity in ants: the emergence of the social representation concept' in '*Information processing in social insects*', pp. 219-237, edited by C. Detrain, D. J. L., and J. M. Pasteels. Birkhäuser Verlag, Basel.

Lessard, J. P., J. A. Fordyce, N. J. Gotelli, N. J. Sanders. 2009. Invasive ants alter the phylogenetic structure of ant communities. *Ecology* 90: 2664-2669.

Lester, P. J., K. L. Abbott, M. Sarty, K. C. Burns. 2009. Competitive assembly of South Pacific invasive ant communities. *BMC Ecology* 9: Article No.: 3.

Lester, P. J., A. Tavite. 2004. Long-legged ants, *Anoplolepis gracilipes* (Hymenoptera: Formicidae), have invaded Tokelau, changing composition and dynamics of ant and invertebrate communities. *Pacific Science* 58: 391–401.

Liang, D., J. Silverman. 2000. 'You are what you eat': Diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften* 87: 412-416.

Librado, P., J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451-1452.

Liu, H., P. Stiling. 2006. Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions* 8: 1535-1545.

Liu, H., P. Stiling, R. W. Pemberton, J. Pena. 2006. Insect herbivore faunal diversity among invasive, non-invasive and native Eugenia species: Implications for the enemy release hypothesis. *Florida Entomologist* 89: 475-484.

Loope, L. L., D. Mueller-Dombois. 1989. 'Characteristics of invaded islands, with speciel reference to Hawaii' in '*Biological Invasions - A global perspective*', pp. 257-281, edited by J. A. Drake, H. A. Mooney, F. Di Castri, K. H. Groves, F. S. Kruger, M. Rejmánek, and M. Williamson. Wiley, New York.

Lowe, S., M. Browne, S. Boudjelas, M. De Poorter. 2000. 100 of the world’s worst invasive alien species: A selection from the Global Invasive Species Database (GISD). *Aliens* **12**: 12.

MA. 2003. Ecosystems and Human Well-being: A Framework for Assessment *in* I. P. Millenium Ecosystem Assessment, Washington, D. C. , ed. Conceptual Framework Working Group of the Millenium Ecosystem Assessment (MA), Island Press, Washington, D. C.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, F. A. Bazzaz. 2000a. Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689-710.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, F. A. Bazzaz. 2000b. Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689-710.

Macom, T. E., S. D. Porter. 1996. Comparison of polygyne and monogyne red imported fire ant (Hymenoptera: Formicidae) population densities. *Annals of the Entomological Society of America* 89: 535-543.

Maginnis, T. L. 2006. The costs of autotomy and regeneration in animals: a review and framework for future research. *Behavioral Ecology* 17: 857-872.

Martin, S., F. Drijfhout. 2009. A review of ant cuticular hydrocarbons. *Journal of Chemical Ecology* 35: 1151-1161.

Martin, S. J., H. Helanterä, F. P. Drijfhout. 2008a. Colony-specific hydrocarbons identify nest mates in two species of *Formica* ant. *Journal of Chemical Ecology* 34: 1072-1080.

Martin, S. J., H. Helanterä, F. P. Drijfhout. 2008b. Evolution of species-specific cuticular hydrocarbon patterns in *Formica* ants. *Biological Journal of the Linnean Society* 95: 131-140.

Martin, S. J., H. Helanterä, K. Kiss, Y. R. Lee, F. P. Drijfhout. 2009. Polygyny reduces rather than increases nestmate discrimination cue diversity in *Formica exsecta* ants. *Insectes Sociaux* 56: 375-383.

Matsui, S., T. Kikuchi, K. Akatani, S. Horie, M. Takagi. 2009. Harmful effects of invasive Yellow Crazy Ant *Anoplolepis gracilipes* on three island bird species of Minami-daito Island. *Ornithological Science* 8: 81-86.

Mayr, E. 1942. *Systematics and the Origin of Species*. Columbia University Press, New York, USA.

McDonald, I. A. W., J. Cooper. 1995. 'Insular lessons for global biodiversity conservation with particular reference to alien invasions' in '*Islands: Biological Diversity and Ecosystem Function*', pp. 189-204, edited by P. M. Vitousek, L. L. Loope, and H. Anderson. Springer Verlag, Berlin.

