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Social Dynamics in Paper Wasps : The case of nest-drifting in *Polistes canadensis*

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Originally published at : Thesis, University of Lausanne

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UNIL Université de Lausanne

Faculté de biologie
et de médecine

Département d'Ecologie et d'Evolution

Social Dynamics in Paper Wasps:

The case of nest-drifting in *Polistes canadensis*

Thèse de doctorat ès sciences de la vie (PhD)

présentée à la

Faculté de biologie et de médecine
de l'Université de Lausanne

par

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Lausanne 2013

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole Doctorale

Doctorat ès sciences de la vie

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Président	Monsieur Prof. Mehdi **Tafti**
Directeur de thèse	Monsieur Prof. Laurent **Keller**
Experts	Monsieur Prof. Michel **Chapuisat**
	Monsieur Prof. Thibaud **Monnin**

le Conseil de Faculté autorise l'impression de la thèse de

Monsieur Thibault Lengronne

Biologiste diplômé de l'Université de Paris Nord, France

intitulée

Social Dynamics in Paper Wasps: The case of nest-drifting in *Polistes canadensis*

Lausanne, le 13 décembre 2013

M. Tafti

pour Le Doyen
de la Faculté de Biologie et de Médecine

Prof. Mehdi Tafti

REMERCIEMENTS

Je tiens tout d’abord à remercier Laurent pour m’avoir accepté au sein de son groupe bien qu’étant un outcast ne travaillant pas sur les fourmis. Merci pour tes conseils, ton efficacité et ta patience. J’ai appris beaucoup au sein de ce groupe et passé de très bons moments à Lausanne qu’ils fussent professionnels ou relationnels.

Je remercie bien évidemment aussi Seirian pour m’avoir également accepté en tant que doctorant. Tu as toujours été à l’écoute et très impliquée dans mes travaux, presque une seconde maman. Merci pour ton aide incommensurable aussi bien au sein du labo que lors du terrain. Merci également à ton mari, Nick, pour son expertise et aide en programmation.

Merci à Michel, Thibaud Monnin et Mehdi Tafti pour avoir accepté de faire partie de mon jury de thèse, pour le temps investi et les conseils apportés.

Merci à tous ceux qui un jouer un rôle direct ou indirect dans l’accomplissement de cette thèse et que j’ai côtoyé aussi bien au sein du DEE que d’IoZ.

Je tiens particulièrement à remercier tous les membres du groupe « fourmis » de Lausanne : Romain, Tim, Christophe, Mic, Joël, Yannick, Eric, Nathalie, Tom, Christine, Anabelle, Mischa, Danielle, Mingkwan, Adria, Akiko, Morgan, Miguel, Colby, Valérie, Nayuta, Michel, Vicky et j’en oublie surement, pour la bonne ambiance apportées au sein du labo mais aussi lors des conférences et pour leur aide.

Merci à France et Nadia pour avoir facilité toutes les tâches administratives, pour le forward de courrier à Londres, les crédits et pour leur disponibilité.

Merci pour ces moments privilégiés à tous les gens du bureau 3212 : Romain, Tim, Sophie, Christophe « El Gladiator », Séverine, Susana, Alia mais aussi aux gerbilles Congrus et Crispus, aux mantes religieuses, aux axolotes et aux fourmis.

Un immense merci aussi à tous les gens ayant apporté de la vie au sein du département mais aussi hors du labo, entre pétanque, bowling, poker et autres soirées plus ou moins arrosées. Pour tout cela un grand merci à Alex, Auré, Tim, Romain, Sophie, Aline, Christophe, Caro A, Caro BC, Helene, Agnès, Cyril, Guillaume, Charlène, Pierre B, Pierre F, Fred, Nathalie, Nadia, Rob, Céline S (x2), Walid, Fabrice, Loïc, Colby, Yannick, Joël, Christine, Zoé, Alan, Lucie, Pauline, Delphine, Elo et pleins d'autres.

Un énorme merci à Emily, Claire, Henry et Solenn pour la bonne ambiance apportée au bureau et dans la vie sur Londres, ces pintes au « Social » et divers pubs … ainsi qu'à mes ancien colocs: Florence & Martin, Harriet et Duncan. Thanks a lot guys !

Je remercie également tout le groupe SEaS et autres membres de l'Institut de Zoologie de Londres : Stéphanie, Elli, Inti, Kate, Ian, Anna, Clare, Patricia, Nathalie, Dada, Trent…. Et j'en oublie.

Merci à toutes les personnes m'ayant aidé tout au long de mes saisons de terrain au Panama: Javier, Stéphanie, les 2 Roberto, Jessie, … Jorge, Gabriel ainsi que toutes les personnes du STRI. Un merci tout particulier à Solenn pour toute son aide et pour avoir su m'endurer pendant près de 8 mois au Panama…

…et merci aussi aux *P. canadensis* de s'être restreint de me piquer plus de 20 fois malgré ce que je leur faisais subir.

Merci à mes vieux potes de Caen et particulièrement St Ouent : Antoine, Jimmy, Nico, Jeannot, Nathan… mais aussi tous les anciens du Master Etho: Jérem, Uriel, Nérik, Anaïs, Violaine …

Enfin, merci à toute ma famille pour leur écoute, leur intérêt et patience et particulièrement mes parents, ma sœur… merci pour le soutien qu'il mont toujours apporté dans mes études et pour avoir toujours été fier de moi.

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“Social wasps are among the least loved insects... Yet, where statistics will not alter a general impression, another approach might. Every schoolchild, perhaps as part of religious training, ought to sit watching a Polistes wasp nest for just an hour... I think few will be unaffected by what they see. It is a world human in its seeming motivations and activities far beyond all that seems reasonable to expect from an insect: constructive activity, duty, rebellion, mother care, violence, cheating, cowardice, unity in the face of a threat -- all of these are there."

W. D. Hamilton (1996)

SUMMARY

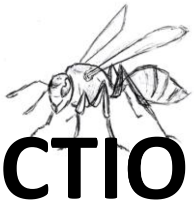
One major transition in evolution is the shift from solitary to social life. Sociality has evolved in a few taxa of the animal kingdom, most notably in the social insects which have achieved the highest level of sociality: eusociality. Colonies of social insects are formed by a reproductive queen, and many non-reproductive or sterile workers who help raise their mother queen's offspring. Kin selection theory explains worker behaviour in terms of the indirect fitness they gain from raising non-offspring kin. It therefore predicts that workers should stay faithful to their natal nests, to which they are the more related. However, in the tropical paper wasps *Polistes canadensis*, high levels of nest-drifting, whereby workers spend time on other neighbouring nests, has been reported. This PhD aimed at understanding the mechanisms involved in this peculiar behaviour as well as its implications for kin selection theory.

I examined nest-drifting through the study of the social dynamics of the tropical paper wasp *P. canadensis*. My results showed that populations of this species of paper wasps are composed of different aggregations of nests. The studied populations showed little limited dispersal (viscosity), despite previous suggestion, but nests within these aggregations were more related to each other than nests outside of aggregations. This suggested that drifters may benefit from indirect fitness when helping on neighbouring nests. Drifting was unlikely to be accidental since we found drifting patterns at various rates and consistently over several time periods during monitoring. Workload (differences in colony-level foraging effort) was also a potential factor explaining nest-drifting in *P. canadensis*. Worker and brood removal experiments revealed that drifters do not respond to any changes in the need for help in the non-natal nests they visit. Drifters thus bias their help in their natal nests, from which they may benefit the most in terms of indirect fitness, before investing in others. Altogether, these results on nest-drifting in *P. canadensis* are consistent and suggest that nest-drifting is an important alternative reproductive strategy, contributing to the indirect fitness benefits gained by non-reproductive wasps. Additionally, this PhD provides information on the genetic structure of paper wasps' populations and demonstrates the role of inactive or lazy wasps as a "reserve worker force", which provides resilience to the colony in the event of worker mortality. More generally, this work further highlights the complex organization and adaptability of individuals in insect societies.

RESUME

Le passage de la vie solitaire à la vie sociale représente une des principales transitions évolutives. La socialité a évolué au sein de plusieurs taxons du règne animal et notamment chez les insectes sociaux qui ont atteint son niveau le plus élevé : l'eusocialité. Les colonies d'insectes sociaux se composent d'une reine, qui monopolise la reproduction, et d'ouvrières, non-reproductrices ou parfois stériles, qui aident à élever la descendance de la reine. Selon la théorie de la sélection de parentèle, les ouvrières augmentent leur fitness (succès reproducteur) non pas à travers leur propre progéniture, mais en aidant des individus apparentés (leur reine) à produire davantage de descendants. Cette théorie prédit ainsi que les ouvrières ont un intérêt à rester fidèles à leur nid natal. Toutefois, chez la guêpe tropicale *Polistes canadensis*, de nombreuse ouvrières visitent d'autres nids que leur nid natal : un phénomène appelé « dérive des ouvrières ». Le but de ce doctorat est ainsi de mieux comprendre les mécanismes impliqués dans ce comportement particulier des ouvrières ainsi que ces implications pour la théorie de la sélection de parentèle.

Nous avons examiné le comportement de dérive des ouvrières à travers une étude des dynamiques sociales chez la guêpe tropicale *P. canadensis*. Mes résultats montrent que les populations de *P. canadensis* se composent en différentes agrégations de nids. Malgré de précédentes suggestions, on n'observe qu'une faible viscosité génétique au sein des populations de *P. canadensis* étudiées. On retrouve toutefois un degré d'apparentement entre nids d'une même agrégation. Ceci suggère que les ouvrières dériveuses sont susceptibles de bénéficier de fitness indirect en aidant les nids proches géographiquement. De plus, ces échanges d'ouvrières ne semblent pas accidentels puisque l'on constate des variations de taux de dérive et puisque les déplacements observés entre nids persistent sur plusieurs périodes de temps. La charge de travail, qui correspond aux différences d'effort de fourragement entre nid visités et natals, est décrite dans notre étude comme potentiel facteur expliquant le comportement de dérive des ouvrières chez *P. canadensis*. Nos expériences de retrait d'ouvrières et de couvain ont révélées que les dériveuses ne semblent pas répondre aux changements de besoins en aide des nids visités. Les ouvrières dériveuses biaisent leur effort en aidant leur propre nid, par lequel elles bénéficient le plus en termes de fitness indirect, avant de se consacrer à tout autre nid. Dans l'ensemble, ces résultats sur la dérive des ouvrières chez *P. canadensis* sont cohérents et suggèrent que ce comportement est une importante stratégie de reproduction alternative chez cette espèce qui contribue à la fitness indirecte de ces ouvrières non-reproductrices. De plus, ce doctorat apporte des informations sur la structure génétique des populations de guêpes *Polistes* et décrit le rôle des ouvrières inactives. Celles-ci semblent servir de réserve en ouvrières apportant du support à la colonie dans l'éventualité d'une perte d'individus. Plus généralement, ce travail met l'accent sur l'organisation complexe et l'adaptabilité des individus dans les sociétés d'insectes.



GENERAL INTRODUCTION

Social Evolution

The transition from solitary to social living is one of the major transitions in evolution (Szathmary and Maynard Smith 1995). Social life, the living together of certain organisms in a balanced and cooperative manner, appears at several key moments, independently, during evolution and shows various levels of specialization and complexity: from mutualistic interactions, collective brood care to highly advanced cooperative societies (Aron and Passera 2000). The highest degree of cooperation found in the animal kingdom is eusociality and its best representative are the social insects with tens of thousands species across the world. They share this particular trait with only few other organisms such as the sponge-dwelling shrimps (Duffy 1996) and naked mole-rats (Burda *et al*. 2000). More recently, the discovery of eusociality in flatworms (Hechinger *et al*. 2011) has raised the idea that eusociality might be more common than generally realized (Newey and Keller 2010). The defining components of eusociality are cooperative brood care within the group, overlap of adult generations and reproductive division of labour or reproductive altruism (Wilson 1971). This last characteristic denotes the capability of certain individuals in the society, the dominant queen(s), to monopolise reproduction whereas the others, the workers, forego their personal reproduction and instead help raise the brood of their dominant (Wilson 1971).

The existence of such non reproductive, and sometimes sterile individuals in some highly eusocial species, presents an obvious challenge to Darwin’s theory of natural selection (Darwin 1859). Indeed, since natural selection favours individuals best fitted to their environment who achieve the greatest reproductive success, this raise the question of how can such behaviour which favour the reproduction of others, while being costly to the actor, be maintained?

An elegant explanation for such cooperative behaviours in insect societies has been provided more than a century later by Hamilton and his theory of "kin selection" or "inclusive fitness" (Hamilton 1964a). The theory states that individuals can pass on their genes to the next generation not only through their own reproduction, but also indirectly through reproduction of relatives (which share common copies of genes) (Hamilton 1964a). Formally, the theory can be encapsulated in the inequality $r*b>c$, commonly called Hamilton's rule. Genes of altruistic behaviour should increase in frequency when the additional reproductive benefit gained by the recipient of the altruistic act (b) multiplied by the genetic relatedness between the recipient to the actor (r) is greater than the reproductive cost to the individual performing the act (b). Applied to eusocial insects, kin selection theory explains how non-reproductive individuals, the workers, can increase their fitness, indirectly, through the reproduction of their mother, the queen.

Nest-drifting behaviour

In many species of social insects, some individuals have been observed moving between nests: a phenomenon known as nest-drifting (or drifting). Nest-drifting can be defined as the behaviour whereby some workers called drifters, spend time in nests other than their own. This behaviour presents an obvious challenge to kin selection theory. Indeed, to pass on their genes, non-reproductive individuals should help raise the more related brood (i.e. their kin). Therefore, in order to maximize indirect fitness benefits, individuals should remain faithful to their natal nest, to whom they are highly related, rather than any other nest, to whom they are less related or even unrelated.

This behaviour has largely been overlooked in the eusocial insect literature and only recently has drifting been considered as a potentially adaptive strategy. To date, nest drifting behaviour has been detected in social wasps, such as stenogastrine wasps (Coster-Longman 1994; Turillazzi *et al*. 1997), polistine wasps (Kasuya 1981; Tsuchida and Itô 1987; O'Donnell 1992; Seppä *et al*. 2002; Sumner *et al*. 2007) and vespine wasps (Akre *et al*. 1976); the social bees (Roubik 1981; Goerzen *et al*. 1995; Peso and Richards 2011) and particularly honeybees (Pfeiffer and Crailsheim 1998; Neumann *et al*. 2000; Paar *et al*. 2002; Nanork *et al*. 2005; Härtel *et al*. 2006; Beekman and Oldroyd 2008; Chapman *et al*. 2009; Wongvilas *et al*. 2010), sweat bees (Michener 1966; Paxton *et al*. 2002; Ulrich *et al*. 2009), and bumblebees (Godzinska 1989; Tasei *et al*. 2001; Birmingham and Winston 2004; Lopez-Vaamonde *et al*. 2004; Birmingham *et al*. 2004; Lefebvre and Pierre 2007; Takahashi *et al*. 2010; Blacher *et al*. 2013). Nest-drifting has also been detected in cooperatively breeding birds, such as bell miners, *Manorina melanophrys* (Wright 2007; Wright *et al*. 2010).

A variety of terms have been used to describe nest-drifting with the terminology usually reflecting the frequency and duration of visits. Certain studies use for instance terms such as "movers", "joiners" or "visitors" to describe individuals permanently moving or just visiting others nests (Seppä *et al*. 2002). However, the more general term of "drifters" seems to be accepted for characterizing individuals shifting group membership to a non-natal group either permanently (Ulrich *et al*. 2009) or intermittently (Lopez-Vaamonde *et al*. 2004; Birmingham *et al*. 2004; Sumner *et al*. 2007).

Levels of nest-drifting appear to vary substantially across species. For instance, drifting is common in natural colonies of honeybees, with around 2% to 5% of workers drifting to non-natal queenright and queenless hives for both *Apis florae* (Nanork *et al*. 2005) and *A. cerana* (Nanork *et al*. 2007). In the bumblebees, around 20-30% of *Bombus terrestris* workers drifted among laboratory colonies where workers were allowed to forage outside buildings or in greenhouses (Lopez-Vaamonde *et al*. 2004; Lefebvre and Pierre 2007; Blacher *et al*. 2013). In other species of bumblebees, such as *B. impatiens*, *B. occidentalis* and *B. hypnorum*, up to 28% of marked individuals drifted to alien colonies (Paxton *et al*. 2001; Birmingham *et al*. 2004). Other reports of high levels of drifting in bees includes the sweat bees with up to 33% of alien bees observed in *Lasioglossum malachurum* (Soro *et al*. 2009) and up to 44% of females with genotypes consistent with other populations in *Halictus scabiosae* (Ulrich *et al*. 2009). In the carpenter bee *Xylocopa virginica,* 50%, and up to 70% of individuals have been observed drifting between nests (Peso and Richards 2011). Similarly high levels of drifting were found in the tropical paper wasp *Polistes canadensis*, where up to 50% of wasps were observed visiting one or more nests in a natural population in Panama (Sumner *et al*. 2007).

Although the occurrence of drifting has been widely investigated across social insects' taxa, only recently have researchers begun to explore the explanations as to why some individuals would decide to move to non-natal nests (Paxton *et al*. 2002; Sumner *et al*. 2007; Ulrich *et al*. 2009; Peso and Richards 2011; Blacher *et al*. 2013).

As pointed out by Peso *et al.* (2011), the term 'drifting' imply a passive phenomenon and have first been used to define individuals experiencing no clear benefit in terms of fitness.

Indeed, in several cases, drifting is clearly only an accidental event, and can be explained by orientation errors coupled with discrimination errors. In the bumblebees, *B. occidentalis* and *B. impatiens*, for instance, the presence of drifters in artificial colonies may have resulted from the high density of nests needed for pollination of agricultural plants in greenhouses (Birmingham *et al*. 2004). Kasuya (1981) already described similar observations of drifters in greenhouses ('glasshouses') in the paper wasp, *Polistes chinensis antennalis* where he suspected wasps to perform navigational errors due to similar topographical features between natal and joined nests. Similarly, specific arrangement with repetitive patterns of densely packed honeybee hives in apiaries can increase drifting (Free 1958; Pfeiffer and Crailsheim 1998; Neumann *et al*. 2000).

However, in more natural situations, fitness benefits may explain why drifters visited non-natal nests. For instance, drifters may gain direct fitness benefits by parasitizing foreign nests. One well-studied example is the social parasitism by worker in bumblebee (Lopez-Vaamonde *et al*. 2004; Birmingham *et al*. 2004; Blacher *et al*. 2013). In various species of bumblebees (*B. terrestris, B. occidentalis, B. hypnorum*), some workers drift in alien conspecific colony and lay male eggs earlier than they would normally do in their natal nest. Since early male would have greater mating success than later male, producing early male may allow these workers to benefit from direct fitness benefits without jeopardizing their colony's overall reproductive output (Lopez-Vaamonde *et al*. 2004). In the honeybee, some workers, mostly young foragers (Free 1958), sometimes infiltrate and lay eggs in other queenright colonies. In *A. cerana*, drifting individuals (5% of individuals in host colonies) seem to have more developed ovaries than individuals from their natal nests, suggesting that they may lay eggs (Nanork *et al.* 2007, Beekman & Oldroyd 2008). Similarly, in *A. florea* queenright colonies, around 2% of the workers from queenright colonies are from alien colonies and may lay eggs (Nanork *et al.* 2005). Such a small number of extra egg-layers is unlikely to have an impact on colony productivity, because workers eat or remove eggs that have been laid by other workers (worker policing). In queenless colonies of *A. cerana*, drifters contribute up to 5.5% of male production (Nanork *et al.* 2007). In queenless colonies of *A. florea*, drifting individuals (4.5% of the workers) are benefiting from infiltrating a colony as they produce 10 times more eggs as they would in their natal nest (Nanork *et al.* 2005). Since drifting individuals seem to increase their direct fitness benefits by laying in other queenless colonies, they are considered as social parasites (Beekman e& Oldroyd 2008).

Likewise, some individuals in the sweat bee *Halictus scabiosae* are also likely to gain direct fitness benefit through drifting, specifically in small and unrelated nests because it increase their chance to inherit the nest in the next breeding season and reduce competition between relatives for the dominant breeding position (Ulrich *et al*. 2009).

Finally, drifting may have also evolved to provide indirect fitness benefits, especially for drifters acting as helpers in the nest they visit rather than as parasite. One particular case is found in the tropical paper wasp *P. canadensis*. In this species, high levels of drifting have been described, whereby females visit nests other than their own and appear to act as helpers on related nests that they visit by performing task such as foraging and brood care (Sumner *et al*. 2007).

The related study performed on *P. canadensis*, which serves as a groundwork for this PhD, aimed at testing the potential hypotheses explaining drifting. Results suggested that the observed levels of drifting was unlikely to be artefact of the technique or of the study population since marked and unmarked wasps show similar behaviours and since drifting have been observed in other populations, at different periods (seasons) and with other monitoring techniques. Drifters were unlikely to be lost foragers making mistakes in nest identity since they seem to visit, on average, closely related nests and also unlikely to be social parasite because none had developed ovaries. The more likely explanation was therefore that drifters benefit from indirect fitness benefits by visiting other nests as they act more as workers (i.e. foraging, raising brood) than queens (Sumner et al. 2007).

***Polistes* paper wasps as a model for understanding the evolution of sociality**

Polistes, species-rich genus of social wasps from the vespid sub-family Polistinae, has long been called a “key genus” for the study of eusocial evolution (Evans 1958). These paper wasps exhibit intermediate colony organization between sub-social species, where grouping and parental care are selected, and highly eusocial species, characterized by cooperative brood care, overlapping generation and morphologically distinct reproductive and worker castes (West-Eberhard 1969), which makes them an excellent system to study the early stages of eusocial evolution. Indeed, *Polistes* wasps belong to the primitively eusocial insects and exhibit all characteristics of eusocial species but their castes are often morphologically indistinguishable (bumblebees and some species of sweat bees are exceptions in the primitively eusocial insects). Such lack of morphological caste differentiation leads to considerable flexibility in the social and reproductive status in these species. All females have the potential to lay eggs but only few do so (West-Eberhard 1969). Generally, one individual becomes the dominant queen while the remaining individuals are subordinate workers (independent-founding). The queen dominates others nestmates through physical aggressions and monopolise reproduction. She spends most of her time on the nest, initiating the building of new cells, but hardly goes out of the nest to forage unlike her subordinate nestmates (Jeanne 1980). Subordinates typically help at the different task necessary for the colony growth but their status is not pre-determined and in some cases they may decide to leave their natal nests and found their own nest.

Further traits have contributed to make *Polistes* paper wasps an excellent model for studying social behaviour (West-Eberhard 1969; Turillazzi and West-Eberhard 1996; Starks *et al*. 2006). First, they are cosmopolitan in their distribution, with a variety of species being found both in temperate and tropical regions. Temperate species are annual with a seasonally enforced diapause whereas tropical species lack this seasonality and therefore found their nests throughout the year. Second, *Polistes* wasps display typically small nests with up to 150-200 individuals (Reeve 1991) and are characterized by small, simply constructed open nests with exposed combs (without envelope) allowing observations of individually marked wasps and the study of colony-wide behaviours (West-Eberhard 1969). Only *Polistes* wasps and few other species of social insects can be observed in the field with such convenience (West-Eberhard 1969).

PhD Aims and Objectives

The aim of this PhD is to use the tropical species *Polistes canadensis* as a model for the study of paper wasps' social dynamics. One particular feature described in this species is the ability of some workers to visit others nest, or drift. My studies mainly focus on this specific case of nest-drifting in *P. canadensis.* We aim at confirming what is known and provide new insights on the mechanisms underlying this behaviour. Indeed, despite the recent growing interest on nest-drifting behaviour in social insects, very little is known about the mechanisms involved in its evolution and maintenance as well as its implications for the kin selection theory.

This PhD is constructed around three main chapters:

The first chapter tests the hypothesis that seasonal constraints can influence population structure, and hence influence wasp behaviour. Differences in colony founding and colony synchrony exist between temperate and tropical species. Such variations in climate and life cycle have been suggested to affect wasp behaviour (dispersal) and therefore their genetic structure (Hamilton 1964b). Moreover, nest-drifting is likely to have derived from a particular population structuring known as viscous population, which refer to increased genetic relatedness of neighbours due to limited dispersal (as discussed in previous studies on *P. canadensis* (Sumner *et al*. 2007)). I therefore examined the population genetics of the tropical species, *P. canadensis* (our main model throughout the thesis), and another well-studied temperate species, *P. dominulus* via the estimation of the genetic differentiation and relatedness. The objectives of this study therefore were to determine potential differences in population structure (genetic differentiation and relatedness) between wasps from temperate and tropical area, investigate the potential viscosity of *P. canadensis* populations and, to further increase our understanding of the structure and dynamic of paper wasps' natural populations.

In the second chapter, I test the hypothesis that the inactivity of workers, whereby some workers do not seem to contribute to helping the colony, and nest-drifting, whereby some workers leave their natal nest to help others, have potential adaptive value in *P. canadensis.*

Inactive workers have been observed in many species of social insects (Schmid-Hempel 1990) including *P. canadensis* (Sumner pers. obs.). Previous studies have suggested that these inactive workers may represent a "reserve worker force" acting as a stand-by group of helpers that can engage in activity when unexpected contingencies arise (Lindauer 1961; Michener 1964; Schmid-Hempel 1990; Robinson 1992). As for nest-drifting, high levels has been reported in *P. canadensis* (Sumner *et al*. 2007). Nests appear to receive foraging help from the workers of nearby related colonies. This may represent an effective strategy for replacing lost helpers if drifters are able to determine the need of the nests they visit. The objectives of this chapter, therefore, was to investigate whether the lazy/inactive workers and/or drifting individuals (from neighbouring nests) were recruited to compensate for the loss of helpers by manipulating the need for help of nests through removal of hard-working foragers. Importantly, I further examined whether the employed strategy allows fitness of helpers to be assured via excess brood being reared to adulthood.

In the third chapter, I test the hypothesis that structured patterns of drifting exist in *P. canadensis* and that inclusive fitness can explain these patterns. To do so, I examine and analyzed in details nest-drifting behaviour in *P. canadensis* by performing a comprehensive Social Network Analysis (SNA) of the drifting patterns in 8 aggregations (or clusters) of nests. The objectives of this study were, first, to describe both temporal and spatial patterns of drifting and explore both within and between aggregation levels of drifting. I then tested whether drifting in *P. canadensis* results from random/accidental events, or rather from structured interactions between nests by investigating variation in intensity (number of events between nests) and consistency over time of nest interactions. Further, I investigated factors influencing fitness payoffs, such as relatedness, proximity and nest-level need for help, which are likely to explain drifting patterns in *P. canadensis*. Lastly, I tested whether changing the level of colony need (obtained from experimental removal of workers and brood) affect drifting patterns across the aggregation, with drifting individuals apportioning their help (number of visits) accordingly.

General approach and methodology

Along the thesis, I investigated the complex organizational system of these primitively eusocial insects, using a multi-disciplinary approach. I used methods such as population genetics analysis, experimental approach using manipulations such as individual and brood removal and social network analysis.

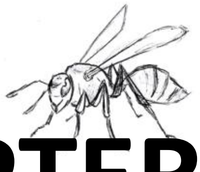
All observations and experiments were performed in natural populations of *P. canadensis* during 2 fieldwork seasons (2009 and 2010), in areas along the canal of the Republic of Panama where the species is common and can be easily manipulated in-situ. Wasps were mostly monitored using automated RFID equipment (see description box below) which record in real time and continuously the movement of wasps (Streit *et al*. 2003; Sumner *et al*. 2007). This sophisticated method of data collection provides more accurate and complete data compared to traditional monitoring methods (manual censuses of paint-marked individuals).