McGlynn, T. P. 1999a. Non-native ants are smaller than related native ants. *The American Naturalist* 154: 690-699.

McGlynn, T. P. 1999b. The worldwide transfer of ants: Geographical distribution and ecological invasions. *Journal of Biogeography* 26: 535-548.

McNatty, A., K. L. Abbott, P. J. Lester. 2009. Invasive ants compete with and modify the trophic ecology of hermit crabs on tropical islands. *Oecologia* 160: 187-194.

Messing, R. H., M. G. Wright. 2006. Biological control of invasive species: solution or pollution? *Frontiers in Ecology and the Environment* 4: 132-140.

Mikheyev, A. S., U. G. Mueller. 2007. Genetic relationships between native and introduced populations of the little fire ant Wasmannia auropunctata. *Diversity and Distributions* 13: 573-579.

Mitchell, C. E., A. G. Power. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* 421: 625-627.

Moller, H. 1996. Lessons for invasion theory from social insects. *Biological Conservation* 78: 125-142.

Monnin, T., C. Peeters. 1999. Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. *Behavioral Ecology* 10: 323-332.

Mooney, H. A. 2005. 'Invasive Alien Species: The nature of the problem' in '*Invasive Alien Species - A New Synthesis*', pp. 1-15, edited by H. A. Mooney, R. N. Mack, J. A. McNeely, L. E. Neville, P. J. Schei, and J. K. Waage. Island Press, Washington, D.C.

Mooney, H. A., E. E. Cleland. 2001. The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America* 98: 5446-5451.

Mooney, H. A., R. N. Mack, J. A. McNeely, L. E. Neville, P. J. Schei, J. K. Waage, eds. 2005. *Invasive Alien Species - A New Synthesis*. Island Press, Washington, D.C.

Moreau, C. S., C. D. Bell, R. Vila, S. B. Archibald, N. E. Pierce. 2006. Phylogeny of the ants: Diversification in the age of angiosperms. *Science* 312: 101-104.

Morrison, L. W. 2000. Mechanisms of interspecific competition among an invasive and two native fire ants. *Oikos* 90: 238-252.

Moulton, M. P., S. L. Pimm. 1986. 'Species introductions to Hawaii' in '*Ecology of biological invasions of North America and Hawaii*', pp. 231-249, edited by H. A. Mooney, and J. A. Drake. Springer, New York.

Myers, J. H., D. Simberloff, A. M. Kuris, J. R. Carey. 2000. Eradication revisited: dealing with exotic species. *Trends in Ecology & Evolution* 15: 316-320.

Nelson, E., G. Mendoza, J. Regetz, S. Polasky, H. Tallis, D. R. Cameron, K. M. A. Chan, G. C. Daily, J. Goldstein, P. M. Kareiva, E. Lonsdorf, R. Naidoo, T. H. Ricketts, M. R. Shaw. 2009. Modeling multiple ecosystem services, biodiversity conservation, commodity production, and tradeoffs at landscape scales. *Frontiers in Ecology and the Environment* 7: 4-11.

Neves, C. S., R. M. da Rocha. 2008. Introduced and cryptogenic species and their management in Paranagua Bay, Brazil. *Brazilian Archives of Biology and Technology* 51: 623-633.

O'Dowd, D. J., P. T. Green, P. S. Lake. 1999. Status, impact, and recommendations for research and management of exotic invasive ants in Christmas Island National Park. Centre for Analysis and Management of Biological Invasions, Monash University, Clayton, Victoria.

O'Dowd, D. J., P. T. Green, P. S. Lake. 2003. Invasional 'meltdown' on an oceanic island. *Ecology Letters* 6: 812-817.

Olesen, J. M., L. I. Eskildsen, S. Venkatasamy. 2002. Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Diversity and Distributions* 8: 181-192.

Oliver, T. H., T. Pettitt, S. R. Leather, J. M. Cook. 2008. Numerical abundance of invasive ants and monopolisation of exudate-producing resources - a chicken and egg situation. *Insect Conservation and Diversity* 1: 208-214.

Oliveras, J., J. M. Bas, C. Gomez. 2005. Long-term consequences of the alteration of the seed dispersal process of Euphorbia characias due to the Argentine ant invasion. *Ecography* 28: 662-672.

Palmer, T. M. 2004. Wars of attrition: colony size determines competitive outcomes in a guild of African acacia ants. *Animal Behaviour* 68: 993-1004.

Panzer, G. W. F. 1799. Faunae insectorum germanicae initia. Deutschlands Insecten. Sechster Jahrgang (Hefte LXI - LXXII. Felsecker, Nürnberg.

Parr, C. L. 2008. Dominant ants can control assemblage species richness in a South African savanna. *Journal of Animal Ecology* 77: 1191-1198.

Passera, L., S. Aron. 1993. Social-Control over the Survival and Selection of Winged Virgin Queens in an Ant without Nuptial Flight - Iridomyrmex-Humilis. *Ethology* 93: 225-235.

Payne, C. M., C. V. Tillberg, A. V. Suarez. 2004. Recognition systems and biological invasions. *Annales Zoologici Fennici* 41: 843-858.

Pearcy, M., M. A. D. Goodisman, L. Keller. 2011. Sib mating wothout inbreeding in the longhorn crazy ant. *Proceedings of the Royal Society B - Biological Sciences* in press.

Pedersen, J. S., M. J. B. Krieger, V. Vogel, T. Giraud, L. Keller. 2006. Native supercolonies of unrelated individuals in the invasive Argentive Ant. *Evolution* 60: 782-791.

Pennanec'h, M., L. Bricard, G. Kunesch, J. M. Jallon. 1997. Incorporation of fatty acids into cuticular hydrocarbons of male and female *Drosophila melanogaster*. *Journal of Insect Physiology* 43: 1111-1116.

Perdereau, E., A. G. Bagneres, S. Dupont, F. Dedeine. 2010. High occurrence of colony fusion in a European population of the American termite *Reticulitermes flavipes*. *Insectes Sociaux* 57: 393-402.

Perrings, C., H. Mooney, M. Williamson. 2010. 'The Problem of Biological Invasions' in '*Bioinvasions and Globalization*', edited by C. Perrings, H. Mooney, and M. Williamson. Oxford University Press Inc., New York.

Perrings, C., M. Williamson, E. B. Barbier, D. Delfino, S. Dalmazzone, J. Shogren, P. Simmons, A. Watkinson. 2002. Biological invasion risks and the public good: an economic perspective. *Conservation Ecology* 6: -.

Pfeiffer, M., H. C. Tuck, T. C. Lay. 2008. Exploring arboreal ant community composition and co-occurrence patterns in plantations of oil palm *Elaeis guineensis* in Borneo and Peninsular Malaysia. *Ecography* 31: 21-32.

Pimentel, D. 1993. 'Habitat factors in new pest invasions' in '*Evolution of Insect Pests*', pp. 165-181, edited by K. C. Kim, and B. A. McPheron. Wiley, New York.

Pimentel, D., R. Zuniga, D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273-288.

Pritchard, J. K., M. Stephens, P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Quek, S. P., S. J. Davies, P. S. Ashton, T. Itino, N. E. Pierce. 2007. The geography of diversification in mutualistic ants: a gene's-eye view into the Neogene history of Sundaland rain forests. *Molecular Ecology* 16: 2045-2062.

Queller, D. C., K. F. Goodnight. 1989. Estimating relatedness using genetic markers. *Evolution* 43: 258-275.

Reeve, H. K. 1989. The evolution of conspecific acceptance thresholds. *The American Naturalist* 133: 407-435.

Rodda, G. H., J. A. Savidge. 2007. Biology and impacts of pacific island invasive species. 2. *Boiga irregularis*, the Brown Tree Snake (Reptilia : Colubiridae). *Pacific Science* 61: 307-324.

Ruiz, G. M., J. T. Carlton, eds. 2003. *Invasive Species - Vectors and Managment Strategies*. Island Press, Washington, D. C.

Ruiz, G. M., T. K. Rawlings, F. C. Dobbs, L. A. Drake, T. Mullady, A. Huq, R. R. Colwell. 2000. Global spread of microorganisms by ships - Ballast water discharged from vessels harbours a cocktail of potential pathogens. *Nature* 408: 49-50.