Tagging: Radio frequency identification tags (passive RFID, 16 bit programming mode (GiS TS-Q5Bee Tags), 18mg (<1% body mass of a typical *P. canadensis* female), 6x2mm) encoded with unique 4-digit identification numbers are glued to the thorax of each collected wasp.

Reader and antenna set-up: Circular antennae (3cm diameter, GiS TS-A37) are attached in front of the nest and detect wasps passing by. Antennae have a tag detection zone of app. 3cm. Each antenna will be connected to a reader (GiS TS-R64) where the time, date and identification numbers of each wasp detected by the antenna will be stored. Antennae + readers are powered by 12V car battery.

Data storage: Readers are removed from the field every 1 or 2 days and connected to a lab computer to extract the data. Up to 1000 data points (wasp movements) per day per nest can be recorded by the readers.

Picture 1. Early post-emergence nest of *P. canadensis*. (Photo credit: Emily Bell)



CHAPTER I

Chapter I

Little effect of seasonal constraints on population genetic structure in eusocial paper wasps

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Paper published in *Ecology and Evolution*

Lengronne T, Leadbeater E, Patalano S, Dreier S, Field J, Sumner S, and L Keller. 2012. Little effect of seasonal constraints on population genetic structure in eusocial paper wasps. *Ecology and Evolution* **2**:2615-2624

ABSTRACT

Climate has long been suggested to affect population genetic structures of eusocial insect societies. For instance, Hamilton (1964b) discusses whether temperate and tropical eusocial insects may show differences in population-level genetic structure and viscosity, and how this might relate to differences in the degree of synchrony in their life cycles or modes of nest founding. Despite the importance of Hamilton's 1964 papers, this specific idea has not been tested in actual populations of wasps, probably due to the paucity of studies on tropical species. Here, we compare colony and population genetic structures in two species of primitively eusocial paper wasps with contrasting ecologies: the tropical species *Polistes canadensis* and the temperate species *P. dominulus*. Our results provide important clarifications of Hamilton's discussion. Specifically, we show that the genetic structures of the temperate and tropical species were very similar, indicating that seasonality does not greatly affect population viscosity or inbreeding. For both species, the high genetic differentiation between nests suggests strong selection at the nest level to live with relatives, whereas low population viscosity and low genetic differentiation between nest aggregations might reflect balancing selection to disperse, avoiding competition with relatives. Overall, our study suggests no prevalence of seasonal constraints of the life cycle in affecting the population genetic structure of eusocial paper wasps. These conclusions are likely to apply also to other primitively eusocial insects, such as halictine bees. They also highlight how selection for a kin structure that promotes altruism can override potential effects of ecology in eusocial insects.

INTRODUCTION

The general theoretical framework for the evolution of eusociality is provided by the concept of inclusive fitness theory (Hamilton 1964a; Hamilton 1964b) which states that individuals can pass on their genes to the next generation not only through their own reproduction, but also indirectly through reproduction by relatives. The key components expected to influence the evolution of reproductive altruism are the relatedness between interacting individuals, and the impact of ecology of this. Differences in relatedness may arise either from individuals being close relatives (e.g., siblings) or population structuring with individuals being genetically more similar to individuals in their deme than individuals in other demes. Colony and population-level genetic structure may also be influenced by ecology. In his seminal paper, Hamilton discussed how the potential interactions of population structure, ecology (specifically, differences in seasonality of temperate and tropical climes) and life-history traits may influence the kin structure required for altruism. Yet, these factors remain little studied as comparative data on closely related species with contrasting ecologies (particularly tropical species) are limited.

In social insects, partitioning of genetic variance is generally organized at the level of the colony. However, life history traits also influence higher-level structuring between colonies, from a local scale (e.g. sub-populations) to larger scale population level structuring. For example, in many species, there is limited dispersal by one of the sexes, which leads to isolation by distance with greater genetic similarity among individuals in colonies close together than in colonies further apart. In ants, where most studies of genetic structure in social insects have been performed, there appears to be a strong association between certain life history traits such as the number of breeders per colony or mode of dispersal, and the degree of genetic differentiation among colonies (Pamilo and Rosengren 1984; Seppä and Pamilo 1995; Ross and Keller 1995; Ross *et al*. 1997). In species with a single reproductive queen per colony (monogyny), young queens typically depart on a mating flight and initiate a new colony on their own after mating. By contrast, in species containing several queens per colony (polygyny), the young queens frequently return to an established colony after mating (Hölldobler and Wilson 1990; Keller 1991). In these species new colonies are frequently initiated by budding, a process whereby queens leave their parental nest with workers to initiate a new colony nearby (short-ranged dispersal). Several studies have revealed that limited dispersal of queens in polygynous species is frequently associated with significant population viscosity (Pamilo and Rosengren 1984; Seppä and Pamilo 1995; Chapuisat and Keller 1999; Giraud *et al*. 2000; Liautard and Keller 2001; Ruppell *et al*. 2003; Zhu *et al*. 2003; Fournier *et al*. 2004; Zinck *et al*. 2007; Seppä *et al*. 2009; Rees *et al*. 2010). By contrast, the long-range dispersal of females in monogynous species usually leads to no significant population viscosity (Chapuisat *et al*. 1997).

In contrast to ants, we know little about how different modes of dispersal and colony founding influence population structure in eusocial wasps. But the potential impact of ecology/climate may be greater in wasps, because they build short-lived, annual colonies rather than long-lived perennial ones. Among the eusocial *Polistinae* wasps, modes of dispersal and colony founding may influence the breeding system (Pamilo *et al*. 1997). Independent founding species (e.g. *Polistes*) are mostly monogynous (Reeve 1991), whereas swarm-founding species, which form new nests by colony fission (e.g. *Polybia*), are usually highly polygynous (Jeanne 1991; Pamilo *et al*. 1997). But dispersal and modes of colony founding might also be associated with ecology, specifically climate (Reeve 1991; Ross and

Carpenter 1991). Hamilton discussed how the interaction of climate (seasonality), life-history traits and population structure might affect the conditions under which altruism can evolve in *Polistes* wasps, where temperate species tend to initiate new colonies without workers, whereas tropical species are more likely to initiate colonies “by swarms” (Hamilton 1964b; West-Eberhard 1969).

Here we provide the first attempt to address this specific discussion by Hamilton. We aimed to compare at a microscale the population genetic structure of *P. dominulus* and *P. canadensis*. These two species of primitively eusocial wasps share many social traits in having a single egg-laying queen and high within colony relatedness, but they differ in their modes of colony founding and colony synchrony. *P. dominulus* is native to Europe, Asia and North Africa (Judd and Carpenter 1996), where its colony cycle is constrained by the seasonality characterizing a temperate climate. Newly, singly mated gynes hibernate in communal shelters of sometimes hundreds of individuals to overcome the harsh winter conditions (Reeve 1991; Dapporto *et al*. 2004). In the spring, the overwintered gynes then disperse and associate with a small number of other females to found new colonies (West-Eberhard 1969; Dapporto *et al*. 2004). It has been suggested that *P. dominulus* reproductives tend to be philopatric (Starks 2003; Dapporto *et al*. 2004), and indeed Hamilton suggested that there would be little effect of diapause on the relatedness of temperate co-foundresses, because they are often observed returning to their natal colony site before co-founding. However, recent genetic studies have revealed considerable variation in colony kin structure among *P. dominulus* co-foundings, with associations containing both related and unrelated females (Queller *et al*. 2000; Zanette and Field 2008; Leadbeater *et al*. 2010). These individuals sometimes lay eggs (Leadbeater *et al*. 2011), which should influence the degree of within-nest relatedness, as documented in several ant species with perennial colonies (Chapuisat *et al*. 1997; Liautard and Keller 2001; Zinck *et al*. 2007).

In contrast, nests founded by the tropical species *P. canadensis* in Panamá generally comprise large associations of females (Pickering 1980), and nest founding takes place throughout the year without a seasonally-enforced diapause (Giray *et al*. 2005). This lack of seasonality may induce asynchronous male production which may lead to some inbreeding because virgin females may be forced to mate with related males from the colony if colonies are relatively isolated, or if no other males are available from neighbouring nests. Colony-level relatedness appears to be similarly high as *P. dominulus* (Sumner *et al*. 2007), and observations suggest that this probably stems from groups of sisters initiating new colonies close to the parental nests, not dissimilar to nest founding by budding in ants (West-Eberhard 1969; Pickering 1980). These life-history traits may lead to some population viscosity and genetic structuring at a relatively small scale. But, in contrast to temperate *Polistes,* there is currently very little genetic data on colony and population structure in tropical *Polistes* (Sumner *et al*. 2007). Thus, it is not yet clear to what extent (if any) these differences in ecology and life-history traits influence population structure and hence the conditions for altruism.

To test the hypothesis that differences in the mode of colony founding and colony synchrony between tropical and temperate colonies should affect the genetic structure of wasp populations, we therefore carried out a comprehensive analysis of genetic structure of both *P. canadensis* and *P. dominulus* populations.

METHODS

Sampled populations

P. canadensis

In July 2009, twenty-six mature, post-emergence (after the emergence of the first batch of workers) colonies were collected from a large natural population on abandoned buildings of over 200 nests located in Punta Galeta, Colón, Republic of Panamá (9°24'08.28''N, 79°52'19.41''W, under ANAM permit #SE/A-33-99, Fig. 1a). The population was subdivided into four aggregations (A1, A2, A3 and A4) in a series of abandoned buildings, each composed of 6 to 10 nests. Within aggregations, nests were separated by 40.1cm to 681.4cm (mean±S.D., 204.2±176.3cm). Aggregations were separated from each other by approximately 25m (A1-A2) to 830m (A3-A4). All females were individually marked and monitored in order to determine the queen’s identity and exclude the possibility of queen turnover. All collected individuals were stored in 95% ethanol for later DNA analyses.

P. dominulus

Twenty-six randomly selected nests were sampled from a large population of over 200 nests in March 2008 around Conil de la Frontera, Cádiz Province, Spain (36°15'10.76''N, 6°03'56.48''W, Fig. 1b). All colonies were on hedges of *Opuntia* cacti. The population was subdivided into three aggregations (B1, B2 and B3), in a series of cacti banks, each containing from seven to ten nests. The distance between nests within aggregations ranged from 43.0cm to 1047.9cm (mean±S.D., 323.1±247.7cm). The mean distance between aggregations (measured from the centre point of each aggregation) varied from 80 to 170m. All females were individually marked and monitored in order to determine the queen’s identity and exclude the possibility of queen turnover. All brood and adults were stored in 95% ethanol.

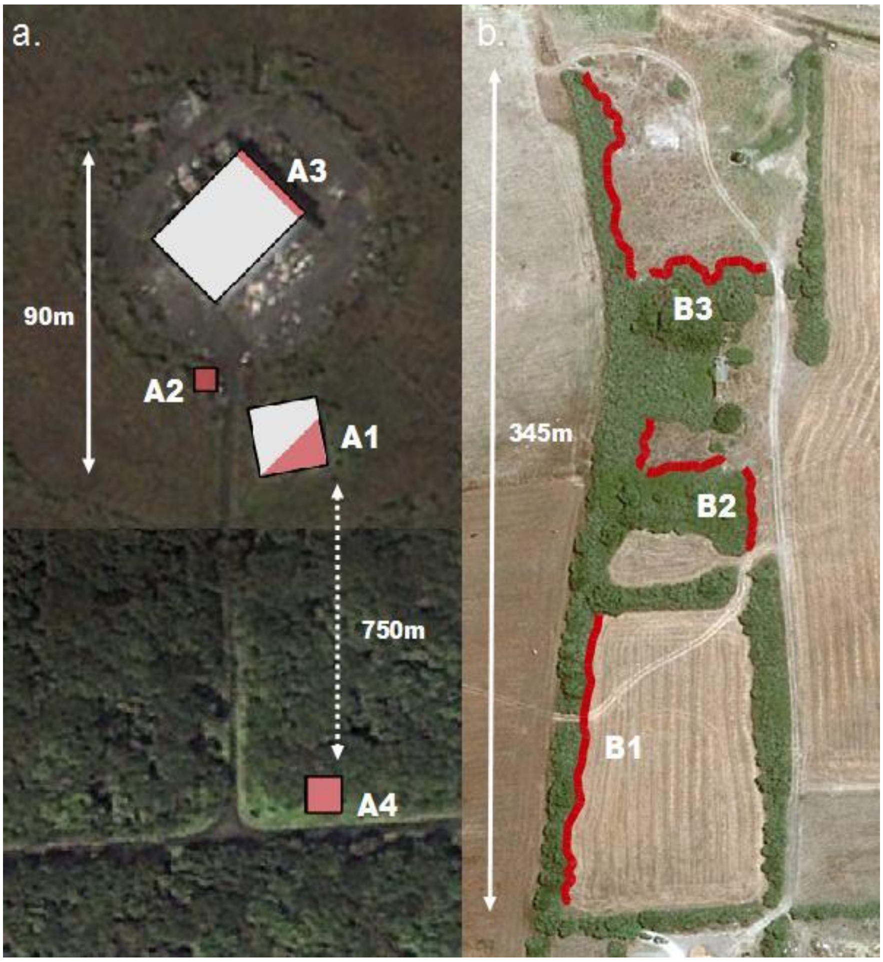


Fig. 1. (a) Map of the sampling sites of *P. canadensis* located in Punta Galeta, Colón, Republic of Panamá (9°24'08.28''N, 79°52'19.41''W). Each rectangle or square correspond to an aggregation. The areas shaded in red represent the distribution of studied nests within the aggregations (A1, A2, A3 and A4). Between A1 and A4, the entire surface area is not shown (for distances see dotted arrow) (b) Map of the sampling sites of *P. dominulus* located around Conil de la Frontera, Cádiz Province, Spain (36°15'10.76''N, 6°03'56.48''W). The red «lines» represent the distribution of studied nests along the hedges of *Opuntia* cacti. Adjacent «lines» forms each of the three aggregations (B1, B2 and B3) (images from Google Earth)

Molecular Methods

Development of markers for P. canadensis

Previous genetic analyses on *P. canadensis* were performed using non-specific markers developed from various related species of *Polistes* (*P. annularis, P. bellicosus,* Strassmann *et al*. 1997, see Sumner *et al*. 2007). To increase the accuracy of our relatedness and genetic differentiation estimates, we developed species-specific microsatellites markers for *P. canadensis*. Seven polymorphic microsatellite loci were isolated and used in the present study (Table 1). The first steps of microsatellite primers development, from DNA extraction to sequencing, were conducted by Ecogenics GmbH (Zurich, Switzerland) based on specimens collected in 2008 in the area of the Panamá City, Republic of Panamá (8°54’17.42”N, 79°34’35.41”W)(under ANAM permit #SE/A-53-08). DNA extractions were performed using one leg per individuals from each of 30 different nests. An enriched library was developed from size selected genomic DNA ligated into SNX forward/SNX reverse-linker (Hamilton *et al*. 1999) and enriched by magnetic bead selection with biotin-labelled $(CT)_{13}$, $(GT)_{13}$, $(AAC)_{10}$ and $(AAG)_{10}$ oligonucleotide repeats (Gautschi *et al*. 2000a; b). Of 528 recombinant colonies screened, 367 gave a positive signal after hybridization. Plasmids from 36 positive clones were sequenced. Primers were designed from 22 inserts using the PRIMER SELECT module in the DNAStar computer program from Lasergene (DNASTAR Inc., Madison, WI, USA), optimized and tested for polymorphism. From the initial 22 designed primers, we then selected 7 highly polymorphic loci (number of alleles > 5) that yield clear amplification products (Table 1).

Genotyping methods

P. canadensis

Because high levels of nests drifting by workers has been documented in the species (Sumner *et al*. 2007), some workers may not be individuals originating from the nest but simply visitors from other colonies. This would potentially reduce colony-level relatedness, if we inadvertently sampled drifters from other colonies. Therefore, our relatedness estimates were based on the genotypes of late stage larvae and pupae. DNA was extracted from 5 pupae or large larvae (only females) from each of the 26 nests. Extraction and purification were carried out with BioSprint96 (Qiagen) using the “Animal Tissue DNA Purification” kit and DNA analysed at the 7 polymorphic loci (Table 1). In total, 129 individuals were genotyped. PCR (polymerase chain reaction) amplifications were performed separately for each locus in 20µL final volume containing 2µL of DNA extract, 2µL of (Qiagen) PCR buffer x10, 2µL of (Qiagen) Q-solution, 0.24µL of dNTPs (2mM), 11.56µL of milli-Q water, 0.2µL of *Taq* polymerase (Qiagen) and 1µL of each *forward* (fluorescently labelled) and *reverse* primers (each 10µM). Amplifications were conducted in a GeneAmp PCR System 9700 (Applied Biosystems). The PCR mix was denatured at 95°C for 5min and cycled 25 to 35 times, depending on the locus, at 95°C for 30sec, at primers annealing temperature (55-60°C) for 30sec and elongated at 72°C for 30sec. A final elongation step at 72°C for 10min followed to complete extension of PCR fragments. Labelled PCR products were analysed on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems) and allele sizes estimated using GENEMAPPER software (Applied Biosystems). Individuals that showed heterozygous alleles for at least one locus were considered as females; individuals that did not fit this criterion were considered as males and excluded from the analysis.

P. dominulus

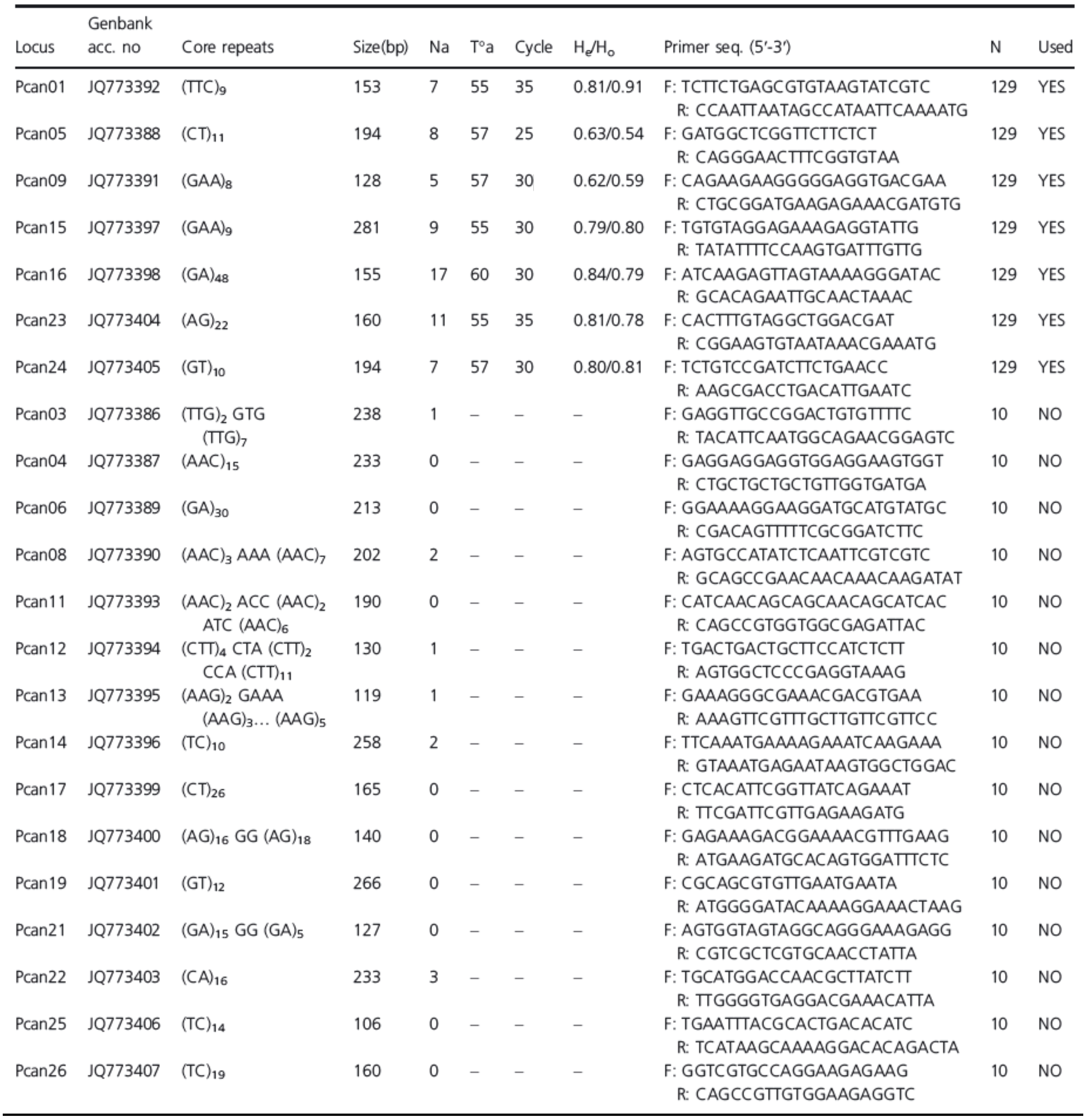
DNA was extracted from 5 pupae or large larvae (only females) from each of the 26 nests. We used a selection of the primer sets previously isolated from *P. dominulus* (Pdom1jc, Pdom2jc, Pdom7, Pdom20, Pdom25jc, Pdom122jc, Pdom127b, Pdom140) (Henshaw 2000; Leadbeater *et al*. 2010) and *P. bellicosus* (Pbe128TAG) (Strassmann *et al*. 1997). In total, 129 individuals were genotyped. Multiplex PCR were carried out on a Peltier Thermal Cycler. Amplifications in a single multiplex mix were performed in 4µL, containing approximately

80ng of template DNA, 0.75µM of the four primer pairs (Pdom1jc, Pdom2jc, Pdom20, Pbe128TAG), 0.375µM of the remaining five primer pairs (Pdom7, Pdom25jc, Pdom122jc, Pdom127b, Pdom140) and 2 µL of PEQlab hot start mix Y (details in Leadbeater *et al.* 2010). A droplet of mineral oil was added to prevent evaporation. Multiplex mix was denatured at 95°C for 15 minutes and cycled 35 times at 94°C for 30 sec, 57°C (annealing temperature) for 90sec and 72°C for 60 sec. Final extension was performed at 60°C for 30 minutes. PCR products were separated by size using a 48-well capillary ABI 3730 sequencer (Applied Biosystems) and visualized using GENEMAPPER software (Applied Biosystems). For further genotyping information see Leadbeater *et al.* 2010.

Genetic analyses

Population genetic structure

Expected and observed heterozygosities were estimated using the program GDA 1.0 (Genetic Data Analysis, Lewis and Zaykin 2001). We tested linkage disequilibrium between pairs of loci and deviations from Hardy-Weinberg equilibrium randomizing 10000 times alleles among individuals within nests using the software GENEPOP 4.0.10 (Raymond and Rousset 1995). To account for the non-independence of nestmate genotypes, a resampling procedure providing unbiased estimates was performed for calculations of deviations from Hardy-Weinberg equilibrium and linkage disequilibrium. A R script was written to randomly select a single individual’s multi-locus genotype from each nest and create 1000 distributions of independent genotypes. Iterations for each test, using the resampled genotype distributions, were performed using the “batch mode” option, available in the command line version of the GENEPOP software.



Locus	Genbank acc. no	Core repeats	Size(bp)	Na	T°a	Cycle	H_e/H_o	Primer seq. (5′-3′)	N	Used
Pcan01	JQ773392	$(TTC)_9$	153	7	55	35	0.81/0.91	F: TCTTCTGAGCGTGTAAGTATCGTC R: CCAATTAATAGCCATAATTCAAAATG	129	YES
Pcan05	JQ773388	$(CT)_{11}$	194	8	57	25	0.63/0.54	F: GATGGCTCGGTTCTTCTCT R: CAGGGAACTTTCGGTGTAA	129	YES
Pcan09	JQ773391	$(GAA)_8$	128	5	57	30	0.62/0.59	F: CAGAAGAAGGGGGAGGTGACGAA R: CTGCGGATGAAGAGAAACGATGTG	129	YES
Pcan15	JQ773397	$(GAA)_9$	281	9	55	30	0.79/0.80	F: TGTGTAGGAGAAAGAGGTATTG R: TATATTTTCCAAGTGATTTGTTG	129	YES
Pcan16	JQ773398	$(GA)_{48}$	155	17	60	30	0.84/0.79	F: ATCAAGAGTTAGTAAAAGGGATAC R: GCACAGAATTGCAACTAAAC	129	YES
Pcan23	JQ773404	$(AG)_{22}$	160	11	55	35	0.81/0.78	F: CACTTTGTAGGCTGGACGAT R: CGGAAGTGTAATAAACGAAATG	129	YES
Pcan24	JQ773405	$(GT)_{10}$	194	7	57	30	0.80/0.81	F: TCTGTCCGATCTTCTGAACC R: AAGCGACCTGACATTGAATC	129	YES
Pcan03	JQ773386	$(TTG)_2$ GTG $(TTG)_7$	238	1	–	–	–	F: GAGGTTGCCGGACTGTGTTTTC R: TACATTCAATGGCAGAACGGAGTC	10	NO
Pcan04	JQ773387	$(AAC)_{15}$	233	0	–	–	–	F: GAGGAGGAGGTGGAGGAAGTGGT R: CTGCTGCTGCTGTTGGTGATGA	10	NO
Pcan06	JQ773389	$(GA)_{30}$	213	0	–	–	–	F: GGAAAAGGAAGGATGCATGTATGC R: CGACAGTTTTTCGCGGATCTTC	10	NO
Pcan08	JQ773390	$(AAC)_3$ AAA $(AAC)_7$	202	2	–	–	–	F: AGTGCCATATCTCAATTCGTCGTC R: GCAGCCGAACAACAAACAAGATAT	10	NO
Pcan11	JQ773393	$(AAC)_2$ ACC $(AAC)_2$ ATC $(AAC)_6$	190	0	–	–	–	F: CATCAACAGCAGCAACAGCATCAC R: CAGCCGTGGTGGCGAGATTAC	10	NO
Pcan12	JQ773394	$(CTT)_4$ CTA $(CTT)_2$ CCA $(CTT)_{11}$	130	1	–	–	–	F: TGACTGACTGCTTCCATCTCTT R: AGTGGCTCCCGAGGTAAAG	10	NO
Pcan13	JQ773395	$(AAG)_2$ GAAA $(AAG)_3$… $(AAG)_5$	119	1	–	–	–	F: GAAAGGGCGAAACGACGTGAA R: AAAGTTCGTTTGCTTGTTCGTTCC	10	NO
Pcan14	JQ773396	$(TC)_{10}$	258	2	–	–	–	F: TTCAAATGAAAAGAAATCAAGAAA R: GTAAATGAGAATAAGTGGCTGGAC	10	NO
Pcan17	JQ773399	$(CT)_{26}$	165	0	–	–	–	F: CTCACATTCGGTTATCAGAAAT R: TTCGATTCGTTGAGAAGATG	10	NO
Pcan18	JQ773400	$(AG)_{16}$ GG $(AG)_{18}$	140	0	–	–	–	F: GAGAAAGACGGAAAACGTTTGAAG R: ATGAAGATGCACAGTGGATTTCTC	10	NO
Pcan19	JQ773401	$(GT)_{12}$	266	0	–	–	–	F: CGCAGCGTGTTGAATGAATA R: ATGGGGATACAAAAGGAAACTAAG	10	NO
Pcan21	JQ773402	$(GA)_{15}$ GG $(GA)_5$	127	0	–	–	–	F: AGTGGTAGTAGGCAGGGAAAGAGG R: CGTCGCTCGTGCAACCTATTA	10	NO
Pcan22	JQ773403	$(CA)_{16}$	233	3	–	–	–	F: TGCATGGACCAACGCTTATCTT R: TTGGGGTGAGGACGAAACATTA	10	NO
Pcan25	JQ773406	$(TC)_{14}$	106	0	–	–	–	F: TGAATTTACGCACTGACACATC R: TCATAAGCAAAAGGACACAGACTA	10	NO
Pcan26	JQ773407	$(TC)_{19}$	160	0	–	–	–	F: GGTCGTGCCAGGAAGAGAAG R: CAGCCGTTGTGGAAGAGGTC	10	NO

Table 1. Characterization of seven polymorphic loci in *Polistes canadensis*, including locus name and Genbank Accession no., repeat motifs (core repeats), size of cloned allele, optimal annealing temperature (Ta), optimal number of cycles and primer sequences. The number of alleles (Na) and observed and expected heterozygosities (He/Ho) are reported for a pooled sample of 129 individuals. The last column indicates whether the primer was used for the present genetic analysis. When Na = 0, no clear amplification were obtained after several runs

Genetic differentiation was quantified for the different levels of biological organisation (individual, nest, aggregation) by conducting a three-level hierarchical F-analysis. We measured Wright's hierarchical F-statistics, using Weir and Cockerham's (1984) method implemented in GDA 1.0. Five thousand bootstrap procedures were performed to give 95% confidence intervals. We also used ARLEQUIN (Excoffier *et al*. 2010) to provide significance for the F-statistics using the non-parametric permutation procedures implemented in the software. In the analyses, nests ($_{\text{NEST}}$) and aggregations ($_{\text{AGG}}$) were considered as two different levels of sub-populations and all sampled individuals as total populations ($_{\text{TOTAL}}$). We estimated F-statistics at different levels: $F_{\text{NEST-AGG}}$ (estimates of genetic differentiation between nests within aggregations), $F_{\text{AGG-TOTAL}}$ (estimates of genetic differentiation between aggregations), $F_{\text{IND-TOTAL}}$ (inbreeding coefficient of individuals relative to the total population).