Sagata, K., P. J. Lester. 2009. Behavioural plasticity associated with propagule size, resources, and the invasion success of the Argentine ant *Linepithema humile*. *Journal of Applied Ecology* 46: 19-27.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, D. H. Wall. 2000. Biodiversity - Global biodiversity scenarios for the year 2100. *Science* 287: 1770-1774.

Sanders, N. J., N. J. Gotelli, N. E. Heller, D. M. Gordon. 2003. Community disassembly by an invasive species. *Proceedings of the National Academy of Sciences of the United States of America* 100: 2474-2477.

Savidge, J. A. 1987. Extinction of an island forest avifauna by an introduced snake. *Ecology* 68: 660-668.

Sax, D. F., J. H. Brown. 2000. The paradox of invasion. *Global Ecology and Biogeography* 9: 363-371.

Sodhi, N. S., L. P. Koh, B. W. Brook, P. K. L. Ng. 2004. Southeast Asian biodiversity: an impending disaster. *Trends in Ecology & Evolution* 19: 654-660.

Stachowicz, J. J., H. Fried, R. W. Osman, R. B. Whitlatch. 2002. Biodiversity, invasion resistance, and marine ecosystem function: Reconciling pattern and process. *Ecology* 83: 2575-2590.

Steiner, F. M., B. C. Schlick-Steiner, K. Moder, C. Stauffer, W. Arthofer, A. Buschinger, X. Espadaler, E. Christian, K. Einfinger, E. Lorbeer, C. Schafellner, M. Ayasse, R. H. Crozier. 2007. Abandoning aggression but maintaining self-nonself discrimination as a first stage in ant supercolony formation. *Current Biology* 17: 1903-1907.

Stys, P., P. Banar, J. Drescher. 2010. A new *Oncylocotis* Stal (Hemiptera: Heteroptera: Enicocephalidae) from Sabah: a predacious species associated with the yellow crazy ant, *Anoplolepis gracilipes* (Smith) (Hymenoptera: Formicidae). *Zootaxa*: 27-42.

Suarez, A. V., T. J. Case. 2002. Bottom-up effects on persistence of a specialist predator : Ant invasions and horned lizards. *Ecological Applications* 12: 291-298.

Suarez, A. V., D. A. Holway, D. S. Liang, N. D. Tsutsui, T. J. Case. 2002. Spatiotemporal patterns of intraspecific aggression in the invasive Argentine ant. *Animal Behaviour* 64: 697-708.

Suarez, A. V., D. A. Holway, N. D. Tsutsui. 2008. Genetics and behaviour of a colonizing species: The invasive Argentine Ant. *The American Naturalist* 172: 72-84.

Suarez, A. V., P. Yeh, T. J. Case. 2005. Impacts of Argentine ants on avian nesting success. *Insectes Sociaux* 52: 378-382.

Sunamura, E., X. Espadaler, H. Sakamoto, S. Suzuki, M. Terayama, S. Tatsuki. 2009a. Intercontinental union of Argentine ants: behavioral relationships among introduced populations in Europe, North America, and Asia. *Insectes Sociaux* 56: 143-147.

Sunamura, E., S. Hatsumi, S. Karino, K. Nishisue, M. Terayama, O. Kitade, S. Tatsuki. 2009b. Four mutually incompatible Argentine ant supercolonies in Japan: inferring invasion history of introduced Argentine ants from their social structure. *Biological Invasions* 11: 2329-2339.

Swanson, T. M. 1995. 'Why does biodiversity decline? The analysis of forces for global change' in '*The economics and ecology of biodiversity decline - The forces driving global change*', pp. 1-10, edited by T. M. Swanson. Cambrisge University Press, Cambridge.

Takahashi, A., S. C. Tsaur, J. A. Coyne, C. I. Wu. 2001. The nucleotide changes governing cuticular hydrocarbon variation and their evolution in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* 98: 3920-3925.

Thomas, M. L., K. Becker, K. Abbott, H. Feldhaar. 2010a. Supercolony mosaics: Two different invasions by the yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. *Biological Invasions* 12: 677-687.

Thomas, M. L., K. Becker, K. L. Abbott, H. Feldhaar. 2010b. Supercolony mosaics: two different invasions by the yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. *Biological Invasions* 12: 677-687.

Thomas, M. L., C. M. Payne-Makrisa, A. V. Suarez, N. D. Tsutsui, D. A. Holway. 2006. When supercolonies collide: territorial aggression in an invasive and unicolonial social insect. *Molecular Ecology* 15: 4303-4315.

Thompson, J. D., D. G. Higgins, T. J. Gibson. 1994. Clustal-W - improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673-4680.

Tilman, D. 1997. Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology* 78: 81-92.

Torchin, M. E., K. D. Lafferty, A. P. Dobson, V. J. McKenzie, A. M. Kuris. 2003. Introduced species and their missing parasites. *Nature* 421: 628-630.

Torres, C. W., M. Brandt, N. D. Tsutsui. 2007. The role of cuticular hydrocarbons as chemical cues for nestmate recognition in the invasive Argentine ant (*Linepithema humile*). *Insectes Sociaux* 54: 363-373.

Tsutsui, N. D., A. V. Suarez, D. A. Holway, T. J. Case. 2000. Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences of the United States of America* 97: 5948-5953.

Ugelvig, L. V., F. P. Drijfhout, D. J. C. Kronauer, J. J. Boomsma, J. S. Pedersen, S. Cremer. 2008. The introduction history of invasive garden ants in Europe: Integrating genetic, chemical and behavioural approaches. *BMC Biology* 6.

van Wilgenburg, E., C. W. Torres, N. D. Tsutsui. 2010. The global expansion of a single ant supercolony. *Evolutionary Applications* 3: 136-143.

van Zweden, J. S., J. B. Brask, J. H. Christensen, J. J. Boomsma, T. A. Linksvayer, P. d'Ettorre. 2010. Blending of heritable recognition cues among ant nestmates creates distinct colony gestalt odours but prevents within-colony nepotism. *Journal of Evolutionary Biology* 23: 1498-1508.

van Zweden, J. S., S. Dreier, P. d'Ettorre. 2009. Disentangling environmental and heritable nestmate recognition cues in a carpenter ant. *Journal of Insect Physiology* 55: 158-163.

Vander Meer, R. K., L. Morel. 1998. 'Nestmate recognition in ants' in '*Pheromone communication in social insects*', pp. 79-103, edited by R. K. Vander Meer, M. D. Breed, K. E. Espelie, and M. L. Winston. Westview Press, Boulder, Colorado.

Vander Meer, R. K. V., S. D. Porter. 2001. Fate of newly mated queens introduced into monogyne and polygyne Solenopsis invicta (Hymenoptera : Formicidae) colonies. *Annals of the Entomological Society of America* 94: 289-297.

Vargo, E. L., C. Husseneder. 2011. 'Genetic structure of termite colonies and populations ' in '*Biology of Termites: A Modern Synthesis*', pp. 321-348, edited by D. E. Bignell, Y. Roisin, and N. Lo. Springer, New York.

Vasquez, G. M., C. Schal, J. Silverman. 2009. Colony Fusion in Argentine Ants is Guided by Worker and Queen Cuticular Hydrocarbon Profile Similarity. *Journal of Chemical Ecology* 35: 922-932.

Vasquez, G. M., J. Silverman. 2008. Intraspecific aggression and colony fusion in the Argentine ant. *Animal Behaviour* 75: 583-593.

Verschuren, D., T. C. Johnson, H. J. Kling, D. N. Edgington, P. R. Leavitt, E. T. Brown, M. R. Talbot, R. E. Hecky. 2002. History and timing of human impact on Lake Victoria, East Africa. *Proceedings of the Royal Society B-Biological Sciences* 269: 289-294.

Vitousek, P. M., C. M. DAntonio, L. L. Loope, R. Westbrooks. 1996. Biological invasions as global environmental change. *American Scientist* 84: 468-478.

Vogel, V., J. S. Pedersen, P. d'Ettorre, L. Lehmann, L. Keller. 2009. Dynamics and genetic structure of Argentine Ant supercolonies in their native range. *Evolution* 63: 1627-1639.

Vogel, V., J. S. Pedersen, T. Giraud, M. J. B. Krieger, L. Keller. 2010. The worldwide expansion of the Argentine ant. *Diversity and Distributions* 16: 170-186.