Isolation by distance

Genetic differentiation ($F_{ST} = F_{\text{NEST-TOTAL}}$) between all pairs of nests was estimated using the software FSTAT 2.9.4 (Goudet 1995). To investigate patterns of isolation by distance, we plotted the transformed genetic distance formula $F_{ST}/(1-F_{ST})$ against the natural logarithm of geographical distances, as proposed by Rousset (1997). Significance of the correlation between genetic and geographical distances was assessed with a Mantel test, implemented in FSTAT 2.9.4 (10000 permutations). We also examined isolation by distance within each aggregation using all nests in the aggregations as the total population for the calculations of pairwise F_{ST}.

Colony genetic structure

Genetic relatedness (r) was also calculated within nests and within aggregations for the two populations of *Polistes* species using Queller & Goodnight's method (1989) which is based on Grafen's (1985) relatedness coefficient. Calculations were performed using the program

RELATEDNESS 5.0.8 by weighting nest equally. Standard errors were estimated by jackknifing over loci.

RESULTS

The number of alleles per locus in *P. canadensis* ranged from 5 (Pcan09) to 17 alleles (Pcan16) with a mean of 8.7 alleles (Table 1). The expected heterozygosities ranged from 0.63 to 0.84 (H_e, all loci: 0.76) while the observed heterozygosities ranged from 0.54 to 0.91 (H_o, all loci: 0.75). In *P. dominulus*, the number of alleles ranged between 5 (25jc) and 36 (Pdom122jc) with a mean of 13.8 alleles. The observed heterozygosities ranged from 0.53 to 0.99 (H_o, all loci: 0.77) and the expected heterozygosities from 0.63 to 0.96 (H_e, all loci: 0.79).

No significant departure from Hardy-Weinberg equilibrium was found in either *P. canadensis* or *P. dominulus* populations (all population, all loci: P>0.05 for all 1000 resampled distributions). Additionally, for both species, we found no evidence of linkage disequilibrium between any pair of loci within populations using Fisher's exact test in GENEPOP. In *P. canadensis*, linkage disequilibrium was detected across 4 pairs of loci (over 21) in only 6% of the 1000 resampled dataset (max: 3.2% between Pcan16 and Pcan24). In *P. dominulus*, 8 pairs of loci (over 36) showed significant linkage disequilibrium in only 8.4% of the 1000 resampled dataset (max: 4% between Pdom7 and Pdom140). All loci were then considered behaving as neutral markers and were kept for the analyses of genetic structure.

Population genetic structure

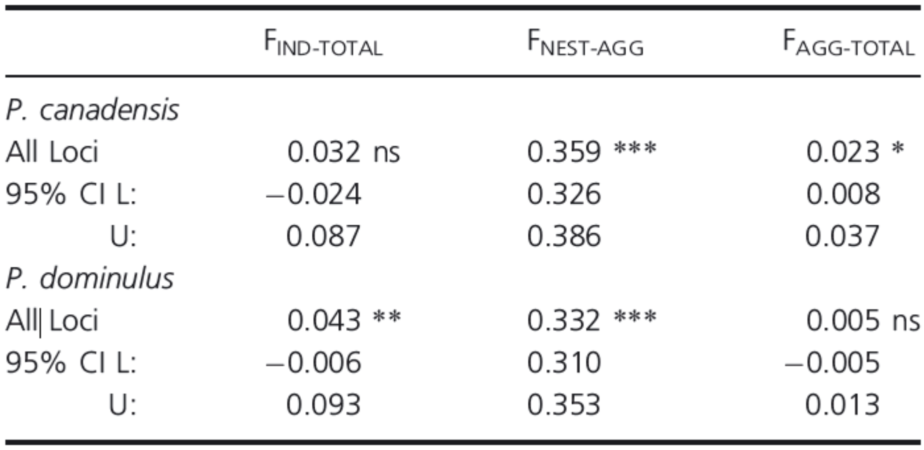
The hierarchical analysis of population structure revealed high genetic differentiation between nests within *P. canadensis* aggregations ($F_{NEST-AGG}$ = 0.359, p<0.0001; L-95% CI:

0.326; U-95% CI: 0.386). A weaker but significant genetic differentiation was also found between aggregations ($F_{AGG\text{-}TOTAL}$ = 0.023, p=0.016, L-95% CI: 0.008; U-95% CI: 0.037). There was no evidence of inbreeding at the population level ($F_{IND\text{-}TOTAL}$ = 0.032, p=0.15; L-95% CI: -0.024; U-95% CI: 0.087), suggesting that mating occurs randomly (see Table 2).

In *P. dominulus*, the hierarchical analysis of population structure indicated a strong genetic differentiation between nests within aggregations ($F_{NEST\text{-}AGG}$ = 0.332, p<0.0001; L-95% CI: 0.310; U-95% CI: 0.353) but no significant genetic differentiation between aggregations ($F_{AGG\text{-}TOTAL}$ = 0.005, p=0.22; L-95% CI: -0.005; U-95% CI: 0.013). The value $F_{IND\text{-}TOTAL}$ was low but significantly greater from zero (0.043, p=0.008; L-95% CI: -0.006; U-95% CI: 0.093) indicating a low level of inbreeding (see Table 2).

Pairwise comparisons between nests showed no significant isolation by distance in *P. canadensis* when nests of the four aggregations were considered simultaneously (Mantel tests, r=0.157; p=0.11; 10000 permutations). There was, however, a significant isolation by distance in one of the four aggregations (aggregation A3, r=0.895; p=0.027). In the three other aggregations there was also a slight positive correlation but it was not significant (A1 r=0.011, p=0.94; A2, r=0.053, p=0.77; A4, r=0.272, p=0.45). Interestingly, aggregation A3 differed from the three others in that nests were located in a relatively open space, whereas nests were clustered inside buildings in the three other aggregations.

In the Spanish population of *P. dominulus* there was a low but significant isolation by distance when considering all nests of the three aggregations (r=0.131; p=0.024). An analysis of each aggregation separately, revealed significant isolation by distance in B2 r=0.330, p=0.014, and B3 (r=0.332, p=0.027), but not in B1 (r=0.009; p=0.97). Although we found only significant isolation by distance in *P. dominulus*, estimations of isolation by distance at both the population level and for each aggregation were relatively low and did not seem to fundamentally differ between the two species.



	$F_{IND-TOTAL}$	$F_{NEST-AGG}$	$F_{AGG-TOTAL}$
P. canadensis			
All Loci	0.032 ns	0.359 ***	0.023 *
95% CI L:	−0.024	0.326	0.008
U:	0.087	0.386	0.037
P. dominulus			
All Loci	0.043 **	0.332 ***	0.005 ns
95% CI L:	−0.006	0.310	−0.005
U:	0.093	0.353	0.013

Table 2. Estimates of inbreeding, genetic differentiation with confidence intervals in *P. canadensis* and *P. dominulus,* and t-test significance between species. Asterisks represent significance of randomization tests performed in Arlequin (*** p<0.001, ** p<0.01, * p<0.05, ns = non-significant).

The mean within-nest relatedness was high (and not significantly different) for both species (*P. canadensis*: r=0.69±0.02; *P. dominulus*: r=0.64±0.01, two-tailed t-test: p>0.05) and close to the theoretical relatedness values between haplodiploid full-sisters (r=0.75). Genetic relatedness at the aggregation level were significantly greater than zero in both species, and higher in *P. canadensis* (r=0.12±0.01) than *P. dominulus* (r=0.07±0.01, two-tailed t-test: p=0.006). This suggests a higher degree of population structuring in *P. canadensis* relative to *P. dominulus*, which parallels the conclusion from our complementary F-statistics analyses.

DISCUSSION

The result of our genetic study provides no support for Hamilton’s statement that the genetic structuring of populations may differ between tropical and temperate areas, at least for the studied species. Overall, there were only small differences in the population genetic structure of *P. canadensis* and *P. dominulus.*

At the colony level, there was a high relatedness among female offspring in both species (*P. canadensis*: r=0.69±0.02; *P. dominulus*: r=0.64±0.01), suggesting that most colonies are headed by one singly-mated queen. These results are consistent with previous studies performed on *P. canadensis* and *P. dominulus* which showed, through observations or genetic analyses, an almost exclusive monopoly of the reproduction by the queen (*P. canadensis* (West-Eberhard 1969; S.S unpublished data) and *P. dominulus* (Zacchi 1998; Queller *et al*. 2000; Zanette and Field 2008). Moreover, contrary to Hamilton's predictions, our colony genetic structure analysis revealed no pronounced level of inbreeding in the tropical species *P. canadensis*. Indeed, we found some evidence of the opposite, with a low level of inbreeding in the temperate species *P. dominulus*.

Our study also revealed only limited population structuring at levels higher than the nest in both species. In *P. canadensis*, the level of genetic differentiation between aggregations was very small ($F_{AGG\text{-}TOTAL}$ = 0.023) while in *P. dominulus* no significant differentiation was detected. In line with these findings there was also a very limited isolation by distance in both species.

In *P. canadensis*, we found no isolation by distance at the level of the population, or in three of the four aggregations studied. Interestingly, however, there was a significant structuring within one aggregation (A3: r=0.895). In contrast to the three other associations, which were located (at least partially) within buildings, the aggregation A3 was in a more open area (overhangs of building). This suggests that topography of the nesting sites may influence the distribution of nests and the dispersal behaviour of individuals. It would be of interest to compare populations of *P. canadensis* within buildings and populations in natural habitat (e.g., trees, open areas) to investigate whether patterns of dispersal are generally influenced by differences in topography.

These results of population structure found in *P. canadensis* are particularly interesting with regard to a recent study which revealed high levels of nest-drifting between closely located nests in a Panamanian population of *P. canadensis* (Sumner *et al*. 2007). This study suggested that visiting individuals may gain indirect fitness benefits by helping raise the brood in closely located nests if nests are genetically similar (viscous population). This hypothesis would seem unlikely in light of the finding of low population structuring and absence of isolation by distance in that species. Although no isolation by distance was detected in the *P. canadensis* population, nests within aggregations seem on average related (r=0.12±0.01) which suggest that, even in non-viscous populations, drifters may still be able to benefit from indirect fitness by delivering help in neighbouring nests. However, it is also possible that isolation by distance was present in past populations of *P. canadensis* and helped promoting the evolution of nest-drifting but disappeared over time due to the availability of human structures which have altered their dynamics of dispersal.

Intriguingly, similar levels of indirect fitness could theoretically be achieved by nest-drifting in *P. dominulus,* where there is a similar level of population structuring to *P. canadensis.* Nest-drifting is difficult to detect using traditional manual censusing methods, as is currently practiced on *P. dominulus.* It is possible that finer-level monitoring (e.g. using radio-frequency identification tags) would in fact detect nest drifting by workers for indirect fitness benefits in *P. dominulus,* if this behaviour is a general phenomenon.

Unlike *P. canadensis*, there was a slight isolation by distance at the population level (r=0.13) as well as population viscosity in two out of the three aggregations in *P. dominulus*. This suggests limited dispersal in these species, with a significant fraction of females initiating new nests in the vicinity of their parental nest.

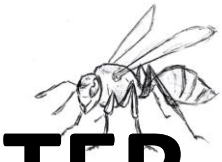
These results on colony and population genetic structure found in *P. canadensis* and *P. dominulus* were determined from data collected from small-scale populations (<1km between aggregations). Significant micro-structuring has been reported in *P. exclamans,* another species of primitively eusocial wasps (Davis *et al*. 1990). In this temperate species, high levels of genetic structure were detected. By contrast, there was no evidence of genetic structuring at a micro-geographic scale in *P. bellicosus, P. carolinus and P. metricus*, which are sympatric to *P. exclamans* (Davis *et al*. 1990).

The use of a small scale in our study was primarily due to the limited number of wasp aggregations for both *P. canadensis* and *P. dominulus*. Additional studies on populations at larger scale as well as in other tropical and temperate species are necessary to unambiguously reject the hypothesis of a difference in population structure between temperate and tropical species. Further investigations on population structure in social insects from different climates may also benefit by examining species living in both tropical and temperate areas. *P. dominulus* may be a suitable model for such comparison as its distribution is widespread across the world. Comparing genetic structure of subtropical populations (with no clear seasonality), into which the species range appears to be expanding naturally (Cervo *et al*. 2000), to native European populations may prove valuable for investigating the potential behavioural and genetic differences. However, one should be cautious because *P. dominulus* has been largely introduced in several countries such as the US, Australia and Chile (Judd and Carpenter 1996). Invasive populations may not reflect the same natural sets of genetic and behavioural features found in native populations, especially because of potential genetic bottlenecks, induced by independent introductions, which may lower the frequency distribution of alleles in the population (Luikart 1998) and induce changes in the population genetic composition. So far, most genetic studies of populations of *P. dominulus* introduced in the US suggest no severe bottleneck with populations having high genetic variability (Johnson and Starks 2004; Liebert *et al*. 2006). However, other populations in the US have already showed behavioural modifications to nest-founding (Liebert *et al*. 2006) as well as the presence of diploid males and triploid females (Liebert *et al*. 2005).

Overall, this study found genetic structure between temperate and tropical species of paper wasps to be far more similar than expected considering the contrasts in life cycles and nest founding behaviour of *P. canadensis* and *P. dominulus*. Strong selection to nest with relatives was revealed by high structuring at the nest level. However, we found low population viscosity and low differentiation between aggregations in both species. This might reflect the effects of balancing selection in promoting emigration outside the natal aggregations and hence avoiding competition between close relatives (Hamilton and May 1977; Taylor 1988; Taylor 1992). Such small differences in population structure between the two species with contrasting ecology indicates that differences in climate with seasonal constraint on the life cycle may not to be such a fundamental factor affecting the genetic structure of populations. Thus, we provide some resolution to the discussions raised by Hamilton on the potential connections between modes of nest founding, seasonality and population structure in his seminal paper on inclusive fitness theory (Hamilton 1964b). Future studies and comparisons with more tropical species are required to further confirm these insights, and to gain a better understanding of the impacts of contrasting life-cycles and seasonality on the population genetic structure of insect societies.

AKNOWLEDGMENTS

We thank Jon Carruthers for technical support with genotyping *P. dominulus* samples. Primer design and genotyping for *P. dominulus* samples were carried out at the NERC Biomolecular Analysis Facility, Sheffield. We are grateful to the Social Evolution group from the Institute of Zoology (ZSL) and Keller, Chapuisat groups (UNIL) for helpful discussions and advices on the analyses and manuscript. We would also like to thank anonymous reviewers for their valuable comments and suggestions to improve the manuscript. This study was supported by grants from the Swiss National Science Foundation (to TL and LK) and NERC grant (NE/G000638/1) to SS.



CHAPTER II

Chapter II

Lazy workers assure fitness in tropical paper wasps

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Paper in preparation for submission in *Nature Communications*

Lengronne T, Patalano S, Dreier S, Keller L and S Sumner. 2013. Lazy workers assure fitness in tropical paper wasps. *In preparation*

ABSTRACT

Insect societies are characterised by the efficiency of their organisation of work. Workers contribute to colony cohesion by performing most of the tasks, allocating help according to changes in social and environmental conditions. However some worker behaviours with no apparent adaptive value remain unexplained. In the large colonied species of tropical paper wasp, *Polistes canadensis*, 25% of workers appear to be ‘lazy’, neither provisioning nor caring for their nestmate brood. Moreover, around 50% of workers drift to non-natal colonies, sharing their help between two or more nests. To understand the possible adaptive significance of lazy workers and drifting workers we manipulated the need for help by removing 30% of the hard-working foragers from each of 22 nests. We monitored subsequent changes in brood survival, and quantified the changes in foraging efforts of nestmate workers and non-nestmate drifters using a combination of manual and automated (radio tagging) monitoring. Lazy workers appear to (fully) compensate for the loss in foraging effort in their nest through the initiation of foraging. Drifters from neighbouring related colonies, however, did not compensate for the loss in worker force, even though they stood to lose inclusive fitness should the brood die. These results provide evidence that apparent ‘lazy behaviour’ by social insects’ workers is adaptive, providing resilience to colonies in the event of worker mortality. Recruitment from apparently lazy nestmates is likely to be a common strategy for assuring fitness in the larger colonied primitively eusocial insects, who can afford such resilience.

INTRODUCTION

The high level of ecological success of insects has been largely attributed to the efficient division of labour of their societies and sophisticated mechanisms to coordinate behaviour within the nest (Wilson 1987). Extensive studies on many different species show that nests can adjust worker task allocation according to the colony/nest needs (Gordon 1986; Robinson 1992; Gordon 1996; Bonabeau *et al*. 1997; Brown and Traniello 1998; Gordon 1999; Fewell and Bertram 1999; Naug 2001). However, many studies found that a high proportion of nestmates remain inactive in the colony (Lindauer 1952; Herhers 1983; Winston and Fergusson 1985; Winston 1987; Dornhaus *et al*. 2008; Jandt and Dornhaus 2009). For example, 30 to 40% of the individuals in colonies of the honeybee *Apis mellifera* are not engaged in any useful work at any given time (Winston 1987; Anderson 2001)(Croft *et al*. 2008). Similarly, in the tropical wasp *Polistes canadensis* a quarter of the individuals do not forage or contribute much to brood care (Sumner unpublished data). The high rate of inactive 'lazy individuals' raises an important puzzle in our understanding of insect colonies (Schmid-Hempel 1990). It has been suggested that these inactive workers may represent a "reserve worker force" acting as a stand-by group of helpers that can engage in activity when unexpected contingencies arise (Lindauer 1961; Michener 1964; Schmid-Hempel 1990; Robinson 1992). Alternatively, the presence of inactive workers may be the outcome of a non-optimal system of task allocation (Dornhaus *et al*. 2008; Klein *et al*. 2008; Jandt *et al*. 2012). It is thought that each individual in a colony has a threshold for particular tasks and that the task performed by a given individual will depend on both its threshold and colony need (Bonabeau *et al*. 1996; Page and Mitchell 1998; Bonabeau 1998; Graham *et al*. 2006; Jeanson *et al*. 2007; Gove *et al*. 2009). On the basis of this model it is conceivable that some individuals would have consistently high thresholds for all tasks and thus remain inactive.

Such inactivity could either be adaptive if these individuals behave as a reserve worker force or be non-adaptive if they do not initiate work when the colony is in real need.

'Lazy' individuals in social insects appear therefore to be widely reported. Yet, it remains unclear why they are tolerated by the colony, and how this affects colony fitness (Jandt *et al*. 2013). Early studies on the honeybee *A. mellifera*, and the paper wasp, *P. metricus* suggested that young and less active workers may respond to changes in colony needs by increasing their activity levels in response to a loss of workers (Dew and Michener 1981; Winston and Fergusson 1985). In another study on the large colonies of the termite *Nasutitermes exitiosus*, groups of unmarked workers (attributed to no task) were recruited to help in response to colony disturbance (Evans 2006). Such studies, indeed, suggest that lazy workers are a reserve worker force.

Further investigations are, however, needed to determine the potential adaptive value of inactive workers. Although, these studies have often hypothesized that this reserve force of workers may be a tool of insect societies to compensate for a potential deficiency of helpers, none have investigated further the potential consequences in terms of fitness. When sudden colony disturbance occurs such as high individual predation, by recruiting inactive workers, a colony may be able to avoid any brood loss (full compensation), allowing to preserve productivity and therefore assure fitness of its members.

Alternative explanations for the presence of such inactive individuals exist in the literature. Inactive workers are also likely to be selfish individuals, resting between tasks (Klein *et al*.

2008), hoarding fat reserves (Schmid-Hempel 1990; Jandt *et al*. 2012) or high-ranked individuals expecting inheritance of the breeding position (Cant and Field 2001).

Another seemingly maladaptive behaviour of workers in social insects is nest-drifting, whereby workers spend time in other nests than their own. According to kin selection theory, workers should stay faithful to their nest to maximize inclusive fitness. Nest-drifting has been described in many species of social insects and most studies revealed that drifting individuals visit other nests either accidentally (Birmingham *et al*. 2004) or to act as a (social) parasite (Lopez-Vaamonde *et al*. 2004). In *P. canadensis*, however, most nests (94%) receive foraging help from the workers of nearby related colonies and over 50% of workers ‘drift’ (Sumner *et al*. 2007). One potential explanation for such behaviour is that drifters may gain indirect fitness benefits by helping raise related brood in nearby nests (Sumner *et al*. 2007). If drifters are able to determine the need for help on the nests they visit, drifting may be a rapid recruitment mechanism for replacing deceased helpers.

Foraging carries high mortality risks due to high levels of predation especially in the tropics (Strassmann 1981). In *P. canadensis*, foragers have a 7% chance of dying per day and so colonies often experience high levels of forager loss (Sumner *et al*. 2007). Both (1) recruitment of ’new foragers’ from a pool of inactive (or lazy workers) and (2) nest-drifting are potential mechanisms by which colonies of *P. canadensis* could react to such loss of the worker force. This species, therefore, offers a unique opportunity to test whether the presence of lazy worker and/or drifting individuals have an adaptive value as a reserve worker force. To test these hypotheses we experimentally removed foragers from nests of *P. canadensis*, and asked whether replacement foragers are recruited from ‘lazy nestmates (hypothesis 1) and/or drifters (hypothesis 2), and whether the employed strategy resulted in the excess brood being reared through to adulthood, as expected if worker fitness is assured (Gadagkar 1990; Field *et al*. 2000; Shreeves *et al*. 2003). We also tested other mechanisms by which nests may compensate for a loss of worker force: increase in foraging effort by remaining foragers, brood recycling and changes in brood development time.

METHODS

Experimental set up

The experiment was conducted on post-emergence nests of small to medium size (wasp number: 14.5±1.2 (s.e.); cell number: 87.4±9.2 (s.e)) from a large population of over 100 nests, dispersed across at least 10 aggregations near Colón, Republic of Panamá (9°24'08.28''N, 79°52'19.41''W) (described in Lengronne *et al*. 2012), in May to July 2010. All wasps from 60 nests were marked with unique number tags or enamel paint spots (n=38 nests) or Radio Frequency Identification (RFID) tags (n=22 nests). Unlike manual tagging, RFID tagging allows accurate quantification of drifting (Sumner *et al*. 2007). The RFID tags (GiS TS-Q5Bee Tags) code a unique identification number for each wasp; 3cm diameter circular antennae (GiS TS-A37) detect any tagged wasp passing within a 3cm radius of it, and time-stamped data is stored in on-site readers (GiS TS-R64) [18]. *Polistes* nests lack an envelope, and so unlike ants and bees (Molet *et al*. 2008; Robinson *et al*. 2008) they have no restricted nest entrance to facilitate RFID monitoring of arrivals and departures. To maximally capture wasp activity, we restricted the area of the nest from which wasps could enter and leave by fixing acetate sheets around the back of the nest. Wasps could therefore enter and leave the nest only via the front (open cell side) (Fig. 1). Two to four antennae (according to nest size) were then spaced across the front of the nest such that at least 80% of the accessible nest area was included in the 'detection zone' (Fig. 1).

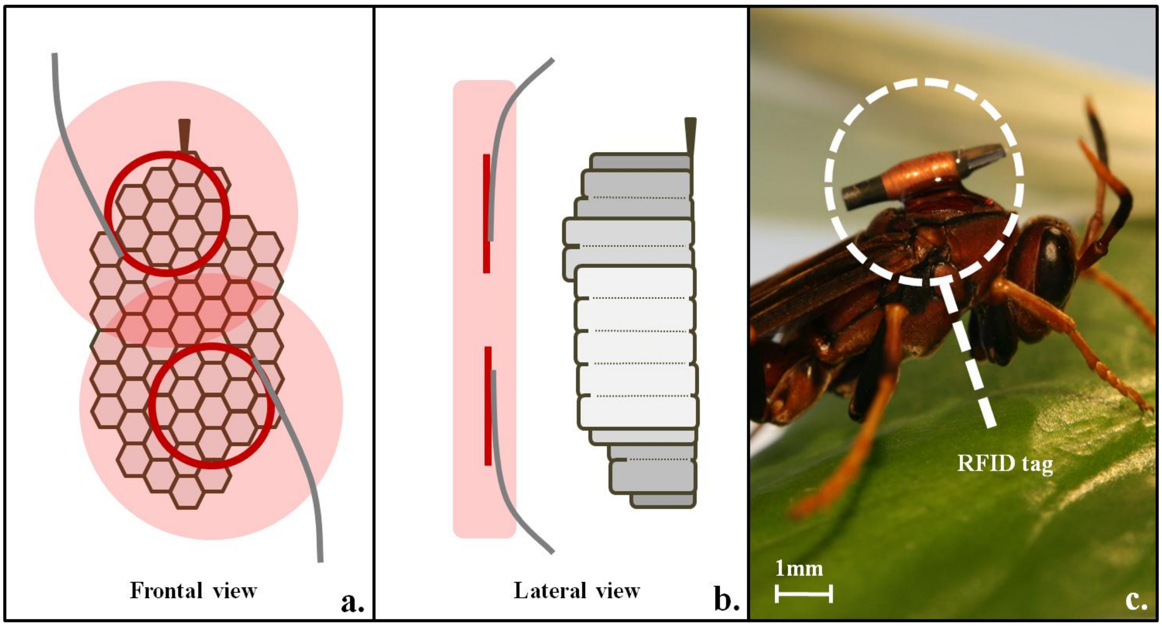


Fig. 1. Frontal (a.) and lateral (b.) views of antenna set-up and detection zone. The 2 dark red circles represent the antennae (a.). The red zone surrounding the antennae represents the range in which tagged wasps are detected. The detection zone covers on average 80% of the nest entry area but does not detect wasps on the nest itself (b). RFID tags were glued onto the thorax of the wasp. Photo credit: Aidan Weatherill (c).