Walters, A. C., D. A. Mackay. 2005. Importance of large colony size for successful invasion by Argentine ants (Hymenoptera : Formicidae): Evidence for biotic resistance by native ants. *Austral Ecology* 30: 395-406.

Wanger, T. C., A. C. Wielgoss, I. Motzke, Y. Clough, B. B. W., N. S. Sodhi, T. Tscharnke. in press. Endemic predators, invasive prey and native diversity. *Proceedings of the Royal Society B - Biological Sciences*.

Ward, D. F., J. R. Beggs, M. N. Clout, R. J. Harris, S. O'Connor. 2006. The diversity and origin of exotic ants arriving in New Zealand via human-mediated dispersal. *Diversity and Distributions* 12: 601-609.

Weeks, R. D., L. T. Wilson, S. B. Vinson, W. D. James. 2004. Flow of carbohydrates, lipids, and protein among colonies of polygyne red imported fire ants, *Solenopsis invicta* (Hymenoptera : Formicidae). *Annals of the Entomological Society of America* 97: 105-110.

Wetterer, J. K. 2005. Worldwide distribution and potential spread of the long-legged Ant, *Anoplolepis gracilipes* (Hymenoptera: Formicidae). *Sociobiology* 45: 1-21.

Wetterer, J. K., J. A. Moore. 2005. Red imported fire ants (Hymenoptera : Formicidae) at gopher tortoise (Testudines : Testudinidae) burrows. *Florida Entomologist* 88: 349-354.

White, E. M., N. M. Sims, A. R. Clarke. 2008. Test of the enemy release hypothesis: The native magpie moth prefers a native fireweed (*Senecio pinnatifolius*) to its introduced congener (*S. madagascariensis*). *Austral Ecology* 33: 110-116.

Whitmore, T. C., J. A. Sayer. 1992. 'Deforestation and species extinction in tropical moist forests' in '*Tropical Deforestation and Species Extinction*', pp. 1-12, edited by T. C. Whitmore, and J. A. Sayer. Chapman & Hall, London.

Williamson, M. 1996. *Biological Invasions*. Chapman & Hall, London.

Williamson, M., A. Fitter. 1996. The varying success of invaders. *Ecology* 77: 1661-1666.

Wilson, E. O. 1971. *The Insect Societies.* The Belknap Press of Harvard University Press, Cambridge, Mass.

Witte, F., B. S. Msuku, J. H. Wanink, O. Seehausen, E. F. B. Katunzi, P. C. Goudswaard, T. Goldschmidt. 2000. Recovery of cichlid species in Lake Victoria: an examination of factors leading to differential extinction. *Reviews in Fish Biology and Fisheries* 10: 233-241.

Worm, B., E. B. Barbier, N. Beaumont, J. E. Duffy, C. Folke, B. S. Halpern, J. B. C. Jackson, H. K. Lotze, F. Micheli, S. R. Palumbi, E. Sala, K. A. Selkoe, J. J. Stachowicz, R. Watson. 2006. Impacts of biodiversity loss on ocean ecosystem services. *Science* 314: 787-790.

XI. ACKNOWLEDGEMENTS

During the years that I worked on this thesis, I experienced the aid and support of many people. With these acknowledgements, I'd like to send my kindest regards to all of the people that helped making this thesis possible.

Nico Blüthgen introduced me to the world of myrmecology and supervised both my Diploma thesis and now this PhD thesis. Thanks for your time, your guidance, your patience as well as your lectures in statistics. The entire **work group** (Insect-Plant-Interactions) is thanked for inspiring work group meetings, which, besides enthusiastic scientific discussions, contained a good deal of humour.

Heike Feldhaar introduced me to the world of population genetics and evolutionary biology. Without your enthusiastic and benevolent criticism, I still wouldn't know how to write a decent manuscript or put together a compelling talk.

Thomas Schmitt introduced me to the immense complexity of chemical communication in social insects, which is something that I would surely like to understand way better than I do now. Thank you for agreeing to co-supervise this thesis.