Monitoring

On the number tag/paint marked nests, we conducted censuses twice a day for 20 days prior to the experimental removal of foragers (see below). When an individual was absent during one census but recorded again subsequently, it was classified as a foraging trip (following methods of Field *et al*. 2000; Shreeves *et al*. 2003). The chance that a wasp was visiting another nest (drifting) rather than foraging was low since drifting tends to take place within aggregations and so we would have detected any drifters on the other nests during the census of that particular aggregation. These census data allowed us to identify which individuals were foragers, which individuals initiate foraging (‘new foragers’) during a given period and to quantify individual foraging efforts (number of censuses an individual was absent, as a proportion of the number of censuses conducted on that nest), over the monitoring periods. All newly emerged (unmarked) individuals detected during manual censuses were carefully marked so they could be included in subsequent censuses.

In the 22 RFID nests (aggregation ‘2010SF’: n=12, aggregation ‘2010MH’: n=10) all individuals were tagged two days prior the start of monitoring. Continuous automated RFID monitoring was conducted from 8am to 6pm per day, allowing identification of foragers and quantification of individual foraging efforts by tagged wasps on their own nest and on any other nests they may visit as drifters. Foragers were defined as individuals that were recorded a minimum of 4 times by the antennae. Foraging effort was estimated as the sum of the number of times each wasp was detected by the antennae divided by two to reflect that one foraging trip is composed of one exit and one entrance on the nest.

In addition to the manual and automated censusing, we conducted night counts of wasps on all nests every 3 days to estimate the total number of wasps present in the nest. Individual-level censuses at night were not possible because these wasps are very lively on the nest at night and disturbance from torch light causes individuals to fly off the nest and be lost.

Manipulation experiment

We manipulated nests by permanently removing 30% of the total number of foragers from half of the nests (‘removal nests’ nR=30; 19 number tagged nest and 11 RFID nests). The remaining nests served as controls, as they were treated the same way as the ‘removal nests' except that all wasps were released immediately after collection ('control nests'; nC=30; 19 number tagged nests and 11 RFID nests). All removed wasps were foragers and none had been detected drifting. Nests were grouped by four manipulation dates (24 May, 10 June, 21 June and 13 July 2010) to facilitate handling of large numbers of wasps, and then assigned randomly to either removal or control. Removing 30% of wasps simulates a loss of helpers (e.g. due to predation or inclement weather) within the natural range (Sumner *et al*. 2007).

Brood in each cell of all number tagged nests (n=38) and 12 of the RFID nests ('2010SF' aggregation) were mapped on manipulation day, and thereafter weekly for 28 days. Each brood was assigned to one of five developmental stages: egg, small larva, medium larva, large larva or pupa (Sumner *et al*. 2010).

We first checked whether the loss of foragers is compensated by comparing the number of brood that were present on manipulation day that survived to pupation (beyond which no provisioning is necessary) between control and removal nests. If colonies compensate for the forager loss, we expect to see no difference in the survival of brood between removal and control nests, irrespective of nest or group size.

We then test all potential mechanisms by which colony may react to the loss of foragers.
Hypothesis 1: Lazy workers who do not forage may initiate foraging (new foragers) to compensate for the loss of individuals. We first quantified the proportion of wasps that did not forage prior to manipulation and classified these wasps as lazy workers. We then quantified the proportion of lazy workers that initiated foraging (or new foragers; calculated

as the number of individuals that were previously non-foragers which initiate foraging over the total number of foragers) within the 5 days pre-manipulation and the 5 days post-manipulation, and compared between control and removal nests (nR=19; nC=19, all number tagged nests).

Hypothesis 2: Drifters may compensate for the loss of individuals by becoming a new drifter, or increasing their helping effort on the nest in need. We compared the number of drifters and the number of visits by drifters between pre-and post-manipulation in both removal and control nests (n=22 RFID nests. nR =11 removal nC =11; ‘2010SF’ and ‘2010MH’ aggregations). Drifting was quantified using RFID nests only since previous work showed that manual censuses are not accurate enough to pick up the majority of drifting events (Sumner *et al*. 2007).

In order to determine the importance of lazy workers and/or drifters, we then tested the other possible mechanisms by which societies may compensate for worker loss. Remaining nestmate foragers may also increase their rate of foraging (Hypothesis 3). We compared foraging efforts of individuals for the five days (nR=25; nC=25, number tagged and RFID nests). Smaller brood may be recycled to feed the larger brood (Hypothesis 4) (Field *et al*. 2000; Shreeves *et al*. 2003). We compared the survivorship of the larger (medium and large larvae) and smaller (eggs and small larvae) brood until pupation between control and removal nests (nR=25; nC=25). Brood may either grow more slowly or pupate earlier to adapt to lower rates of food provisioning in removal nests (Hypothesis 5). We compared the time taken for each brood present at manipulation to reach the next developmental stage in control versus removal nests, using the weekly brood censuses (nR=25; nC=25).

Data analysis

A GLMM analysis was used to confirm brood survivorship after forager loss, with "brood surviving until pupal stage" as response variable (Model 1; binomial errors), "treatment" (removal or control group) and "nest size" (total number of brood) as explanatory variables, and "aggregation", "time of removal" and "nest" as random variables. During the experiment some removal and control nests became infected with a brood parasite (possibly from the Sarcophagidae family (Diptera)). Presence or absence of "parasitism" was included as a potential explanatory variable.

Hypothesis 1 and 2: We used a GLMM analysis determine whether lazy workers initiate foraging (new foragers) to compensate for the loss of individuals and whether drifters increase their helping effort to compensate for the loss of individuals. We use the "change in the proportion of new foragers (Model 2; Hypothesis 1), the "change in the number of drifters at the nest level" (Model 3.1, Hypothesis 2) or "change in the number of visits by existing drifters" (Model 3.2, Hypothesis 2) between pre- and post-manipulation as a response variable (normal errors); "treatment", "nest size" and "parasitism" as potential explanatory variables; and "aggregation" and "time of removal" as random variables.

GLMM analyses were also performed to determine whether remaining foragers on the nest increase their rates of foraging to compensate for the reduced worker force (Hypothesis 3), using "change in the number of visits at the nest level" (Model 4) between pre- and post-manipulation as response variables. All explanatory and random variables were as described in Mechanisms 1 and 2. To test whether small brood are recycled (Hypothesis 4), we performed the same GLMM analysis as the Model 1 (see above) but for small and large brood separately (Model 5.1 and 5.2). Finally, to compare the developmental time of each brood stage between removal and control nests (Hypothesis 5), we used unpaired t-tests. Each GLMM was performed in 'R' using the lme4 package (R development Core Team 2010). We report significance levels for the minimal adequate model after sequential removal of non-significant terms.

RESULTS

Of the 60 nests initially monitored, 50 (83%) survived until the end of the experiment giving the following samples sizes: nR=27 removal nests and nC=23 control nests. There is usually a single egg-layer in *P. canadensis* nests (Lengronne *et al*. 2012): a single dominant queen could be identified on each of our nests as the individual who was on the nest the most time. Control and removal nests showed no significant differences in nest attributes, such as size (number of cells, brood, and wasps) and quality (Table 1).

	n	group size	brood number	cell number	nest quality
RFID controls	11	17.50±9.87	85.00±38.91	95.67±46.87	0.91±0.11
RFID removals	11	17.33±10.91	98.33±37.17	107.83±41.99	0.92±0.10
p-value (t-test)	-	p=0.31	p=0.64	p=0.58	p=0.16
Number tagged controls	12	8.67±5.61	60.83±42.49	60.91±42.61	0.99±0.01
Number tagged removals	16	10.69±4.70	68.06±37.86	69.43±37.37	0.97±0.05
p-value (t-test)	-	p=0.98	p=0.56	p=0.65	p=0.85

Table 1. Mean (±s.d.) wasp, brood and cell number, and nest quality (measured as the ratio of brood:cell number, as presence of empty cells suggests parasitism and/or disease) in removal and control nests for both RFID and number tagged data. T-test significance of comparison between control and removal nests are given for both RFID and number tagged nests.

We first checked that the colony was in some way compensating for the loss of workers, such that the reduced colony size still reared the full cohort of brood through to adulthood. At the time of manipulation, there was a positive linear relationship between group size (number of wasps on each nest at night census) and brood size (Fig. 2 - Pearson's correlation r=0.79, p<0.001, n= 50 nests).

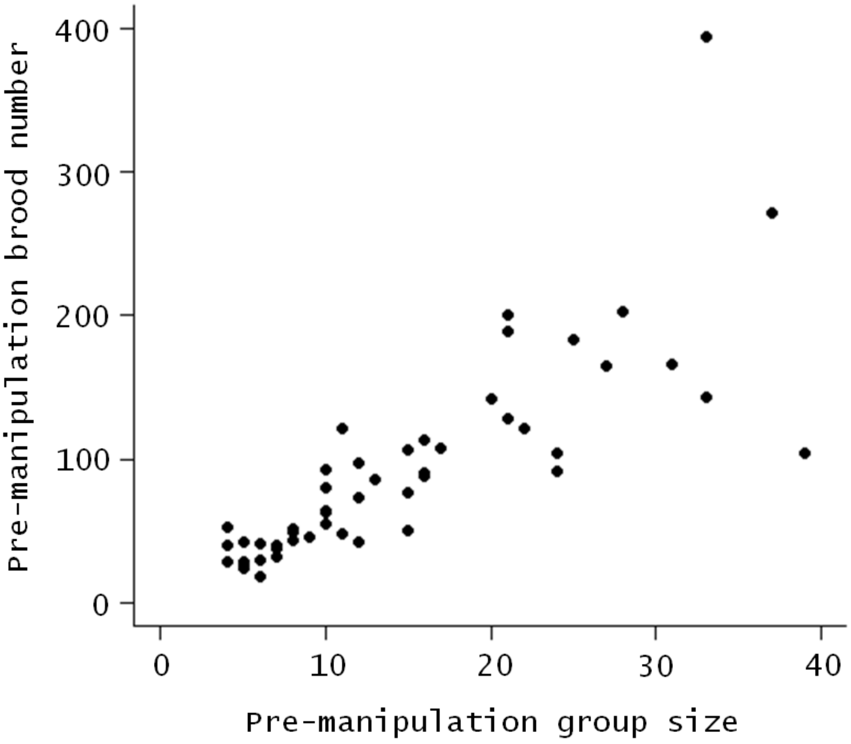


Fig. 2. Positive linear correlation between the number of brood and group size pre-removal (Pearson's correlation *r*=0.79, *p*<0.001, n=50)

Thus, if there is no compensation we expect that loss of 30% of individuals would result in a 30% reduction in number of brood surviving to adulthood. We found no significant difference in the number of brood surviving to pupation between removal and control nests after manipulation (Model 1, GLMM; nR=22, nC=18; treatment: t=-0.36, p=0.72; nestsize: t= -

0.06, p= 0.95; parasitism: t= -0.86, p= 0.35), which suggests a colony-level compensation for the forager loss (Fig. 3).

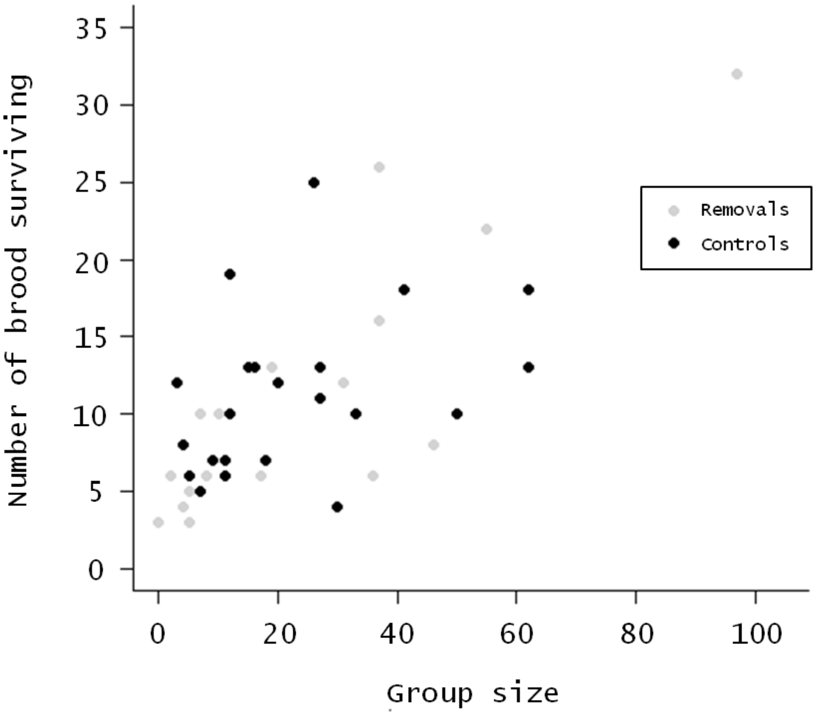


Fig. 3. No difference in the number of brood surviving to pupation between removal nests, where 30% of foragers were removed and control nests (GLMM Model 1, *p*=0.72).

We then went on to test our key hypotheses that lazy and/or drifting workers were responsible for compensating and assuring fitness when the worker force is lost. Firstly, we found that loss of foragers appears to stimulate non-foraging nestmates (lazy workers) to start foraging (Hypothesis 1). The change in proportion of lazy workers that had initiated foraging (or new foragers) during the five days after manipulation compared to the five days before, was significantly affected by treatment (Model 2, GLMM; nR=16, nC=12; treatment t=2.34, p=0.03; nest size t=-0.63, p=0.53; parasitism t=-0.66, p=0.52; Table 2, Fig. 4). This suggests that non-foragers in removal nests were more likely to switch to foraging in removal nests rather than controls.

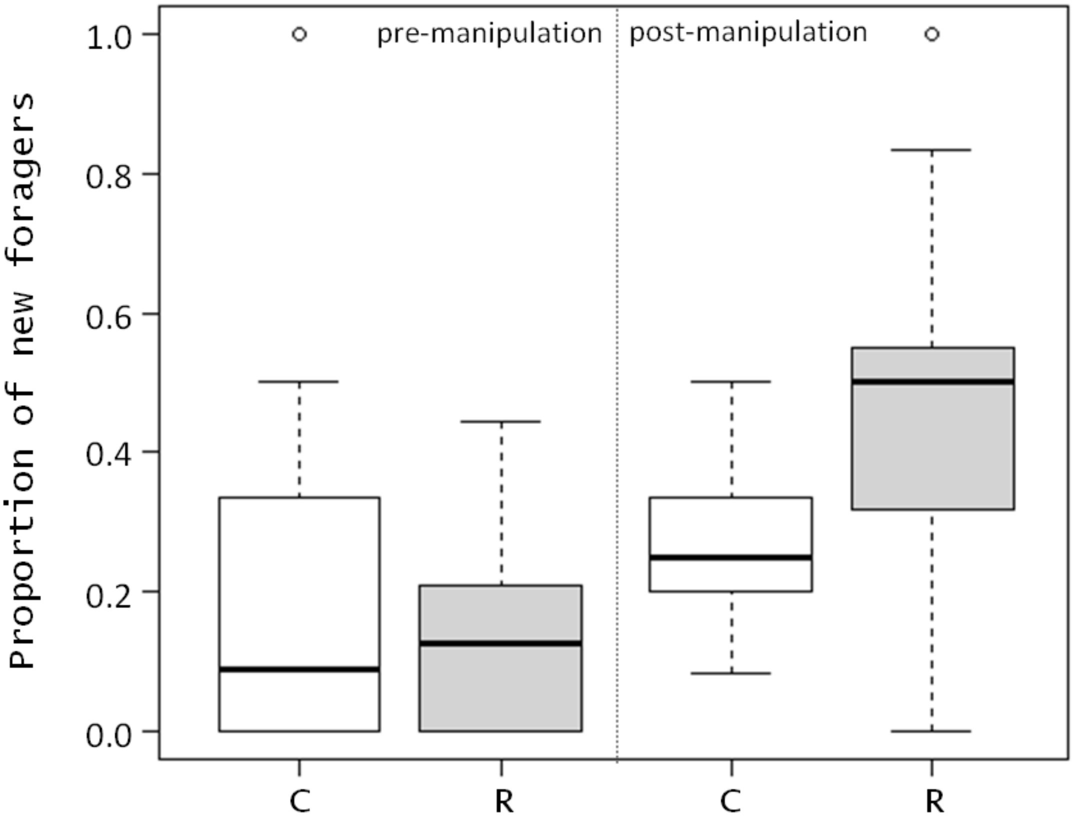


Fig. 4. Proportion of new foragers pre- and post-manipulation of control and removal nests (n=28; all number tagged nests) (C: control nests; R: removal nests).

Conversely, drifters do not appear to be compensating for loss of workers (Hypothesis 2). We found no evidence that the number of drifters visiting nests increased in response to the treatment (Model 3.1, GLMM; nR=11, nC=11; treatment: t=0.78 , p=0.44; nestsize: t=0.24, p= 0.77; parasitism: t=-0.41 , p= 0.68; Table 2) nor that the number of visits from existing drifters increased after manipulation (Model 3.2, GLMM; nR=11, nC=11; treatment: t=-0.34, p=0.73; nestsize: t=0.28 , p= 0.78; parasitism: t=-0.88 , p= 0.39; Table 2). These results suggest that drifters do not adjust their helping effort to compensate for the loss of forager effort on the nests they visit.

In order to confirm that lazy workers are the main mechanism by which colonies are robust to loss off workers, we tested several alternative hypotheses. We found no evidence that existing foraging nestmates increased their foraging effort to compensate (Hypothesis 3), nor were there any signs that brood were consistently recycled (Hypothesis 4), or changed their development (Hypothesis 5). The change in the number of trips per nestmate forager after manipulation was not significantly affected by treatment, nest size, nor parasitism (Model 4, GLMM; manual and RFID data; nR=27, nC=23; treatment: t=-0.12, p=0.90; nestsize: t=-2.21, p=0.03; parasitism: t=-0.13, p=0.90). Both small and large brood are equally likely to be reared after loss of foragers (Model 5.1, GLMM; small brood: nR=22, nC=18; treatment: t=-1.31, p=0.19; nestsize: t=-0.02, p=0.99; parasitism t= -2.07, p=0.04) (Model 5.2, GLMM; large brood: nR=22, nC=18; treatment: t= -0.39, p= 0.7; nestsize: t= -1.06, p= 0.29; parasitism t= 0.36, p= 0.72; Table 2). Finally, we found no evidence that brood developed more slowly or pupated earlier in removal nests than control nests (transitions from SL to ML; ML to LL; LL to Pupa: p>0.4; Table 2).

These results suggest that lazy workers assure fitness for nestmates, to whom they are closely related. Moreover, these workers compensate for the loss of worker force on their nests, providing assurance of inclusive fitness for colony members. Conversely, drifters do not seem to compensate for the loss of workers on non-natal nests.

Hypothesis	Model	Data description	Removals - pre	Removals - post	Controls - pre	Controls - post	p	Nests
n/a	1	Brood survivorship	n/a	23.58±4.25	n/a	26.00±6.35	0.72	RFID +number tagged
n/a	1	Total number of brood	77.43±8.01	93.67±12.84	71.12±11.68	83.82±16.59	0.70 †	RFID +number tagged
1	n/a	Number of foragers	12.81±2.14	9±2.07	16.26±3.40	13.83±2.69	0.26 †	RFID +number tagged
1	2	Proportion of new foragers	0.13±0.04	0.45±0.06	0.21±0.09	0.27±0.03	0.03 *	Manual
2	3.1	Number of drifters	4.8±0.89	3±0.60	3.54±0.61	1±0.30	0.44	RFID (including 2010MH)
2	n/a	Number of foraging trips per drifter	2.49±0.42	3.17±1.68	1.3±0.38	1.54±0.80	0.65 †	RFID (including 2010MH)
2	3.2	Number of foraging trips by drifters per nest	13.72±4.34	8.91±3.61	6.09±2.86	2.45±1.59	0.73	RFID (including 2010MH)
3	4	Number of foraging trips per nestmate forager	13.18±3.86	10.03±3.21	8.52±3.81	6.99±2.51	0.90	RFID +number tagged
3	n/a	Number of foraging trips by nestmates per nest	167.44±53.75	81.88±29.99	167.48±92.18	89.26±32.76	0.95 †	RFID +number tagged
4	5.1	Number of small brood	50.24±5.33	7.24±1.90	43.88±6.23	11.35±2.47	0.19	RFID +number tagged
4	5.2	Number of large brood	17.14±2.29	19.14±2.72	17.12±3.85	20.35±2.72	0.7	RFID +number tagged
5	n/a	Developmental time of brood (SL to ML) in weeks	n/a	1.131±0.057	n/a	1.208±074	0.41	RFID +number tagged
5	n/a	Developmental time of brood (ML to LL) in weeks	n/a	1.126±0.063	n/a	1.133±0.091	0.94	RFID +number tagged
5	n/a	Developmental time of brood (LL to P) in weeks	n/a	1.406±0.103	n/a	1.332±0.090	0.61	RFID +number tagged

Table 2. Summary of results (mean±s.e.) including p-value for treatment from tested models. “Removals/Controls – pre” refers to the state of the nests on the day of manipulation (e.g. brood number), or in the pre-manipulation monitoring period (e.g. rates of foraging). “Removals/Controls – post” refers to the state of the nests at the end of the experiment (e.g. brood number), or in the post-manipulation monitoring period (e.g. rates of foraging). “p” refers to the p-value for the Treatment effects, from GLMM/t-tests given in the text. † Test not shown in main text. * Significant p-value.

DISCUSSION

Our study provides evidence that worker inactivity in *P. canadensis* has an adaptive function, providing resilience to colonies in the event of worker mortality.

After experimental removal of 30% of the foragers on 22 nests, brood were reared to adulthood as successfully as brood in 18 unmanipulated nests. Compensation for the loss of foragers is assured through adjustments in helping effort by the lazy workers, which shift from non-foraging to foraging behaviour. In the literature, similar patterns have been found in social insects where inactive workers are used as a 'reserve worker force' which engages in activities, such as nest defence and food collection when unexpected conditions arise (Schmid-Hempel 1990; Robinson 1992). Support for such strategies has been found in various studies on honeybees (Seeley 1982; Kolmes 1985; Winston and Fergusson 1985), leaf-cutting ants (Wilson 1983; Waddington and Hughes 2010), termites (Evans 2006) as well as social wasps (O'Donnell and Jeanne 1990; Theraulaz and Deneubourg 1994). This study provides one of the first clear evidence of a 'reserve worker force' in *Polistes* wasps. One comparable example may be found in *Polistes metricus*, where it was suggested that young and relatively less active wasps were likely to replace older active foragers after removal or when they become less active (Dew and Michener 1981).

However, to our knowledge, this is the first experimental study demonstrating that these groups of lazy individuals in insect societies responds to changes in colony needs (here by switching from non-foraging to foraging) in order to assure colony fitness (avoiding any brood loss). These findings add further important empirical evidence that indirect fitness plays a key role in driving helping behaviour in primitively eusocial insects.

Recruitment from apparently lazy nestmates is likely to be a common strategy in the larger colonied primitively eusocial insects, who can afford such resilience. The costs to the group of losing non-foraging helpers (if they start foraging) may be relatively low in *P. canadensis* because of the ready supply of maturing pupae on post-emergence nests. For example, in our experiment each nest gained on average a newly emerged female (potential forager) every 2.4 days (0.41±0.04 newly hatched pupae per day; estimated from manual censuses, mean±s.e.). Moreover, the cost of losing helpers is likely to be less in species like *P. canadensis* as they have large groups (14.5±1.2 individuals/nest; this study) relative to other species. For instance, in the tropical hover wasp *Liostenogaster flavolineata* (3.93±0.24 individuals/nest; Field *et al*. 2000) existing non-foraging helpers do not initiate foraging when foragers are removed (Field *et al*. 2000), perhaps because the costs of losing valuable helpers from a small group may be too great or because non-foragers may inherit the nest in the near future. Interestingly, however, *L. flavolineata* recruited new extra workers from their emerging worker force. Thus, adoption of new foragers where available – either from an existing adults nestmates or from soon-to-emerge nestmates – appears to be an important strategy to respond to sudden changes in colony needs in primitively eusocial wasps.

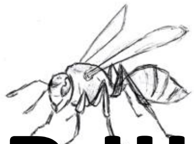
Drifting is thought to be a strategy for gaining inclusive fitness benefits in this species, because drifters appear to preferentially visit (and provision) nests to which they are more closely related (Sumner *et al*. 2007). Interestingly, our study suggests that drifters do not respond to the changing needs of the non-natal nests they visit, as they did not increase their individual helping efforts on manipulated nests which had lost 30% of their foragers. Drifters gain much higher indirect fitness by helping on their natal nest (r(drifter to brood on natal nest) = 0.56±0.135) than the related nests they visit (r(drifter to brood on other nests visited) = 0.19±0.071; Sumner *et al*. 2007). Thus, there may be a fitness benefit to drifters to focus their response to the needs of their natal nest before helping more distantly related nests. Another explanation is that drifters are not spending enough time on non-natal nests to be able to detect (or receive information on) and respond to subtle changes in need for help: drifters spend on average 73,6±4.8% of their time on their natal nest, and compared to 26.4 % of their time on non-natal nests (n = 83 drifters, this study). Drifting may therefore be a coarse-scale behaviour that reduces the cost of investing all helping effort in a single nest in the unstable environments typical of tropical wasp populations, where nests and their brood experience high mortality rates (Jeanne 1979; Strassmann 1981). It remains to be seen as to what the real adaptive value of drifting behaviour in this species is.

Although we manipulated the need for help on our nests, existing foragers did not increase their foraging rates, probably because of the high mortality costs of foraging Cant and Field 2001; Shreeves *et al*. 2003). Indeed, how hard a helper works is likely to influence her survival because foraging carries high mortality risks due to high levels of predation in the tropics (Strassmann 1981). Since forager in *P. canadensis* has a 7% chance of dying per day (Sumner *et al*. 2007), for these individuals, the increased morality risks may not outweigh the inclusive fitness benefits of rearing the extra brood. Likewise, brood were not recycled to compensate for the lower amount of food provisioning resulting from the loss of foragers. This result differs from other studies on social wasps where helpers were removed (Field *et al*. 2000; Shreeves *et al*. 2003). This difference in the behavioural responses may have been influenced by the stage in the colony cycle from which we performed the experiment. Our study focused on post-emergence nests (after worker emergence) whereas other studies (e.g. *Polistes dominulus*, Shreeves *et al*. 2003) examined foundress nests where new helpers were not yet emerging. Thus, in this latter case, brood recycling is the least costly strategy to compensate for lower amount of food provisioning. It is therefore possible, that the mechanisms for assuring fitness may change over the nest cycle of a species, with the first line of defence being recruitment of new helpers if available, and otherwise brood recycling.

In order to cope for sudden changes in colony need, rapid action is required by social groups to preserve colony productivity. Our study highlights how primitively eusocial insect societies may solve this problem through adapted behaviours, which depend on the relative costs and benefits of assuring indirect fitness and allow to respond dynamically for the changes in social environment/structure. Our study brings us one step forward in understanding the function of the peculiar behaviours we observe in animal societies. Although these behaviours may, at first sight, appear to be maladaptive, they often have an adaptive function and may play an important role in the evolution and/or maintenance of sociality.

ACKNOWLEDGEMENTS

We thank the Smithsonian Tropical Research Institute for hosting the field work, particularly Jorge Morales, Stanley Heckledon and the staff at Galeta field station, Colon, Panama. We also thank Javier Hurtado and Roberto Lombardo for assistance in the field. The field-work was conducted under research permits granted by ANAM (#SE/A-65-10; #SEX/A-44-10). This study was supported by grants from the Swiss National Science Foundation (to TL and LK) and a NERC New Investigator grant (NE/G000638/1 to SS).



CHAPTER III

Chapter III

Social network analyses of nest-drifting behaviour in a primitively eusocial wasp

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Paper in preparation for submission in *Behavioral Ecology*

Lengronne T, James R, Patalano S, Dreier S, Keller L and S Sumner. 2013. Social network analyses of nest drifting behaviour in a primitively eusocial wasp. *In preparation.*

ABSTRACT

A potential challenge to kin selection theory is nest-drifting behaviour, whereby nest members spend time on nests other than their own natal one. In the primitively eusocial wasp *Polistes canadensis*, high levels of nest drifting have been reported, where workers appear to spend time in two or more related nests, where they benefit from indirect fitness by helping raise brood. This particular helping behaviour has not been fully described yet in this species and more information on its structuring and mechanisms are required. Understanding the relationship between nests and aggregations of nests but also the factors affecting drifting are also of particular importance since they may consequently influence the relative fitness. In this study, we performed a comprehensive social network analysis to examine the structure of drifting patterns and determine whether inclusive fitness benefits can explain nest drifting in populations of *P. canadensis*. Our analysis reveals that drifting patterns are generally structured within aggregations and that exchanges of wasps between nests are unlikely to derive from accidental events (random) since we found strong variation in the intensity of the between-nests relationships (number of events) as well as some consistency of the nest interactions over time. Furthermore, we found that the proximity (i.e. distance) and the amount of help needed (i.e. workload) are factors influencing fitness payoffs which may explain patterns of drifting. Experimental removal of brood and wasps performed within aggregations to modify this need in help from specific nests revealed that the quality and needs of the natal nest are determining factors that influence helping decisions of drifters. This study provides further evidence of the complexity of helping behaviour and adaptability of social insects and illustrates the potential of adopting a social network approach to understand complex dynamics of groups such as nest-drifting in the primitively eusocial wasp *P. canadensis*.

INTRODUCTION

Sociality is a widespread phenomenon in the animal kingdom and is considered as one of the major evolutionary transitions of life (Szathmary and Maynard Smith 1995). Eusociality in the eusocial insects - ants, termites, some bees and wasps - represents one of the highest levels of social organization. However, eusociality can be regarded as an evolutionary paradox since some individuals (workers) forego reproduction and instead help raise the offspring of others (queens). Hamilton’s inclusive fitness theory provides a theoretical framework for understanding the evolution of such levels of cooperation (Hamilton 1964a; Hamilton 1964b). Inclusive fitness theory states that individuals may transmit copies of their genes not only by reproducing themselves (direct fitness), but also by helping relatives that share the same genes (indirect fitness). Kin selection theory is the keystone for our modern understanding of social evolution (Bourke and Franks 1995).

A potential challenge to kin selection theory is nest-drifting behaviour, whereby nest members spend time on nests other than their own natal one. Recent studies of nest membership, reproduction and behaviour suggest that drifting is more prevalent in insect societies than previously thought. It has been reported in several species of wasps (Akre *et al*. 1976; Kasuya 1981; Tsuchida and Itô 1987; Turillazzi *et al*. 1997; Seppä *et al*. 2002; Sumner *et al*. 2007), also see Uddin and Tsuchida 2012) and bees (Roubik 1981; Pfeiffer and Crailsheim 1998; Neumann *et al*. 2000; Paxton *et al*. 2002; Paar *et al*. 2002; Lopez-Vaamonde *et al*. 2004; Birmingham *et al*. 2004; Nanork *et al*. 2005; Härtel *et al*. 2006; Ulrich *et al*. 2009; Takahashi *et al*. 2010; Peso and Richards 2011; Blacher *et al*. 2013). Explanations for nest-drifting differ across species. Whilst some cases of drifting are clearly accidental (e.g. among densely packed bee nests in greenhouses (Birmingham *et al*. 2004)), in other cases they may be selected for as drifters often may gain fitness benefits either directly through egg laying as social parasites (Neumann *et al*. 2000; Paxton *et al*. 2002; Lopez-Vaamonde *et al*. 2004; Nanork *et al*. 2007; Takahashi *et al*. 2010), or indirectly by acting as helpers on related nests (Sumner *et al*. 2007).

There are therefore two pertinent issues arising from these studies. Firstly, since drifting often carries some fitness benefits, it is likely to be an important component of inclusive fitness theory that has previously been overlooked (Sumner *et al* 2007). Secondly, it may not be correct to always view an insect nest as an independent unit, but rather a nest may be subset of a larger, interconnected network of several nests across an aggregation, and moreover, aggregations may be interconnected across a (meta)population. Understanding the relationships and interactions between nests within aggregations and between aggregations across populations in species that drift is important as it may influence the relative fitness consequences of social behaviour. This is particularly true if specific events (e.g. loss of workers or brood due to predation or disease) in one nest affect fitness payoffs in other nests in the wider connected community.

The aim of this study is to use social network analyses to investigate the significance of nest drifting in the primitively eusocial paper wasp *Polistes canadensis* which is found in the neotropics. Like all primitively eusocial species, queen and worker castes in *Polistes* are behaviourally distinct but morphologically similar, with workers generally retaining the ability to become opportunistic egglayers (Giray *et al*. 2005; Sumner *et al*. 2006; Sumner *et al*. 2010; Ferreira *et al*. 2013). Non-reproductive workers gain high indirect fitness benefits by helping on their natal nest because they are closely related to the brood (Sumner *et al*.

2007; Lengronne *et al*. 2012). High levels of nest drifting have been reported in *P. canadensis,* where up to 50% of the workers appear to spend time in two or more related nests, where they help raise brood (Sumner *et al.* 2007). Moreover, there is a low (but significant) level of population viscosity in this species, which means clusters of neighbouring nests are related (Lengronne *et al*. 2012). Thus, workers in this species are thought to reap indirect benefits by drifting to neighbouring nests, as well as helping on their natal nest. Moreover, almost all nests appear to produce and receive drifters (Sumner *et al*. 2007), suggesting that populations of this species consist of aggregations of interconnected nests that exchange nest members rather than the traditionally viewed ‘distinct’ social insect nest.

These studies raise two outstanding questions that have important implications for how we calculate inclusive fitness payoffs. Firstly, is nest drifting a responsive behaviour whereby individuals adjust their helping effort in response to changes in fitness payoffs? If so, patterns of drifting between nests within an aggregation should show dynamic changes across the whole aggregation such that any change in the fitness payoffs on one nest should cause changes in the network dynamics of the whole aggregation. This would suggest that individual nest ‘units’ (i.e. the nest carton, brood and natal adults) within an aggregation are not independent, such that changes in the behaviours of individuals on one nest will have repercussions at the aggregation level. Secondly, if nests are not independent units, then perhaps aggregations are not either: it is not known if drifting is limited to within an aggregation, or whether spatially segregated aggregations are connected via drifters, forming small-scale metapopulations of spatially distinct sub-populations that interact. These questions are important as they would mean that the scale of the fitness landscape shaping social behaviour may not be restricted to just the nest level, as traditionally viewed for most social insects.

Here we use social network analyses to determine what the unit of social organisation is in *P. canadensis*. Social network analyses are commonly used to describe and explain interactions among individuals or groups in the social sciences (Wasserman and Faust 1994). Recently, they have been used to better understand animal societies (Croft *et al.* 2008), including those of social insects (Fewell 2003; Naug 2008; Thenius *et al*. 2008; Bhadra *et al*. 2009; Naug 2009; Blonder and Dornhaus 2011; Moreau *et al*. 2011; Jeanson 2012). Social networks analyses are a potentially powerful tool for describing and explaining patterns of nest drifting, with drifting rates and directions forming the interactions ('edges') between networks of nests ('nodes') within a population.

We use social networks to first describe temporal and spatial patterns of drifting between and within aggregations. Next we test three specific hypotheses. First, we investigate whether the drifting patterns observed in *P. canadensis* represent random movements of wasps between nests or not. If drifters are making decisions to drift (for example, based on relative fitness payoffs), we then expect drifting patterns within aggregations to be spatially non-random, such that some nests receive or accept more drifters (or higher rates of drifting) than other nests (hypothesis 1). Second, we investigate whether patterns of drifting are constant over short time scales when extrinsic conditions that may influence nest-specific fitness payoffs (such as group size, brood number) are relatively stable (hypothesis 2). Third, we investigate whether factors influencing fitness payoffs (i.e., the relatedness between drifters and the nests they visit, the distance between nests, and the nest-level need for help measured as the work load experienced by workers on a given nest) can explain patterns of drifting (hypothesis 3). Finally, we experimentally manipulated the nest-level need for provisioning help to test the hypothesis that changing conditions at the nest level cause changes in drifting patterns across the aggregation (hypothesis 4).

METHODS

Experimental Set up

The field site consisted of 93 small to medium sized post-emergence nests (19.6±1.4 average wasps per nest (±S.E.)) clustered in 2 populations found in abandoned buildings near Panamá City (2005 (n=33 nests, 2 main buildings + 1 small building); described in Sumner *et al.* 2007; 8°54’44’’N, 79°33’47’’W), and Colón (2009 (n=32 nests, 4 main buildings), 2010 (n=28 nests, 2 main buildings); described in Lengronne *et al.* 2012; 9°24’08.28″N, 79°52’19.41″W (Lengronne *et al.* 2012)), Republic of Panamá (Fig. 1).

All wasps on these nests were captured and fitted with Radio Frequency Identification (RFID) tags) (methods follow Sumner *et al.* 2007). The RFID equipment consists of the tags (passive tags; GiS TS-Q5Bee Tags), which code unique identification numbers, 3cm diameter circular antennae (GiS TS-A37) that detect tagged wasps passing within a 3cm radius of it, and readers (GiS TS-R64) which stored the date, time and unique identification number of each wasp as it passed within the detection range of the antenna (Sumner *et al.* 2007). *Polistes* nests lack an envelope, and so unlike ants and bees (Molet *et al.* 2008; Robinson *et al.* 2009) they have no delimited nest entrance, hence complicating the monitoring of each arrival and departure.

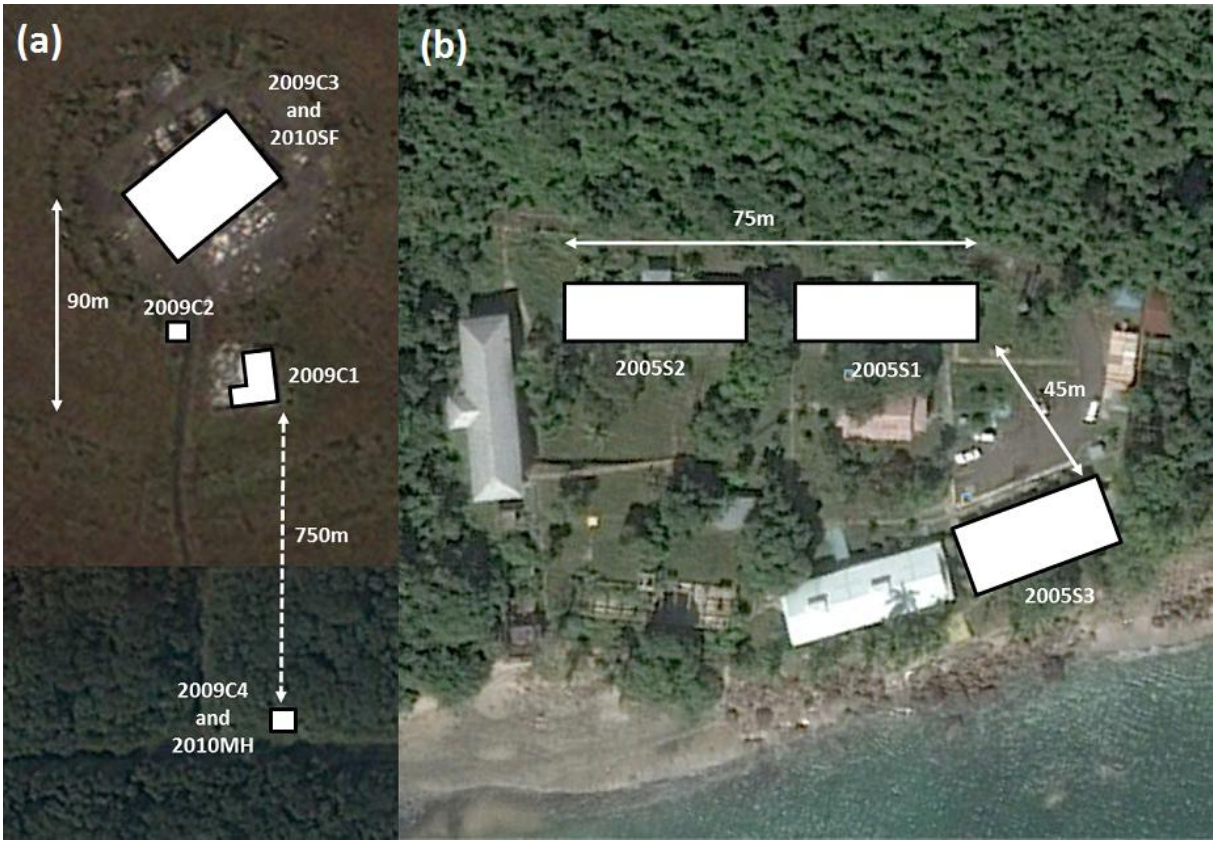


Fig. 1. Maps of the experimental sites where *Polistes canadensis* nests were studied. Each white rectangle corresponds to an aggregation/building. Site (a) is located in Punta Galeta, Colón, Republic of Panamá (9°24'08.28"N, 79°52'19.41"W). Between aggregation 2009C1 and 2009C4, the entire surface area is not shown (for distances see dotted arrow). Site (b) is located on the coast near Panamá (8°54'44''N, 79°33'47''W) (images from Google Earth).

To maximally capture wasp activity we therefore restricted the area of the nest from which wasps could enter and leave by fixing acetate sheets around the back of the nest. Wasps could thus enter and leave the nest only via the front (open cell side). Two to four antennae (according to nest size) were then spaced across the front of the nest such that at least 80% of the accessible nest area was included in the 'detection zone'. Continuous automated RFID monitoring was conducted from 8am to 6pm. Censuses of the numbers of wasps were performed every 3 days at night to estimate group size, and brood were mapped every week in each aggregation to provide estimate of nest size and worker:brood ratio for each nest.

Wasps from both sites (Panama City and Colón) were collected at the end of the experiment for molecular analyses. Samples were genotyped for estimating relatedness using 6 non-specific markers (Pan109, Pan117, Pan63, Pan93, Pan120 and Pbe411) developed from various related species of *Polistes* (*P. annularis, P. bellicosus,* Strassmann *et al*. 1997; see Sumner *et al.* 2007) for the 2005 data and 7 specific markers (Pcan01, Pcan05, Pcan09, Pcan15, Pcan16, Pcan23 and Pcan24; see Lengronne *et al.* 2012) for the 2009 and 2010 data. Although nests and wasps were removed at the end of the experiment, some tagged wasps foraging during collection time may not have been captured.

Data Analyses

For all network analyses, we used the software package UCINET which includes all sort of tools for the analyses and visualization (NetDraw) of social network (Borgatti *et al*. 2002). We first used the 2005 population which was monitored continuously over a 5 days period to identify natural subsets of nests which exchanged drifters more than other sets of nests. To do this we used a Hierarchical Cluster Analysis using dissimilarities and the WTD_AVERAGE Method (average between all pairs). We also performed the same analysis on the 2009 data, where nests were not monitored simultaneously, but had substantial periods of overlapping monitoring (Fig. 2). The cluster analysis starts by assigning each item (nests) to its own cluster and let the distances between the clusters equal the mean number of visit (dissimilarities) between nests (undirected links)). The pair of clusters with the highest number of visits is then merged into a single cluster, and new numbers of visits are computed between the new cluster and each of the old clusters (using the average number between pairs of nests). These steps are repeated until all nests are clustered into a single cluster forming the clustering tree.

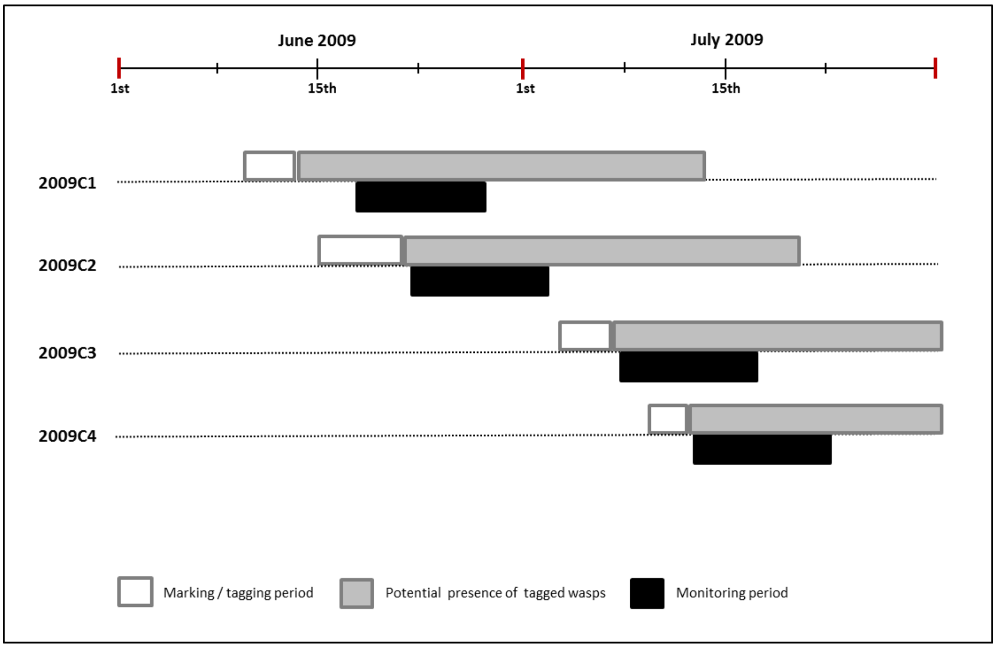


Fig. 2. Timetable of the marking period, monitoring period and period of potential tagged wasps' presence for the 4 aggregations studied in June and July 2009.

We then examined the levels of drifting present within our aggregations by providing descriptive measures such as drifting rates to confirm that drifting is widespread phenomenon in *P. canadensis* populations and investigated the connectivity within and between aggregations.

The degrees of connectivity between nests within aggregation (n=87 nests) were calculated using the edge density measures (number of edges (links/relation between pairs of nests) present in the network over total possible number of edges (if all nests of the network are connected)) of each network. A GLM analysis was performed to investigate factors influencing potential density variation between aggregations. Edge density (n=87 nests) was used as a response variable. We used aggregation size and aggregation shape (length:width in meters) as explanatory variables.

Hypothesis 1: To investigate whether drifting patterns are non-random, we compared the observed drifting networks (of each aggregation) with random networks created with “Random” option in UCINET. The simulated random networks had the same number of connections or edges (same density – see above) as the drifting network but equally distributed edges (interactions/links/connections) across the network. The comparisons between the observed and random networks were performed using dyadic QAP correlation (using 5000 permutations) in UCINET.

Drifting activity at nest and wasp level was studied by looking at the frequency of drifters and drifting events for all nests (n=87 nests). If drifting results from accidental events, we expect levels of drifting to be relatively stable across nests with low rates of drifting. We also investigated whether nests tended to specialize in being drifter sources or sinks by looking at the variation in the frequency of in- and out-degree (n=87 nests). Specifically, we calculated the difference in the percentage between in- and out-degree at the nest level.

We performed a GLMM to detect the potential factor that could explain variation in in- and out-degree using the net in- and out-degree per nest (in-degrees minus out-degrees) as response variable, group size and the presence of parasites as explanatory variables and “site” (aggregation) and “time” (year of experiment) as random variables. If drifting is non-random, we expect its patterns to vary across nests such that some nests receive or accept more drifters than other nests.

Hypothesis 2: To investigate whether drifting patterns fluctuated over time (i.e., over 4 monitoring periods of 5 days) we tested whether edges (i.e. directional links = number drifting events from nest A to nest B) were consistent over time in the 2005 control aggregations, 2005S1 and 2005S2 (where all tagged nests were monitored simultaneously).

Hypothesis 3: To study the potential role of factors influencing drifting patterns we used a GLMM analysis. Number of drifting events between pairs of nests (n=87 nests) was used as a response variable. We used “relatedness”, “distance” (between nests, in meters) and “workload” (estimated as the difference in worker:brood ratio between each pair of nests (i.e. (brood number of nestA * the groupsize of nestB) / (brood number of nestB * the groupsize of nestA)) as explanatory variables, and “site” (aggregation) and “time” (year of experiment) as random variables. Relatedness was calculated by using the program RELATEDNESS 5.0.8 (which is based on Grafen's (1985) relatedness coefficient) and weighting nests equally (Queller and Goodnight 1989).Standard errors were estimated by jackknifing over loci.

Hypothesis 4: To determine whether drifters could actively detect changes in need for help, and respond to these changes we manipulated two variables (see Table 1). First, to increase the nest-level need for help, we permanently removed 30% of the foragers on 14 nests (3 nests in 2009 (1 in 2009C1, 1 in 2009C2 and 1 in 2009C3) and 11 in 2010 (6 in 2010SF and 5 in 2010MH)). Second, to decrease the nest-level need for help, we permanently removed 30% of the brood on 9 nests (3 nests in 2009 (1 in 2009C1, 1 in 2009C2 and 1 in 2009C3) and 6 in 2010 (6 in 2010MH)). Large brood (at least 60% of medium and large larvae were preferentially removed as they represent the most valuable brood, and require the greatest helping effort to raise. Cells that had contained the removed brood were also removed to prevent wasps perceiving empty cells as a decrease in “queen quality” or nest quality – e.g.

from brood parasitism. Control nests were nests from unmanipulated aggregations (7 nests from 2009C4 and 33 nests from the 2005 populations). We did not use unmanipulated nests from aggregations where we manipulated worker or brood number as controls because one of our hypotheses is that a change in conditions on one nest in a connected aggregation will influence drifting/helping effort on the nests to which they are connected. We then quantified for each nest the change in in-degree (number of detections of non-nestmate wasps coming to the nest) and out-degree (number of detections of individuals drifting to another nest). The prediction was that the in-degree of nests where need for help was increased (i.e. those where foragers were removed) would increase and the out-degree decrease. Conversely, the in-degree of nests where need for help was decreased (i.e., those where brood was removed) would decrease and the out-degree increase. To test whether there was a difference in in- and out-degree, we performed chi-squared tests in R. We also carried out binomial test to examine whether these changes in in- and out-degree are different from random changes.

RESULTS

In total, 1599 wasps were tagged across the 93 nests studied. Four hundred and twenty wasps were tagged in 2005 from 27 nests, 665 tagged in 2009 from 32 nests and 619 tagged in 2010 from 28 nests. (mean±s.e., 19.6 ±1.4 per nest). Of tagged wasps, 1009 were recorded at least once. Overall, a total of 30,249 records (one detected arrival/departure of wasp) were generated, of which 2,563 (8.5%) were drifting events.

	2009C1	2009C2	2009C3	2009C4	2010SF	2010MH	2005S1	2005S2
Treatments (manipulated nests)	WR(1nest), BR (1nest), C	WR(1nest), BR (1nest), C	WR(1nest), BR (1 nest), C	C	WR(1/2), C	WR(1/3), BR(1/3), C	C	C
Nest number per treatment	WR :1 BR :1 C :4	WR :1 BR :1 C :8	WR :1 BR :1 C :7	WR :0 BR :0 C :7	WR :6 BR :0 C :6	WR :5 BR :6 C :5	WR :0 BR :0 C :20	WR :0 BR :0 C :11
Number of nests	6	10	9	7	12	16	20	11
Area of site	40	9	45	56	45	56	250	380
Nest density	0.15	1.1	0.2	0.12	0.27	0.29	0.08	0.03
Aggregation shape (length : width)	8 :5	3 :3	15 :3	8 :7	15 :3	8 :7	28 :10	35 :10
Drifting rate	35.1	35.1	31.8	13.6	33.6	28.1	25.9	43.5
Density of edges (directed) (premanipulation)	0.40	0.42	0.21	0.19	0.31	0.12	0.05	0.06

Table 1. Treatment performed (WR: Worker removal; BR: Brood removal; C: Control) with the proportion /number of nests on which each manipulation was performed (in brackets), number of nest per treatment, total number of nests monitored, approximate are of site (in m²), nest density (number of nests over are size), aggregation shape (length:width in meters), drifting rate (expressed as a percentage of wasps on the nest that were detected drifting (i.e. number of drifters over the total number of monitored wasps on a nest)) and edge density (number of edges present between nests over total possible number connections between nests) of each the 7 studied aggregations.

Social structuring of populations

The cluster analysis in UCINET revealed the presence of 4 distinct clusters of connected nests on 2005 and 5 clusters in 2009 (Fig. 3). The clusters of connected nests were usually located on the same building (although wasps occasionally moved between buildings). In the 2009 population, 3 out of 5 clusters regrouped nests from the same building (2009C1, 2009C2 and 2009C4). The 2 remaining cluster regrouped nests from another building (2009C3). In the 2009 population, 3 clusters regrouped nests from one single large building (2005S1), whereas the remaining cluster regrouped nests from another large building (2005S2). Since most clusters regrouped nests from the same building, we therefore used buildings as aggregation units in the analyses. This gives us a number of 6 different aggregations of nests: 2 main aggregations in the 2005 population (2005S1 and 2005S2) (Building 3 having only 2 nests), 4 aggregations in the 2009 population (2009C1 to 2009C4), from which we add 2 aggregations, 2010SF and 2010MH, which correspond to the two buildings studied at separate time periods in 2010 (see Table 1). The average distance between nests within an aggregation (7.7±0.3m; mean ±s.e.) was significantly smaller than the distance (298.4±127.1m) between aggregations (unpaired t-test, p=0.0001, n=91 nests).