Maja Babis, **Jana Bühler**, **Janina Eulenburg**, **Stephan Rinke** and **Deborah Schweinfest** were students that I had the honour to supervise and which produced valuable results that proved to be vital for my research. Thanks for your effort and the good time, both here in Würzburg and in Borneo!

Karl-Eduard Linsenmair, **Dieter Mahsberg** and **Brigitte Fiala** are generally thanked for their passionate involvement in the Department of Animal Ecology & Tropical Biology as well as for valuable suggestions and discussions.

Wolfgang Rössler and **Flavio Roces** let me set up more or less permanent camp in the facilities of the Department of Behavioural Physiology & Sociobiology, even when status as guest scientist had long run out.

Birgit Bünger, **Susanne Linke** and **Irina Stahl** provided help with all kinds of paperwork, envelopes, fax machines, bills, registrations and bookings as well as a good chat every once in a while.

Adrienne Gerber-Kurz, **Annete Laudahn** and **Karin Möller** taught me a great deal about how to keep ants and how to deal with microsatellites.

I would like to thank the entire staff and especially the PhD students of the Departments of Animal Ecology & Tropical Biology and Behavioural Physiology & Sociobiology for helpful discussions, assistance, criticism, suggestions and an overall great time.

Datin Maryati Mohamed, **Maklarin bin Lakim**, **Arthur Chung** and **Sylvia Alsistu** facilitated my field work in Borneo and are thanked for the numerous times that they agreed to help me with my official matters.

Justinah Parantis and all the members of the Butterfly farm in Poring were of great help in Poring by letting me use large parts of the Butterfly Farm for my experiments.

Kamin Gebok was of immense help in Poring, not only by being a generous landlord or knowing people and making things happen, but also by letting me live in the most comfortable bamboo houses I've ever lived in.

Eugene Tan and the entire staff of **Uncle Tan's** offered invaluable assistance during my studies, including accommodation, hardware and a hut to set up a field laboratory. Not to mention an unforgettable jungle camp in the last remnants of the Kinabatangan sweetwater swamp forest, where there's much more to do than 'just' watch Bornean wildlife. HBS!

The following people are thanked for inspiring scientific communication and/or collaboration: **Kirsti Abbott**, **Serge Aron**, **Petr Baňař**, **Catherine Berney**, **Andrew Cline**, **Dennis O'Dowd**, **Kai Dworschak**, **Heikki Helanterä**, **Sigfrid Ingrisch**, **Milan Janda**, **Robert Junker**, **Laurent Keller**, **Petr Klimes**, **Alain Lenoir**, **Christine La Mendola**, **Florian Menzel**, **Juliane Mooz**, **Shyama Pagad**, **Floyd W. Shockley**, **Andrew V. Suarez**, **Pavel Štys** and **Darren Ward**.

Last but not least, I'd like to thank **my entire family** and **my friends** for believing in me and always supporting my passion for the natural world.

Those that I may have inadvertently forgotten to mention are hereby thanked for their help as well as their understanding and forgiveness.

Publikationen

Feldhaar H, **Drescher J**, Blüthgen N (2006). Characterization of microsatellite primers for the invasive ant species *Anoplolepis gracilipes*. ***Molecular Ecology Notes*** 6: 912-914

Drescher J, Blüthgen N, Feldhaar H (2007). Population structure and intraspecific aggression in the invasive ant species *Anoplolepis gracilipes* in Malaysian Borneo. ***Molecular Ecology*** 16: 1453-1465

Drescher J, Blüthgen N, Schmitt T, Bühler J, Feldhaar H. (2010). Societies drifting apart? Behavioural, genetic and chemical differentiation between neighboring supercolonies in the yellow crazy ant *Anoplolepis gracilipes*. ***PLoS ONE*** 5(10): e13581.

Stys P, Banar P, **Drescher J** (2010). A new *Oncylocotis* (Hemiptera: Heteroptera: Enicocephalidae) from Sabah: A predacious species associated with the Yellow Crazy Ant, *Anoplolepis gracilipes* (Hymenoptera: Formicidae). ***Zootaxa*** 2651: 27-42

Drescher J, Feldhaar H, Blüthgen N (2011). Interspecific Aggression and Resource Monopolization of the invasive ant *Anoplolepis gracilipes* in Malaysan Borneo. ***Biotropica*** 43: 93-99