Drifting was detected in all 8 studied aggregations (pre-manipulation data from 2009C1 to 2009C4, 2010SF, 2010MH, 2005S1 and 2005S2). On average 30.8%±3.1 of the wasps (n=831 wasps, 91 nests) were drifters. . At the nest level, 92.7% ± 0.1 (66.7-100%) of nests in each aggregation received or produced drifters (85.8% ± 0.2 of the nests received and 71.1% ± 0.3 of these nests produced drifters), indicating that drifting is a general phenomenon and not restricted to particular nests. from 13.6 to 43.5% (Table 1)

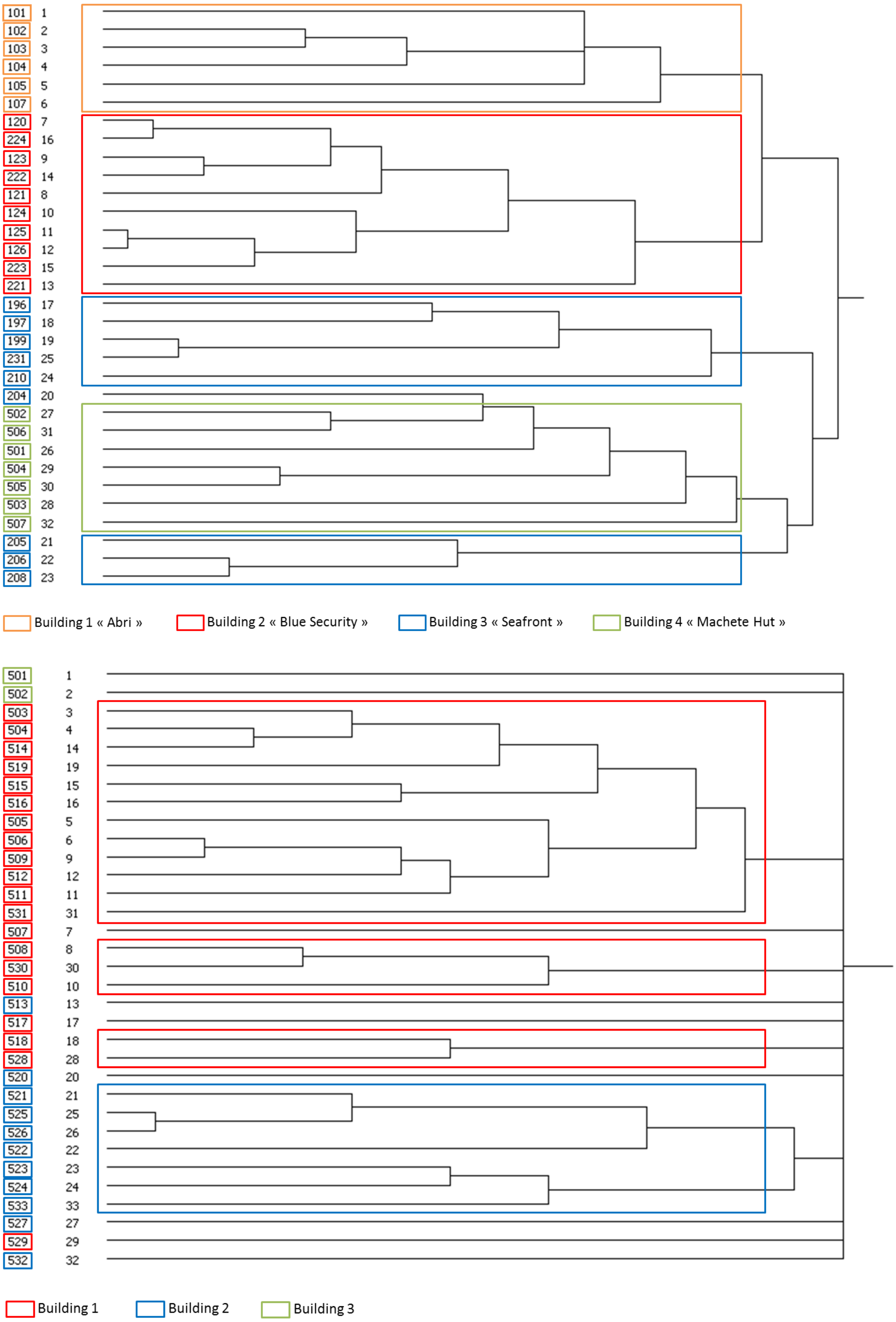


Fig. 3. Dendograms of the hierarchical cluster analysis performed in UCINET for the 2005 and 2009 populations of *P. canadensis*. Numbers on the left are nest labels. The coloured squares around nest labels represent the buildings in which nests are found (see legend). Numbers on the right are rows of the drifting data of each nest on the data matrix used for this analysis (e.g. drifting data of nest 501 in the 2005 data (second tree) were in row 1 of the data matrix).

Connectivity between and within aggregations

There was a low level of drifting between aggregations (Fig. 4) for the 2009 data (2009C1 to 2009C4), where all 4 aggregations are closely located and were studied with some overlapping time periods. The edges between aggregations 2009C2 and 2009C3, and 2009C4 and 2009C3 were due to a single drifter for each pair of aggregations (3.0±0.85 visits; 1-6 visits per drifter). Each of these drifters visited only one nest in another aggregation (Fig. 4a).

In the two 2005 aggregations 2005S1(blue), 2005S2(red), and 2005S3(yellow) (third building regrouping only 2 studied nests) where nests are also closely located and studied simultaneously, we also detected low level of drifting with directed edges (one-way interactions) from 2005S1 and 2005S3 towards aggregation 2005S2 (20 days of monitoring)(Fig. 4b). Edges were also attributed to a single drifter for each pair of aggregation and each of these drifters visits only one nest in the other aggregation (range: 1-3 visits per drifter). For the 2010 aggregations we were unable to look at between aggregations drifting since both 2010SF and 2010MH were monitored at 2 different time periods.

Aggregations also differed in the extent to which nests within them were connected. Edge density (number of observed edges between nests over the total possible connections within an aggregation), which provides a measure of connectivity varied across aggregations (see Table 1) and was significantly affected by the size of the aggregations (GLM, aggregation size: z= -2.65, p=0.037) but not by aggregation shape (length:width; GLM, aggregation size: z= 0.04, p=0.97).

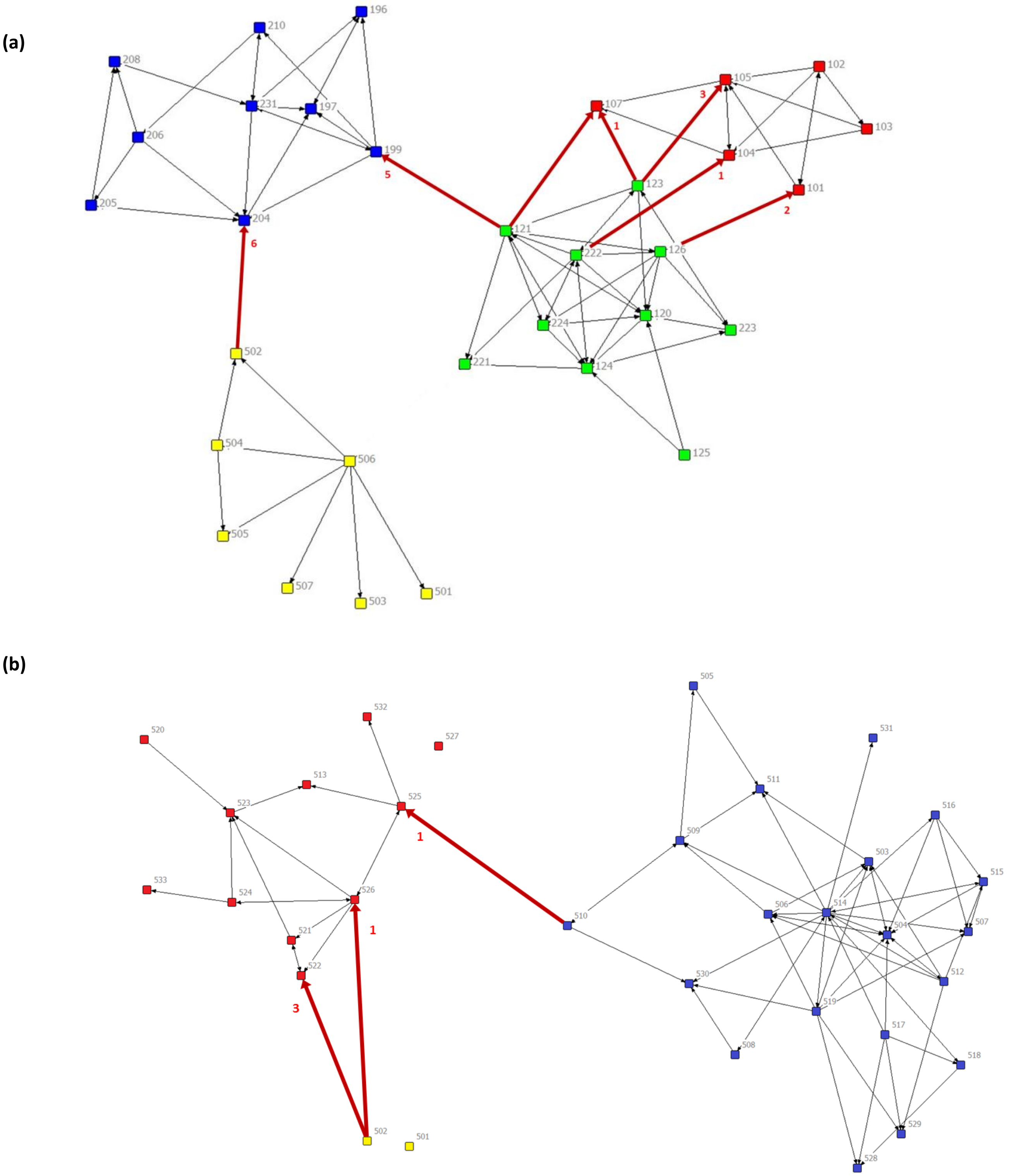


Fig. 4. Social network of (a) the four 2009 aggregations and (b) three 2005 aggregations (equivalent to buildings). Coloured squares (nodes) represent nests (numbers in grey are nest labels; nests from the same aggregation are colour coded). Arrows (directed edges) represent drifting events between nests. Edges highlighted in red correspond to drifting events occurring between aggregations. The number of drifting events between nests is only given between aggregations edges (see numbers in red). Distances between aggregations are not in scale.

Hypothesis 1: networks of drifters are non-random

The comparison of the observed drifting network with simulated random networks revealed that the drifting patterns we observed were unlikely to be random. Indeed we found no significant correlation between our simulated random networks and the observed drifting network (QAP correlation between networks, 5000 permutations, all p>0.05) for each of the 8 studied aggregations. Drifting is unlikely to be accidental events due to discrimination errors since we found either high and low level of drifting rates across nests (Fig. 5). However, we found mostly low drifting rates with 43% of nests having less than 10% of wasp recorded as drifters. Surprisingly, we found that 19.3% of nests show extreme levels of drifting rates (>60% of records were from drifters). We have to be cautious with this high levels since they may be artefacts – e.g. a wasp which was in fact drifting when she was tagged, is therefore assigned to the wrong natal nest.

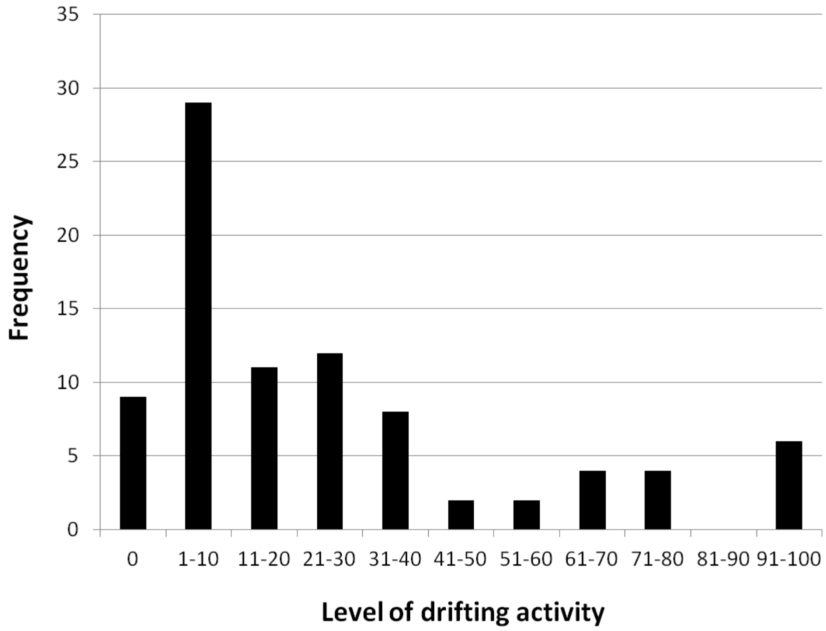


Fig. 5. Frequency distribution of level of drifting activity per nest (drifting rate in %: number of in- and out-degree from drifters over total number of events for each nest) (Shapiro-Wilk test for normality, p=0.02, data have a non-normal distribution)

Variation was also found in the frequency of in- and out-degree such that not all nests are either source or sink of drifters (Fig. 6). The GLMM analysis revealed that specialization as source or sink nests (estimated as the difference between the in- and out-degrees) was influenced by the size of the nest (GLMM, group size: z= -2.58, p<0.015) with larger nests being more likely to be source nests (specialized in out-degrees). Importantly, the presence of parasites did not significantly influenced the likelihood of nests being sources or sinks (GLMM, parasitism: z= -0.40, p<0.69), suggesting that drifters are not actively distributing their efforts in non-natal related healthy nests (n=93 nests).

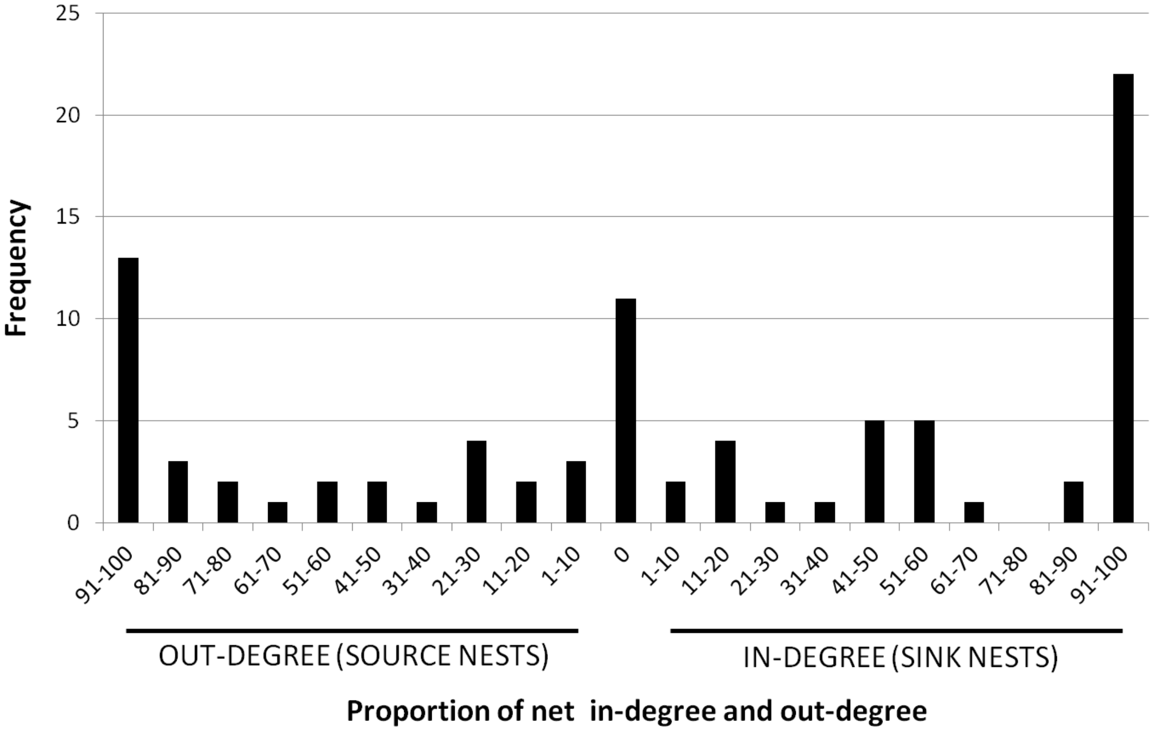


Fig. 6. Frequency distribution of the difference in proportion between in- and out-degree at the nest level. (Shapiro-Wilk test for normality, p=0.16, data have a normal distribution). The nests on the left side have higher proportion of out-degrees recorded, nests in the right have higher proportion of in-degrees recorded; nests at 0 have the same level of in- and out-degrees)

Hypothesis 2: Drifting patterns are consistent over time

We found evidence of persistent edges in the 2005 aggregations which reflect the occurrence of regular and frequent visits between specific pairs of nests, consistent over time (5-20 days). Over the entire monitoring period, 6 pairs of nests had persistent edges (8.2% of observed edges). We also found 12 edges (16% of observed edges) that were persistent for at least 3 of the 4, 5-day monitoring periods, and 22 edges (30% of observed edges) that were persistent for at least 2 of the 4 monitoring periods (Fig. 7 and Fig. 8). The remaining 51 edges were more transient with detections occurring during only one monitoring period. Persistent edges correspond to a higher level of drifting between nests than transient edges, with on average 4.3±0.7 drifting events in any pair of nests (compared with 2.4±0.6 for more transient edges; unpaired t-test, p=0.04), and up to 31 drifting events for a pair of nests in a single monitoring period (max value =31 drifting events for persistent edges and 18 drifting events for transient edges.).

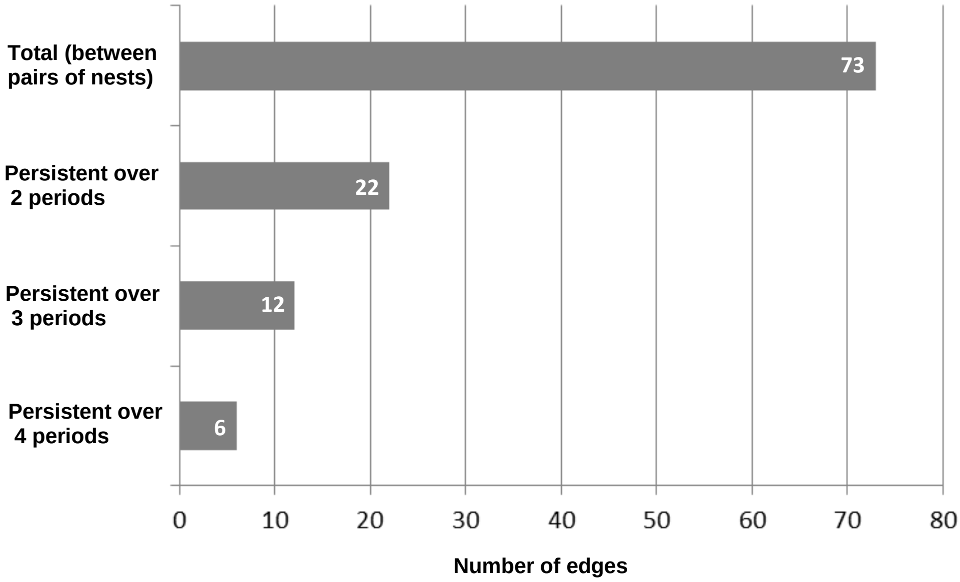
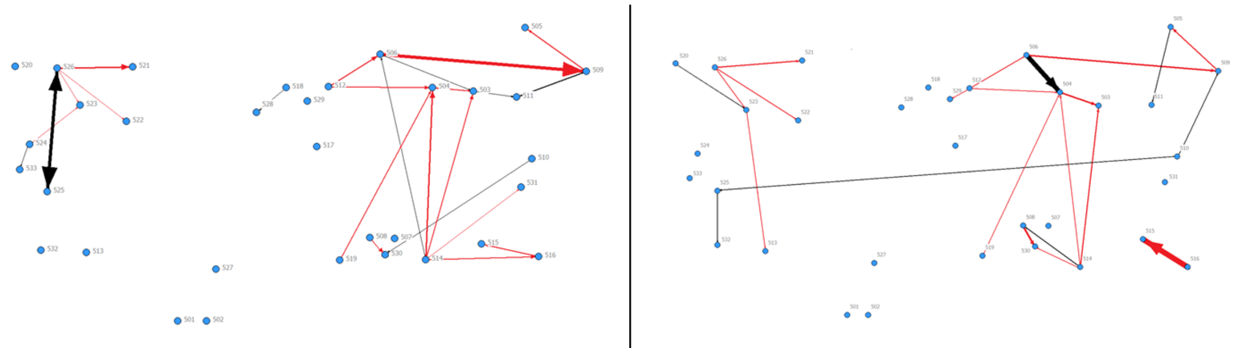


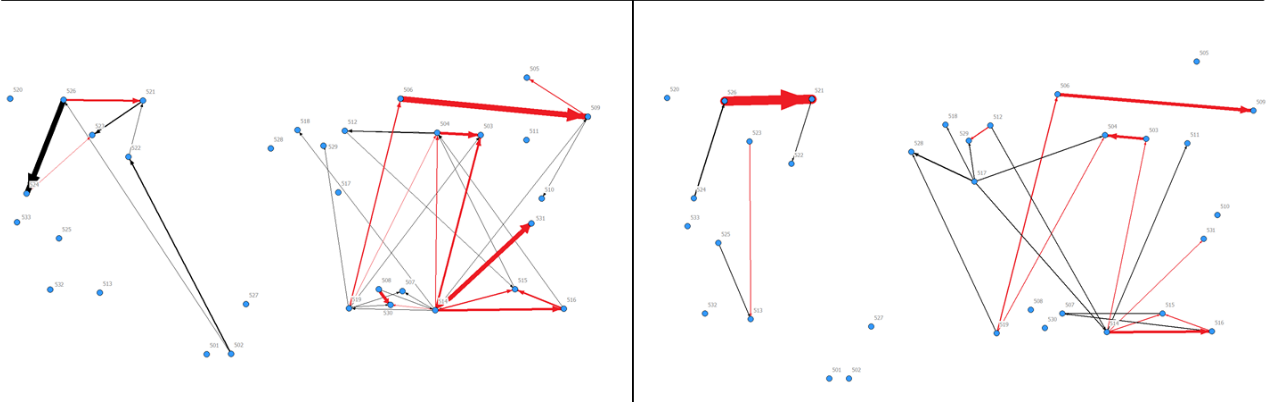
Fig. 7. Total number of edges and number of persistent edges over 2, 3 and 4 monitoring periods detected between each pairs of nests in the 2005 data.

Hypothesis 3: Factors influencing drifting patterns

Of the three explanatory terms investigate in the model two significantly influenced drifting rates. The first was “workload” (difference in worker:brood ratio between each pair of nests: i.e. the relative need for helping effort between a pair of nests) (GLMM, workload: z= 34.01, p<0.001). . The other was the “distance” between nests, such that drifters were more likely to visit neighbouring nests than those further away (GLMM, distance: z=-7.16, p<0.001). By contrast, relatedness did not significantly affect drifting rates (GLMM, relatedness: z=-1.05, p=0.29).



Monitoring period 1 - day 1 to 5 **Monitoring period 2 - day 6 to 10**



Monitoring period 3 - day 11 to 15 **Monitoring period 4 - day 16 to 20**

Fig. 8. Social networks of drifting events between nests from the 2005 aggregations for each of the 4 monitoring period. Edges in red represent persistent drifting events over time (edges observed at least in two monitoring periods). Black edges are drifting events observed only on 1 monitoring period (edges non-persistent over time).

Hypothesis 4: Drifters alter their helping effort on the nests they visit in relation to any changes in the need for help on these nests.

After experimental removal of wasps, wasps are responding strongly in their out-degree, (visitations away from the natal nest) but not in their in-degree (visitations to non-natal nests). We found that 12 out of the 14 nests altered their out-degree in the direction expected (i.e. decreased in-degrees) and only 2 out of the 14 nests altered their in-degree in the direction expected (i.e. increased in-degrees). Both in- and out-degree changes differ significantly from random (binomial test: out-degree, p=0.013; in-degree, p=0.013; Table 2), and from each other (chi-square tests: $\chi^2=11.57$, df=1, p<0.001).

Although this is true when wasps are removed, responses from drifting wasps seem to differ when brood is removed. After experimental brood removal, we found that 6 out of the 9 altered their out-degree in the direction expected and 4 out of the 9 nests altered their in-degree in the direction expected. In- and out-degree changes were not significantly different for nests where brood were removed (Fisher test (for small values), p=0.64) and both did not differ from random changes (binomial test: out-degree, p=0.5; in-degree, p=1; Table 2). This suggests that wasps are not responding strongly in their in- and out-degree when brood is removed.

As for the control nests (nests in the unmanipulated aggregations 2009C4 and 2005S1 and 2005S2), 26 of the 40 nests showed changes in in-degree after manipulation. We found a similar pattern for out-degree, with 21 out of the 40 nests showing changes. These changes did not differ significantly from random as expected for both in- and out-degree (binomial test: out-degree, p=0.08; in-degree, p=0.87) (Table 2). Moreover, we find no significant difference in the change in in- and out-degree among control nests (chi-square test: χ^2=0.89, df=1, p=0.35).

	Expected		Observed	
	in-degree	out-degree	in-degree	out-degree
Worker removal	significant ↑	significant ↓	2/14 nests ↑ - p=0.013*	12/14 nests ↓ - p=0.013*
Brood removal	significant ↓	significant ↑	4/9 nests ↓ - p=0.50 ns	6/9 nests ↑ - p=1 ns
Control	no change / random → non-significant	no change / random → non-significant	14/40 nests → p=0.87ns	19/40 nests → p=0.08ns

Table 2. Expected and observed changes in in- and out-degree for removal nests (worker and brood removal) and control nests. Proportion of nests following the expected change are shown. Upward arrows indicate an increase and downward arrows a decrease in in- or out-degrees; Straight arrows represent no change. For both observed in- and out-degree changes, the significance of the binomial test used to test if changes are different from random are given.

DISCUSSION

Using our comprehensive social network approach, we are able to examine the complex structure of nests in populations of the primitively eusocial wasps *P. canadensis*. Our analyses revealed the existence of distinct clusters of connected nests. These aggregations of nests were tightly associated with the building on which the nests were built. Although our studied populations are found within man-made environment, their structure in aggregations (high density of nests) greatly resembles those found in natural environment, such as populations nesting in cave (Pickering 1980) or under branches and leaves from large trees (e.g. *Cecropia* trees)(Sumner *et al*. 2010; pers. obs.).

The structure of *P. canadensis* populations seem to be centred around these aggregations in which nests exchange wasps. However, a few individuals have also been observed drifting among aggregations. These interactions represent only few visits (between 1 and 6 detections) and are known as weak links in social network analysis. Weak links are not exclusive to between-aggregation interactions but are also commonly found within each aggregation. The precision of data offered by our monitoring methods (continuous RFID monitoring) allow us to detect such weak links and collect all the interactions present in our population of *P. canadensis*. Weak links have long been studied in various social systems but rarely in animal systems (Krause *et al*. 2009). They are usually important links for the cohesion of social networks and often have a different meaning of interactions compared to strong links (Granovetter 1983). In our study, they may correspond to assessment visits by drifters where they assess the general quality of the nests before any investments. However, further investigations are needed to confirm this assumption.

As reported in previous studies (Sumner *et al*. 2007), there was a high rate of drifting with a percentage of drifting varying between 13.5% and 45.3% depending on the aggregation. Such high levels of drifting exceed most natural drifting records in other social insect species. For instance in natural colonies of the honey bees *Apis florea* and *A. cerana*, only 4-5% of the individuals within a colony are drifters (Nanork *et al*. 2005; Nanork *et al*. 2007). More similar levels are found in natural and artificial colonies of bumblebees, with around 20-30% of bees drifting to other colonies (Paxton *et al*. 2001; Lopez-Vaamonde *et al*. 2004; Birmingham *et al*. 2004; Blacher *et al*. 2013).

Although many studies suggest that drifters may benefit from fitness by visiting others nests (Neumann *et al*. 2000; Paxton *et al*. 2002; Lopez-Vaamonde *et al*. 2004; Nanork *et al*. 2005; Sumner *et al*. 2007; Takahashi *et al*. 2010; Blacher *et al*. 2013), it is generally not clear whether visits from drifters are deliberate processes or derive from navigational and discrimination errors. In our study of the primitively eusocial wasp *P. canadensis* several lines of evidence suggest that drifting is not accidental. Firstly, drifting patterns found in *P. canadensis* are unlikely to be accidental events considering the variation in drifting rates we found between nests. If drifting was resulting from errors, we would expect levels of drifting to be low since accidental visits are likely to be occasional. Secondly, we found that some interactions between nests (or edges) were consistent over several periods of time (in at least 2 over the 4 monitoring periods of 5 days). This further suggests that drifters appear to be making clear decisions to visit these specific nests over repeated time periods (up to 4 times 5days) and that the drifting patterns we observed are not random. Thirdly, our GLMM analyses revealed that the rate of drifting was influenced by two factors. The first was the relative amount of help needed (workload) between the pair of nest studied. This suggests that drifters apportion their visits and therefore their helping effort in response to the relative needs of their natal colony and neighbouring colonies. The second factor influencing drifting patterns was the distance between nests. Drifters were more likely to visit neighbouring nests than those further away within the aggregation. Interestingly, relatedness did not explain drifting patterns as suggested in a previous study (Sumner *et al*. 2007). However, in this previous study, they did not take into account the distance between nests within aggregations but only compared relatedness between drifters and adult from the same aggregation (i.e. drifters group) and between drifters and adults from outside the studied aggregation (non-drifters group).

Since proximity and relatedness are inversely related in this species (Lengronne *et al*. 2012), helping close neighbours still enables drifters to assure that their effort will be directed towards related nests.

Although GLMM analyses such as those performed in this study allow to test large amount of data, they assume that all data are independent. Since nests are interconnected, a potential caveat of our analysis is that we did not account for this non-independence of data. It would be interesting to repeat our analyses using improving social network tools such as QAP (quadratic assignment procedure) linear regressions which account for the non-independence (Krackhardt 1988) using the same response and explanatory variables and compare to results of our GLMM.

Another interesting finding, from our brood and worker removal experiments, was that drifters' responses were more influenced by changes occurring in the natal nest (source nest) than non-natal nests (sink nests). There are two potential explanations for this finding. First, wasps spend more time on their natal nest than their drifter nests (see section I) and thus may have better access to information on the needs of the nest. Second, since within-nest relatedness (mean relatedness±s.e.; 0.69±0.02) is known to be higher than between-nest relatedness (mean relatedness±s.e.; 0.12±0.01) in *P. canadensis* (Lengronne *et al*. 2012), drifters may be maximising fitness by adjusting help for the brood which offer them the greatest indirect fitness payoffs (i.e. close relatives).

Our study provides new insight on the mechanisms involved in nest-drifting behaviour in *P. canadensis.* We reveal that drifting is unlikely to be accidental in *P. canadensis* and that drifters are making clear decisions in visiting specific nests. Nest-drifting was assumed to be challenging kin selection theory since some workers were observed visiting neighbouring nests instead of focusing their help solely on their natal nest (to whom they are the most related). We provide evidence that drifters appear to favour help in their natal nests before investing in others. Drifters are likely to invest in closely located nests only when conditions of their natal nest are favourable. Considering the structure of nest aggregations in *P. canadensis*, by visiting neighbouring nests, drifters may increase their chance to invest their help in related nests from which they will gain indirect fitness benefits. With the availability of range of new tools such as social network analyses we are able to improve our understanding of intricate dynamics of groups such as nest-drifting in the primitively eusocial wasp *P. canadensis* and to further highlight the complexity of helping behaviour and adaptability of social insects.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swiss National Science Foundation (to TL and LK) and a NERC New Investigator grants (NE/G000638/1) and Royal Society Research grant to SS.

GENERAL DISCUSSION

The findings of this PhD shed light on the explanations for nest-drifting in *P. canadensis* and revealed some of the mechanisms involved in this behaviour. Essentially, the results showed that nest-drifting occurs mostly within aggregations of related nests and is likely to be a strategy whereby wasps can benefit from indirect fitness by helping in neighbouring nests when their effort in the natal nest is less needed. Additionally, this PhD provides information on the genetic structure of paper wasps' populations and demonstrates the role of inactive (lazy) wasps as a "reserve worker force" which provides resilience to the colony in the event of worker mortality.

More generally, this thesis contribute to a better understanding of the social dynamics of tropical paper wasps and further highlights the complex organization and adaptability of insect societies as well as the plasticity of their behaviours. Although, this work constitutes a solid background on nest-drifting and its mechanisms, further work is required to identify all the mechanisms involved and entirely elucidate the potential adaptive value of nest-drifting in the various species it has been reported.

In the first chapter, I compared colony and population genetic structures in two species of primitively eusocial paper wasps with contrasting ecologies: the tropical species *P. canadensis* and the temperate species *P. dominulus*. This study revealed that seasonal constraints on the life cycle of primitively eusocial wasps have only little effect on population structure, contrary to what were suggested in previous studies (Hamilton 1964b). Indeed, genetic structures of *P. canadensis* and *P. dominulus* were very similar. For both species, the high genetic differentiation between nests suggests strong selection at the nest level to live with relatives, whereas low population viscosity and low genetic differentiation between nest aggregations might reflect balancing selection to disperse, avoiding competition with relatives. Evidently, in order to support these results, further work on the population structure of other temperate and tropical of species of *Polistes* is required.

Moreover, nest-drifting in *P. canadensis* has previously been suggested to have evolved from viscous populations (Sumner *et al.* 2007). Indeed, by helping neighbouring nests in viscous populations, drifters would benefit from indirect fitness by visiting the closer and therefore genetically more similar nests. My study data revealed no (or little) isolation by distance in *P. canadensis*. However, nests within our studied cluster of nests were on average related which suggest that, even in non-viscous populations, drifters may still be able to benefit from indirect fitness by delivering help in neighbouring nests.

The population structure analysis in this study was performed on a population of wasps leaving in semi-natural environment such as the overhangs or ceilings of abandoned buildings. Although, at first sight, they may resemble those from more natural population (caves/trees), the topography of the nesting sites are likely to be a factor influencing the distribution of nests and therefore the dispersal behaviour of individuals. Nests may be more confined within walls in comparison with aggregations observed in trees for instance. It would therefore be of interest to duplicate this study of population structure in more natural, open populations of *P. canadensis* and compare with our results to determine whether differences in population structure exist between open and restricted nest places. The same impact of the topography may relate to the level of drifting we find in our populations of *P. canadensis*. Since the density of nests may be higher within buildings, wasps may be more likely to visit neighbouring nests than in more open areas where travel distance may be larger.

In the second chapter, I investigated whether nest-drifting and inactivity of workers have potential adaptive value in *P. canadensis*. This study revealed that lazy or inactive workers appear to fully compensate for the loss in foraging effort in their nest through the initiation of foraging. Lazy behaviour in *P. canadensis* workers appears to be adaptive providing resilience to colonies in the event of worker mortality. Moreover, via their “recruitment”, inactive workers allow fitness of helpers to be assured. Drifters from neighbouring related colonies, however, did not compensate for the loss in workers even though they stood to lose inclusive fitness should the brood die.

It would be interesting to investigate to what age group, if any, the drifters belong. Age or temporal polyethism, whereby workers change tasks with age, has been reported in many social insects (Robinson 1992) but only evidence for rudimentary temporal polyethism has been found in *Polistes* wasps so far (Giray *et al*. 2005; Shorter and Tibbetts 2008; Torres *et al*. 2013). Likewise, only little is known about these lazy workers. It would also be interesting to investigate whether lazy workers belong to a certain age group. Many of the lazy wasps we monitored in this work are likely to be recently emerged wasps. Young wasps are usually seen inactive on the back of the nest for a few days (pers. obs.).

In the third chapter, I examined the complex structure of nests in *P. canadensis* and investigated drifting patterns to determine on what scale drifting occurs and what are the mechanisms involved. This study revealed that drifting is mainly centred around specific aggregations of nest which exchange wasps. Very few visits were detected between aggregations. An important finding of the study is that drifting patterns in *P. canadensis* are unlikely to be accidental. We find variation in the level of drifting between aggregations with up to 45% of wasps detected as drifters. Such high levels of drifting are similar to previous studies where up to 52% of wasps were drifters (Sumner *et al.* 2007). Additionally, interactions between nests were consistent over time. This further suggests that drifters appear to be making clear decisions to visit these specific nests over repeated time periods (up to 4 times 5 days) and that the drifting patterns we observed are not random.

Furthermore, by removing brood and workers from nests, we revealed that the response from drifters to changes in colony need was more pronounced in their natal nest than any other nests. Nest-drifting has been considered as a behaviour which potentially challenges the kin selection theory since some workers spend time in other nest than their natal one. Unlike what was previously assumed, this PhD revealed that nest-drifting is in fact in accordance with the kin selection theory since drifters in *P. canadensis* favour their help in the natal nests, and therefore the nest where they may benefit the most in terms of fitness. When no help is needed in their natal nest, they may invest in other neighbouring nests. Considering the structure of these wasp populations, these neighbouring nests are likely to be related nests from which they may benefit from indirect fitness by helping in collecting resources.

Finally, this study provides evidence of two factors influencing the drifting patterns of *P. canadensis*. The first factor is the relative amount of help needed (workload) between the pair of nest studied. The other factor influencing drifting patterns is the distance between nests. Interestingly, relatedness was not a factor explaining drifting patterns as suggested in a previous study (Sumner *et al*. 2007).

However, further potential factors are likely to strongly influence the rate of drifting. One such factor is nest quality. Since parasites are frequent in the tropics (Strassmann 1981), future studies should investigate the number of parasites and type of parasites present on the natal or visited nests of drifters and how it affects drifting. The type of parasites present within the nests is likely to have different impact on *P. canadensis* dynamics between nests. From our field observations, we found that the flesh flies (from the Sarcophagidae family) that parasitized the 2010 populations have very strong impact since their larvae were highly mobile inside the nest, creating tunnels between cells to feed on *P. canadensis* larvae and pupae. Additionally, their emergence as an adult fly resulted in the destruction of a large portion of the nest cells. Previous work on *P. canadensis* has already suggested that nest-drifting in this species may be a risk-spreading strategy whereby workers maximize their inclusive fitness by reducing the risk that all their investment is lost through whole-nest predation (Sumner *et al.* 2007).

Queen quality may also be an influential factor. Indeed, previous studies on other species of *Polistes* such as *P. dominulus* (Liebig *et al*. 2005), suggest that workers are able to assess the fertility of the queen by directly monitoring her reproductive output and to react accordingly by starting laying eggs. In the context of nest-drifting, we may expect drifters to prefer their visit on nests where the queen has a high reproductive output from which they may maximize their indirect fitness benefits. Monitoring of the queen reproductive output (i.e.

number of egg laid per day) may be of importance to study the potential impact on worker movements between nests.

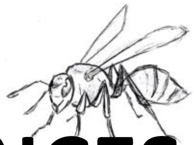
Nest-drifting behaviour has received a surge of interest in a variety of social insect species recently, revealing that there is still much to learn about this behaviour. Drifters of *P. canadensis* are assumed to be exclusively helpers since they have been observed returning with food on the visited nests (pers. obs). Moreover, in a previous experiment performed in *P. canadensis*, the authors also found that drifters behaved more as workers than queens since they were foraging and not egglaying (Sumner *et al.* 2007). However, drifting in many species has been suspected to provide direct fitness benefits (Lopez-Vaamonde *et al*. 2004; Birmingham *et al*. 2004; Ulrich *et al*. 2009; Blacher *et al*. 2013) with drifters laying eggs on visited nests. It would be interesting to collect eggs in nests where drifters have been observed and use molecular tools to investigate whether some of the eggs arise from drifters. Although drifters in *P. canadensis* appear to help in the nest they visit, some of them may try to lay some eggs of their own or be queuing to later compete to inherit the queen position in order to gain direct fitness benefits. Behavioural observations of drifters, examinations of their hierarchical status in the nest they visit and observations of ovary development would be necessary to provide more information on this topic. Unfortunately, the methods adopted in this PhD which mostly made use of the RFID equipment did not allow us to easily observe and capture drifters and therefore to perform such analyses.

Studies on nest-drifting in *P. canadensis*, (Sumner *et al.* 2007; and this PhD) suggest that movements of wasps between nests are likely cooperative actions with drifters helping in visited nests and hence benefit from indirect fitness. To my knowledge, *P. canadensis* is the only species, so far studied, to show such a particular case of drifting in natural conditions.

Cases where workers visit other nest(s) are also frequent in ants (Debout et al. 2007). Most of them involve specific types of colony structures called “supercolonies” or “polydomous” colonies (reviewed in Debout et al. 2007). Such ant colony systems have revealed the substantial social complexity occurring in ant colonies compared to other species of eusocial insects where no similar degree of social organization has been identified. Polydomous colonies are characterized by a more or less complex network of interactions between nests, generally including, among others, movements of brood and sexuals (Debout *et al.* 2007). Polydomy is represented in more than 166 species as referred in the list available in Debout *et al.* 2007. Polydomous nests embody various forms and may contain either one queen (monogynous) or several queens (polygynous). The common characteristic of polydomous colonies is the arrangement of spatially separated nests (with distance between them larger than the distance between the chambers of one nest) which house both workers and brood (Debout *et al.* 2007). Ants travelling between nests are therefore helpers that are likely to gain indirect benefits by visiting closely located and related nests. A specific form of polydomy includes unicoloniality or supercolonies and is associated with low genetic population structure. Supercolonies are usually referring to large scale population of introduced ants (e.g. fire ants, Argentine ants). These interactions between polydomous colonies in ants resemble nest-drifting behaviour as found in *P. canadensis*. Indeed, workers are moving between nests and seem to be helpers in the neighbouring nests. However, one particular difference which can be observed is that, in polydomous colonies, there is usually no aggression between workers, whereas in aggregations of nests in *P. canadensis,* not all wasps are allowed to land on other nests and only drifters seem to have the potential to move freely between their natal and visited nest(s).

Future research should focus on such phenomenon of drifter discrimination/recognition and investigate at a more proximate level, the mechanisms explaining how they are able to drift in these other nests. One particular question is how drifters are tolerated on the nests they visit. *Polistes* wasps have effective nestmate recognition system, in that they usually are highly aggressive against non-nestmate wasps. Performing tests in situ, such as exposing nests with familiar and unfamiliar nestmates and drifters, and monitoring the behaviour of nest members toward the presented wasps would be of interest to test this question. Potential hypotheses are that drifters may be particular type of wasps accepted in many nests or simply wasps with odours matching or similar to those of neighbouring nests. An interesting follow-up of this experiment would be to compare the chemical profiles of drifters and wasps of the host colonies to examine potential chemical similarities.

Finally, another likely explanation for the acceptance of drifters within host nests is that drifters are likely to provide the visited nest with resources, such as food or water, while returning from a foraging trip. Bringing such important resources to the host colony is likely to decrease the threshold for aggressive responses from host nestmates towards drifters and facilitate their acceptance within the nest.



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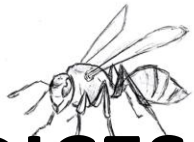
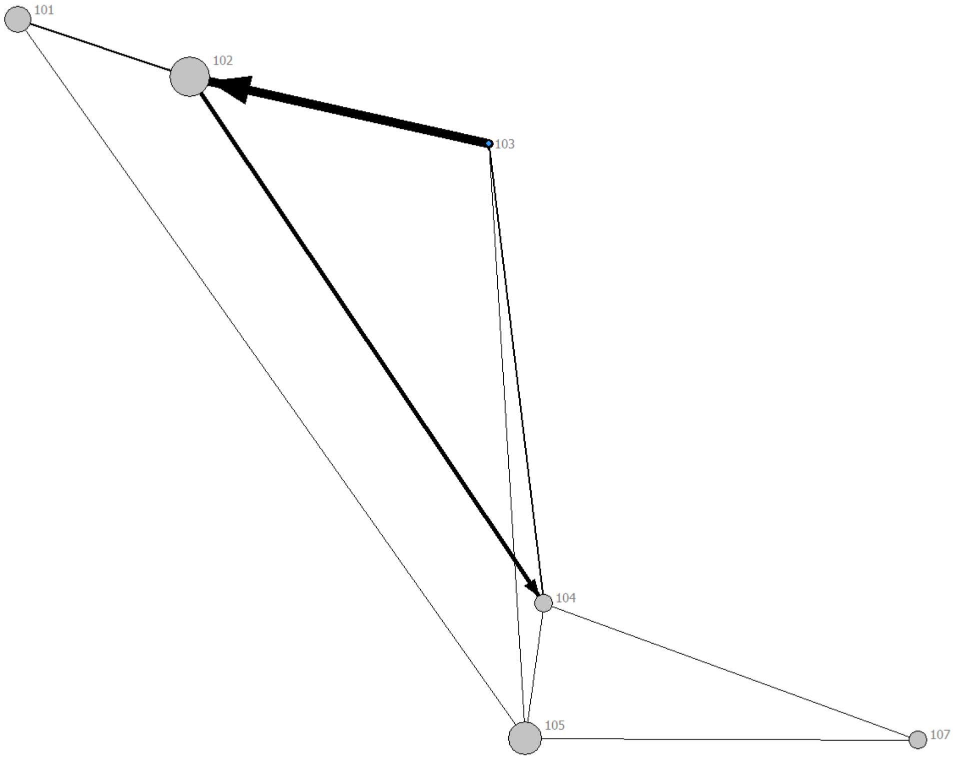


APPENDICES

APPENDIX 1: AGGREGATION NETWORKS

Nodes represent nests and their size represent the size of each nest (=total brood number). For a few nests, nest size was not available (see blank/white nodes). Nest label are numbers in grey, blue or red depending on the manipulation performed. Grey nodes are nests where no manipulation were performed (these include pre-manipulation nests). Red nodes are nests where brood was removed, and blue nodes are nests where wasps were removed (see chapter 3). Directed edges represent the direction and number of drifting events between nests (the bigger the arrow heads and lines, the higher the number of drifting events).

Network of aggregation 2009C1 pre-manipulation (5 days monitoring).



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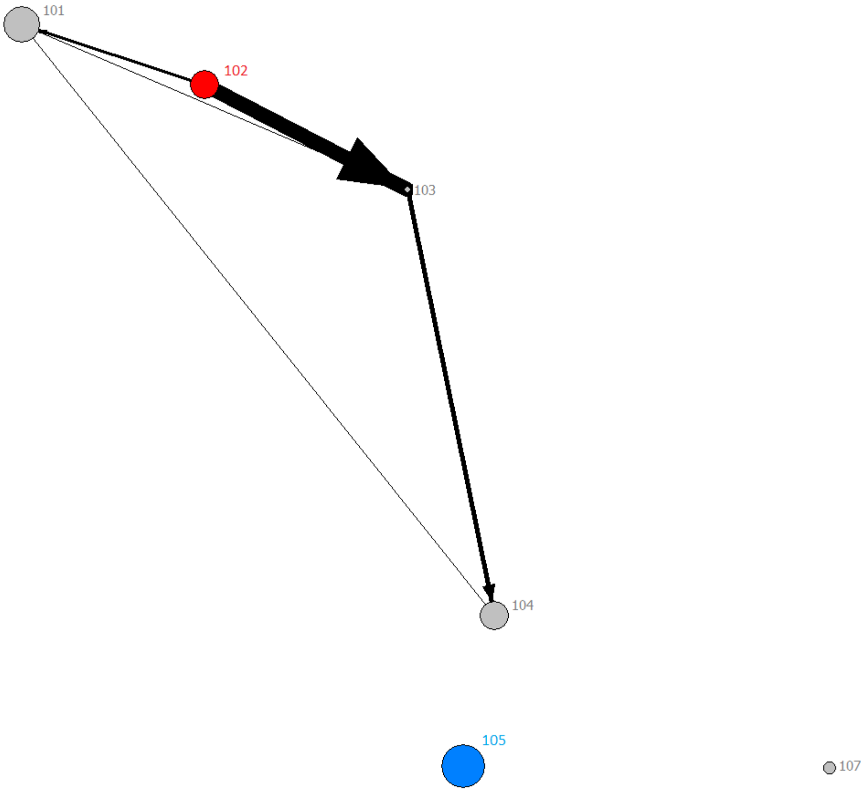

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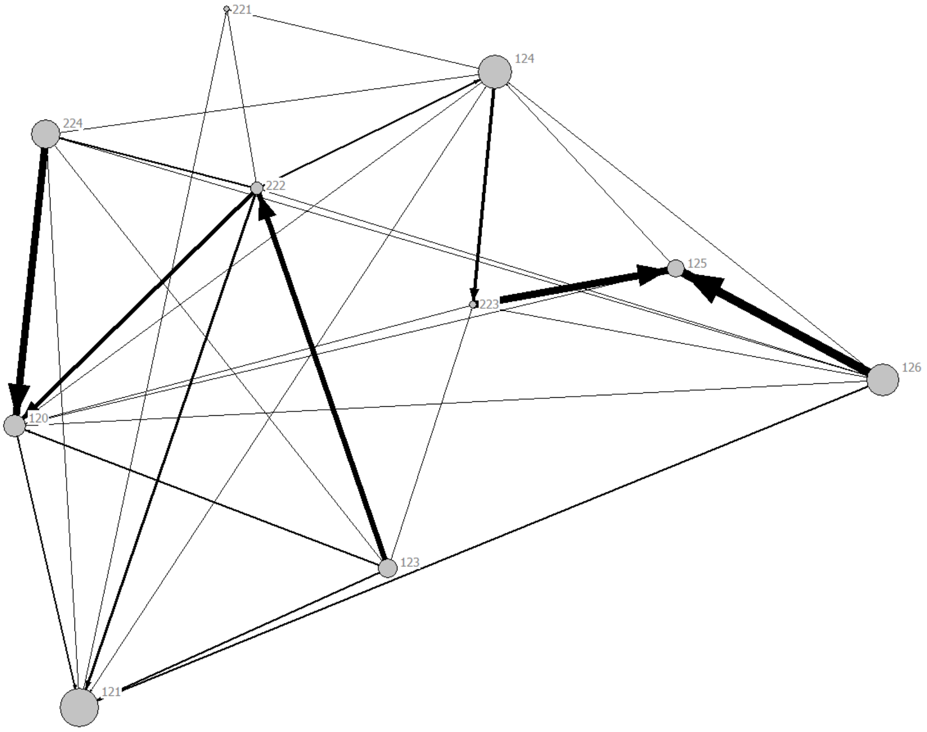

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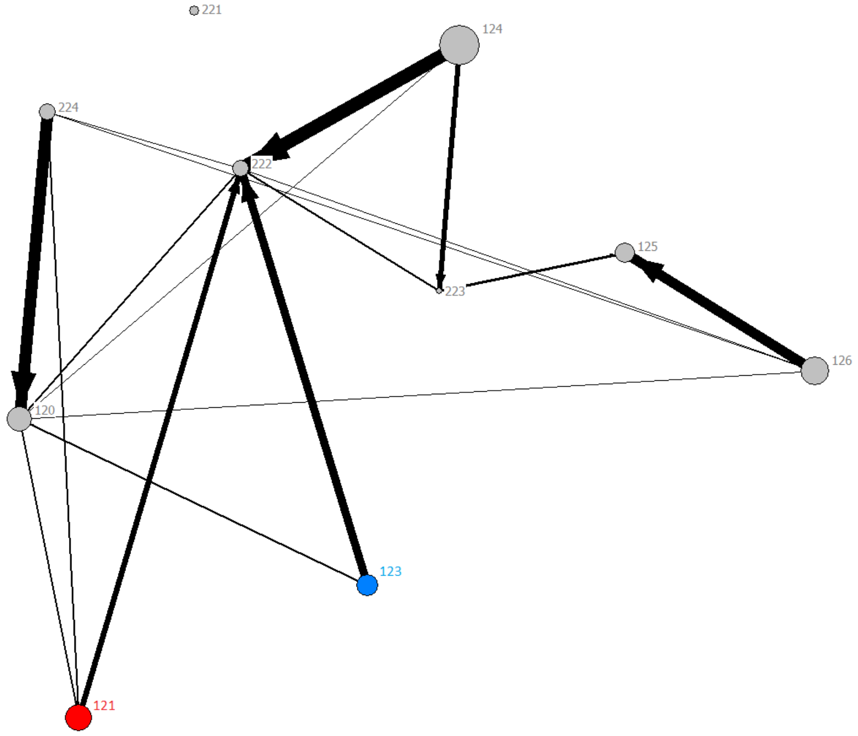

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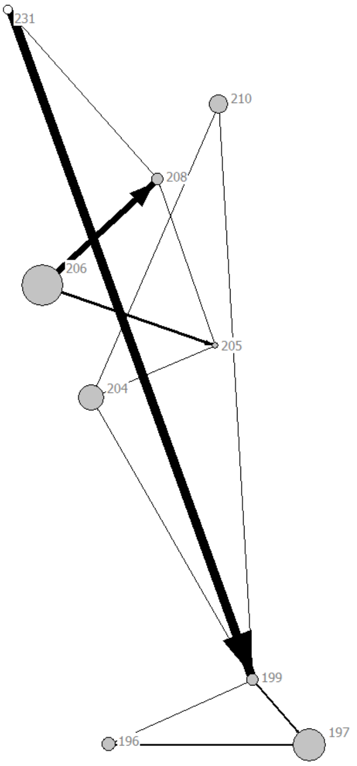

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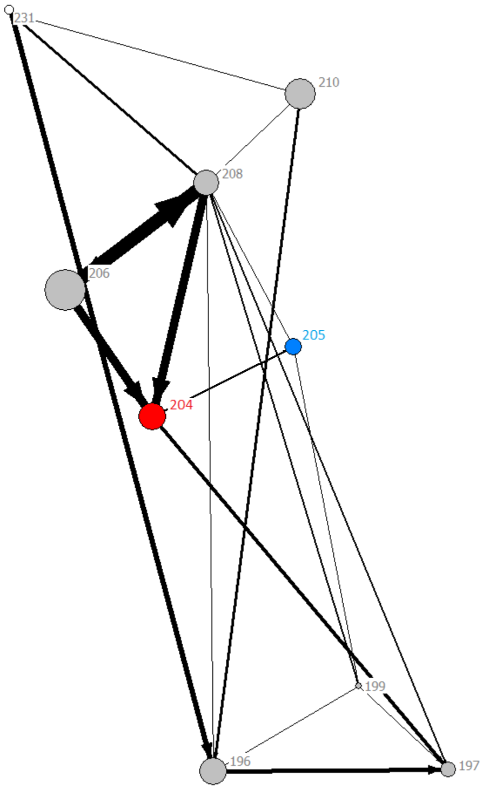

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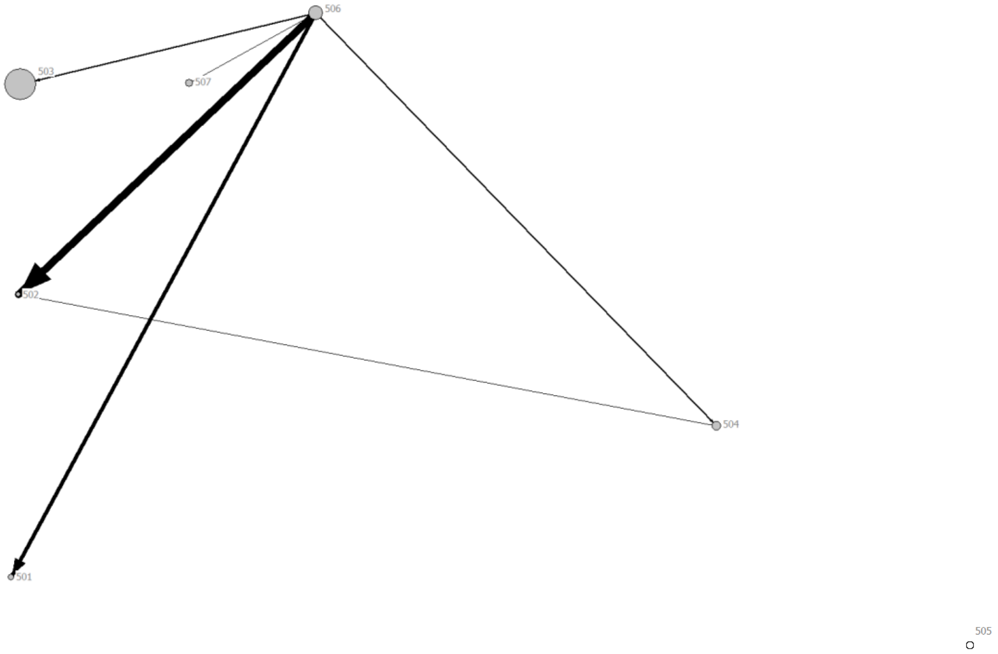

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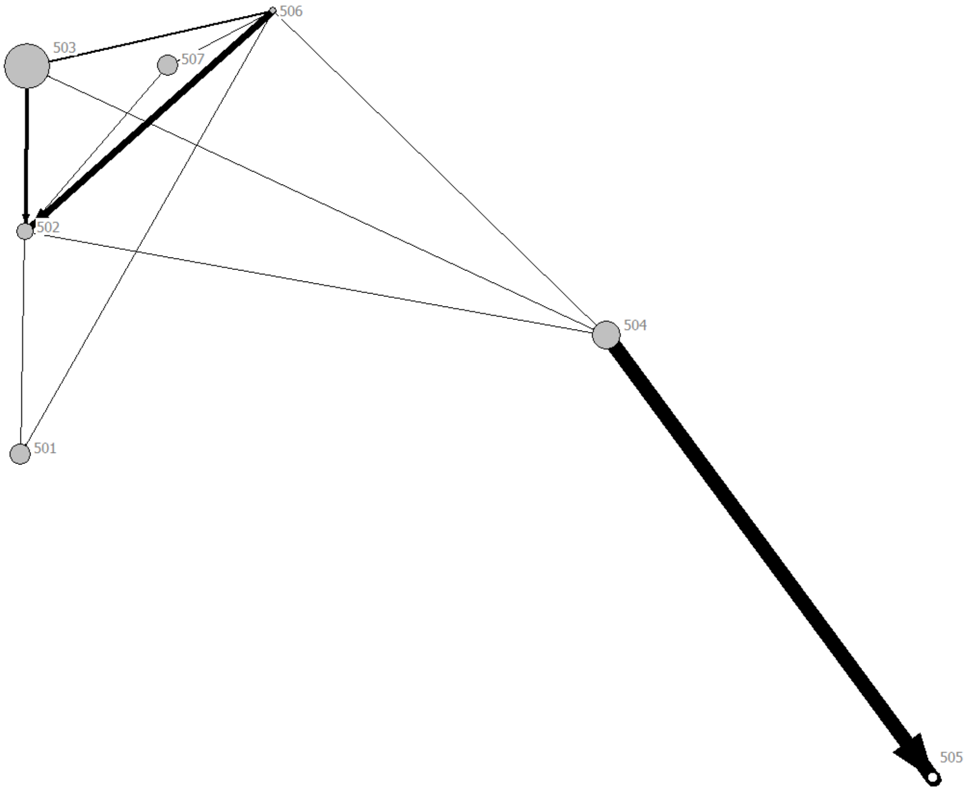

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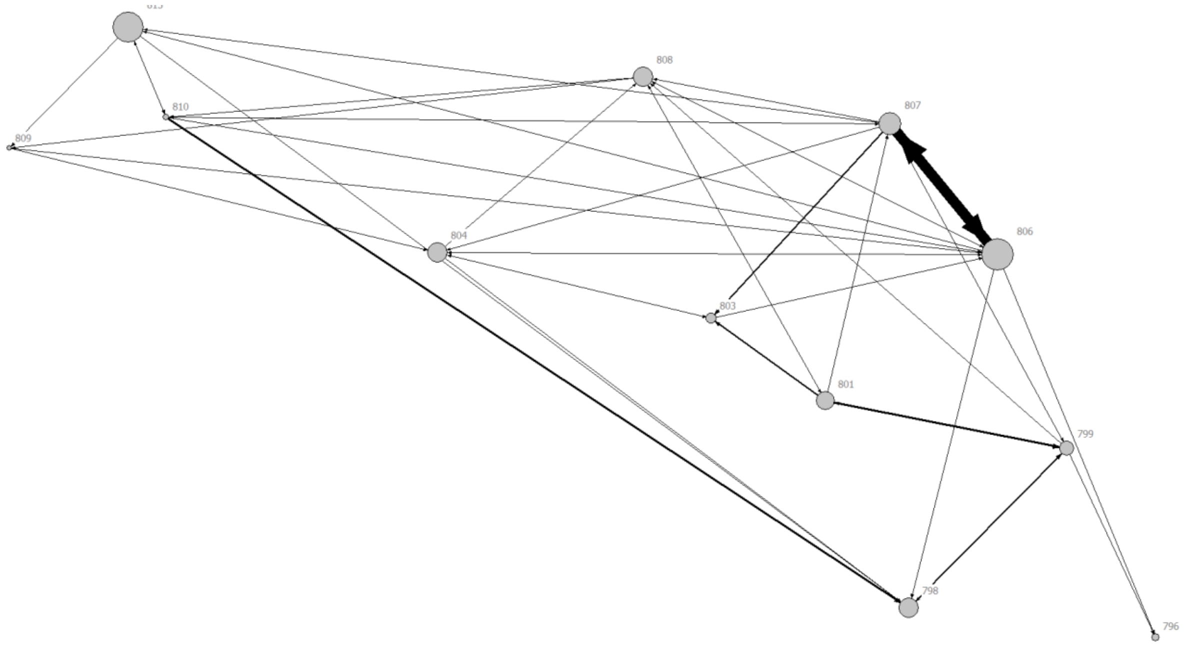

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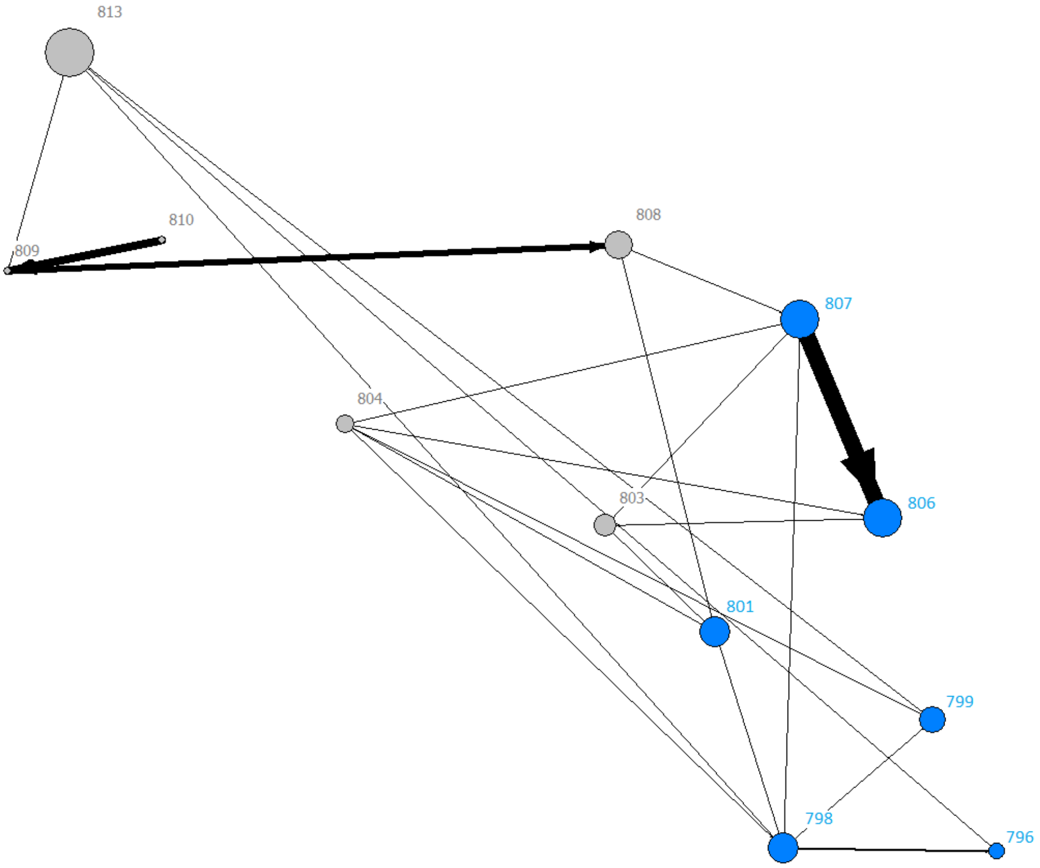

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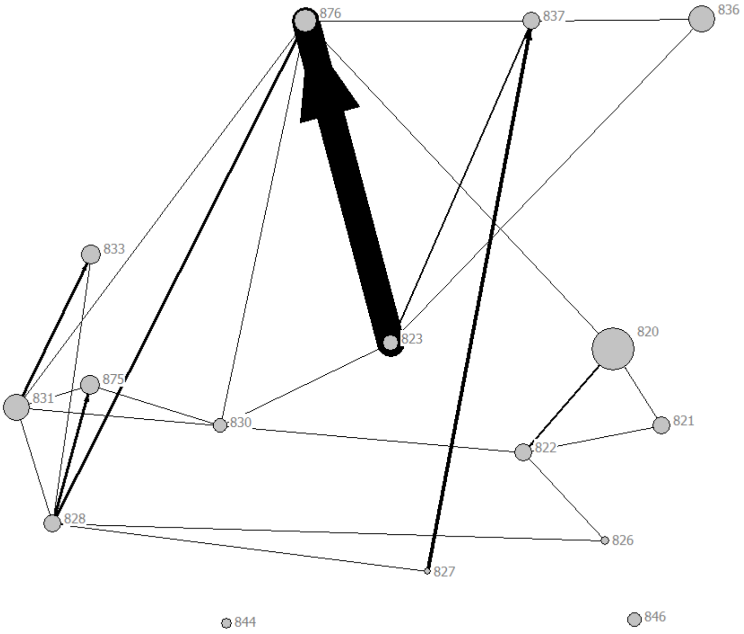

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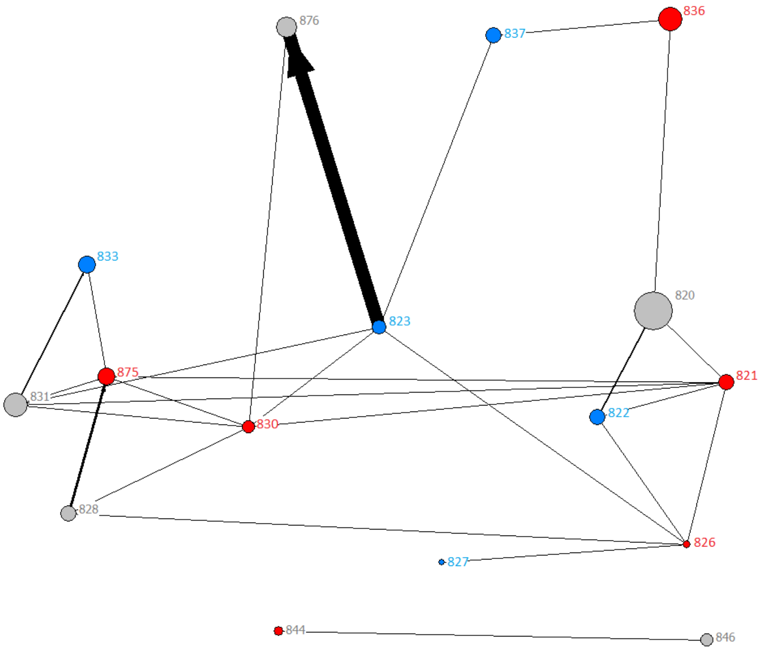

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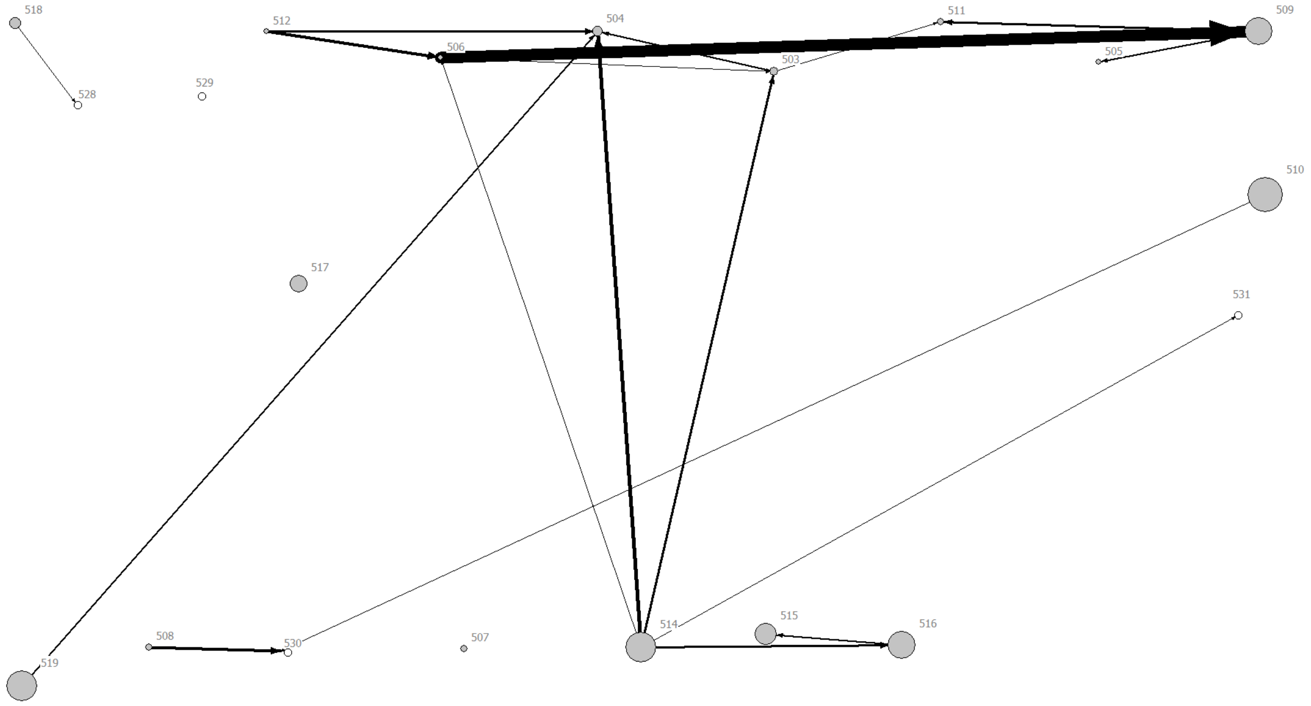

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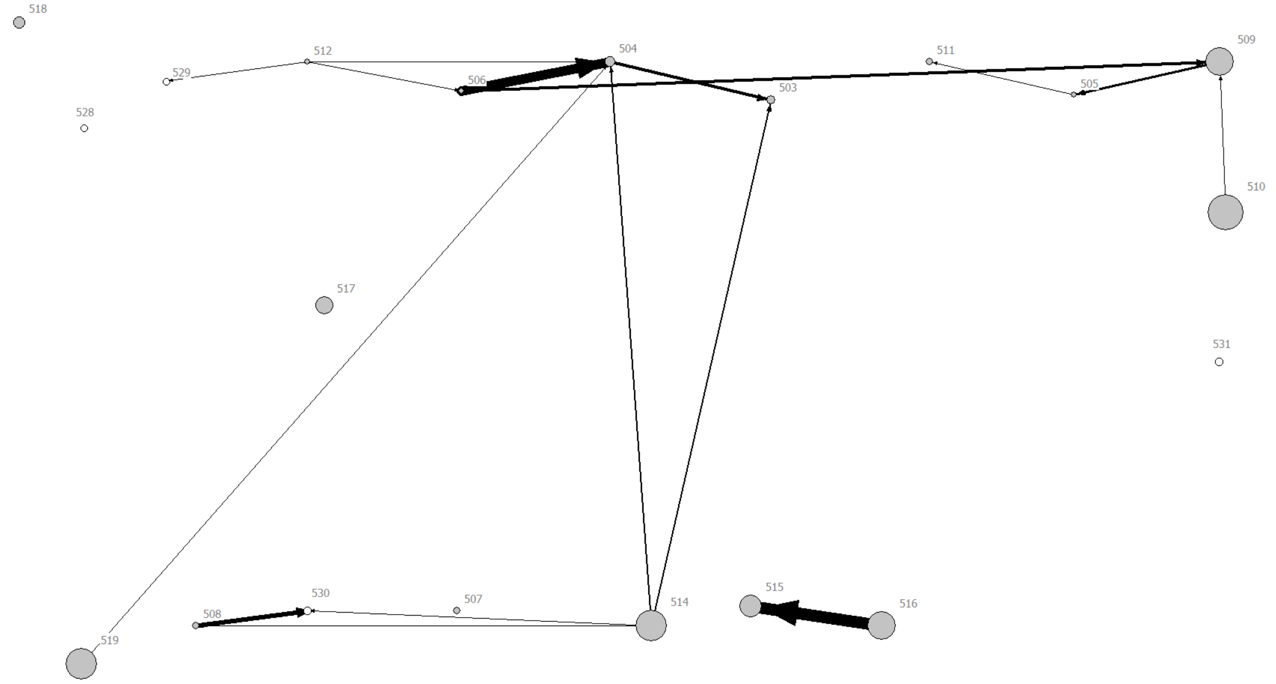

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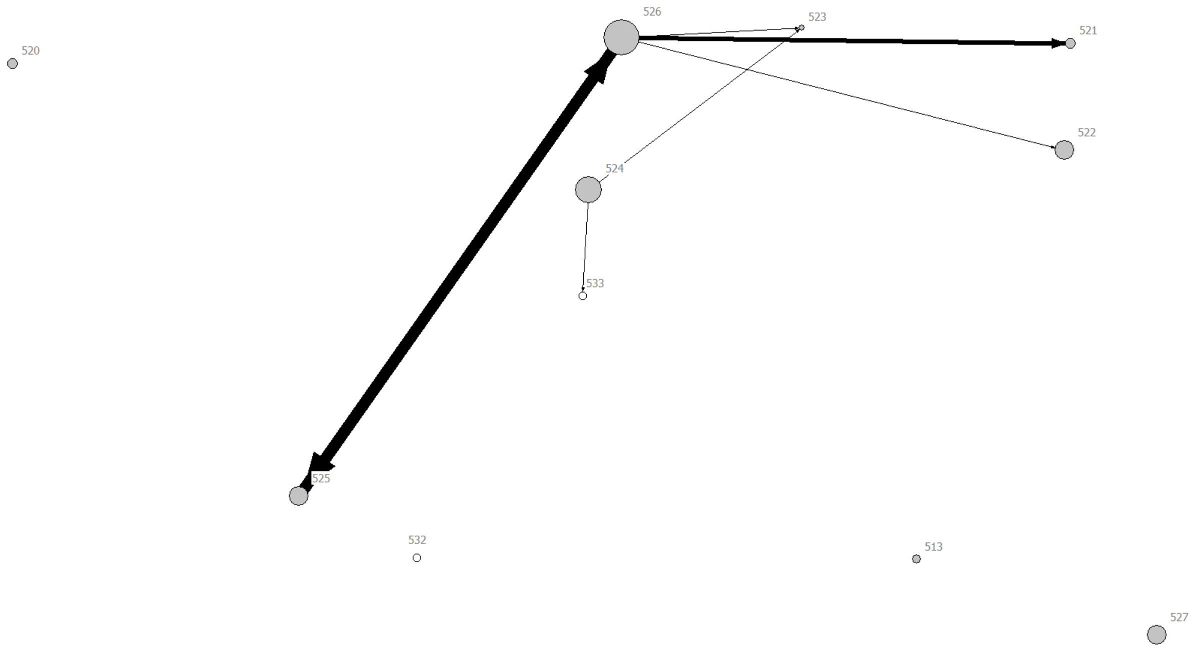

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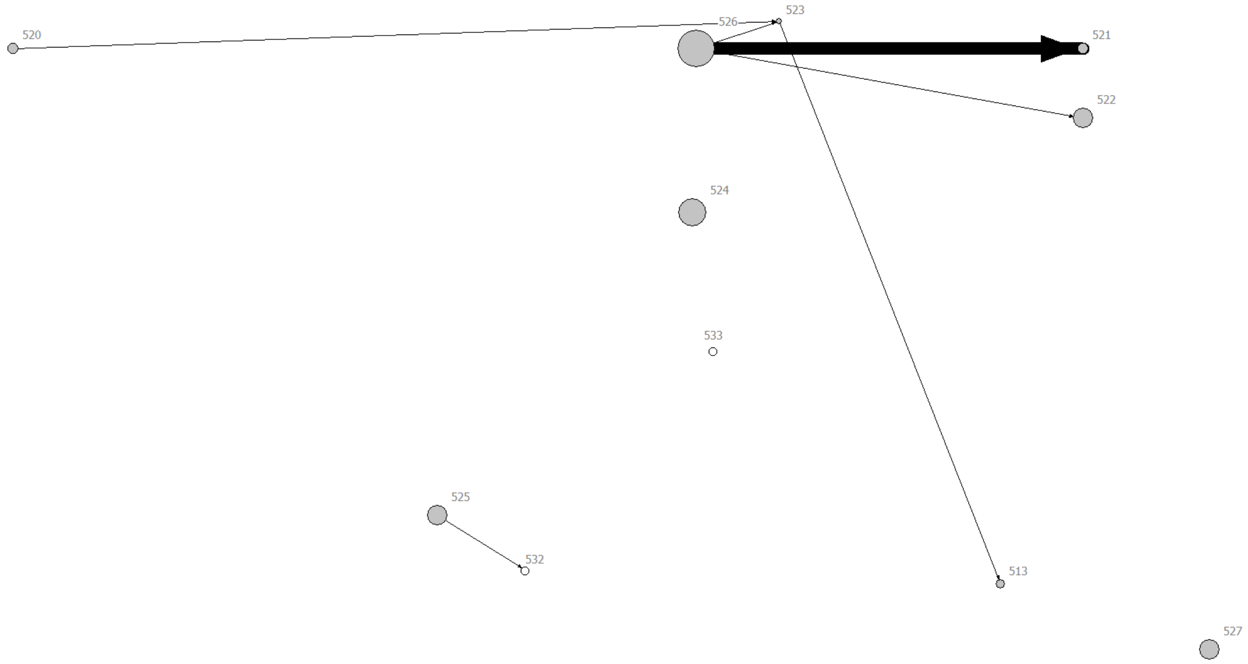
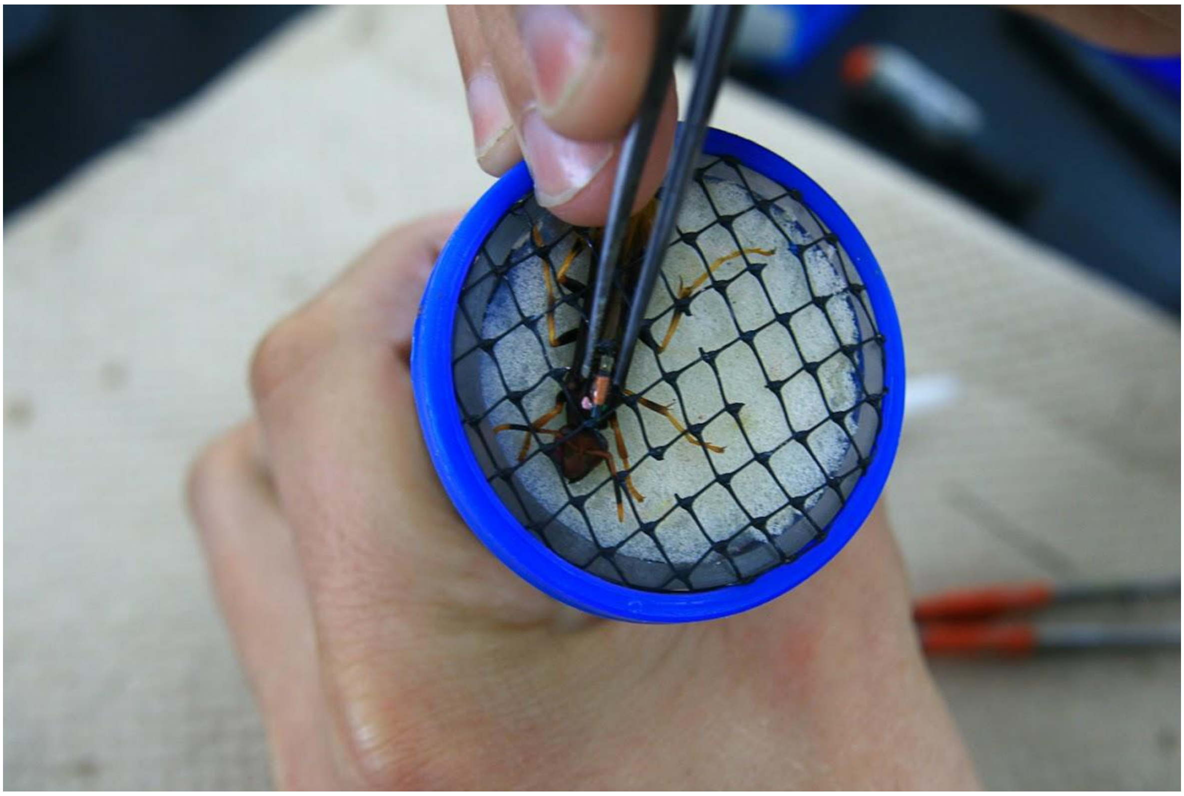


APPENDIX 2: FIELD PICTURES

Picture 2. Mature (red-eyed) *P. canadensis* wasp resting on a leaf (Photo credit: Solenn Patalano).



Picture 4. Tagging of a *P. canadensis* wasp with a RFID tag. The wasp is encaged to facilitate the correct positioning of the tag and thus avoiding any disorientation during flight (Photo credit: Solenn Patalano).

Picture 3. Marking of a *P. canadensis* wasp with a number tag in the field (Photo credit: Solenn Patalano).

Picture 5. Radio-tagged wasps in a post-emergence nest of *P. canadensis*. The wasp in the centre is laying an egg.

Picture 6. Visit of a drifter. A *P. canadensis* wasp (on the left with blue tag) left her nest and is on her way to land on a foreign nest (inhabited by wasp with green tags) (Photo credit: Solenn Patalano)

Thèse de Doctorat déposée le 8 octobre 2013,

défendue devant le Jury de thèse le 13 novembre 2013,

et défendue publiquement le 13 décembre 2013

Lausanne, 2013