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In this PhD thesis, we investigate the aggregation and cooperation mechanisms in the phytophagous mite *Tetranychus urticae* and more particularly to how they adjust their social organization in function of the environmental conditions and their needs. *T. urticae* is considered as a major pest and most of research done was on its control whereas its social behaviours is poorly understood. However, *T. urticae* has several interesting and necessary prerequisites to a social organization (group living, building of a common web, heterogeneous distribution).

We observed that grouped mites produced significantly more eggs and faecal pellets (silk production indicator) per mite per day. Moreover, single mites had a higher death rate than mites living in groups. We assumed that mites could benefit from the silk production of other individuals to invested resources in other activities such as eggs or faecal pellets production.

Our work also showed an influence of the genetic composition of the group on the spatial distribution of individuals. For instance, the interindividual distances increases with inbreeding. Furthermore, we observed a recruitment process for the whole plant infestation, that allows the mites to form large aggregates in a same part of the host-plant and to benefit from group living advantages (e.g. Allee effect, dilution effect, common web building).

Finally, we showed a modulation of the attraction to silk according to age of silk and to the level of starvation. Indeed, fed females were more attracted by fresh silk, while starved females were more attracted by zones without silk. Fed individuals would look for the presence of silk/conspecific whereas starved individuals would avoid silk to decrease the risk of competition.

This work contributed to define the social status of *T. urticae* and to understand the group-living mechanisms present in this species. It underlines also the key role played by the genetic background of individuals on their spatial distribution and their life history traits, which let us suppose a kin discrimination ability in that species.

Guillaume Le Goff, was born in Quimperlé (France) in 1982. He studied biology in University of Rennes 1 and was graduated in 2005 (distinction). Next he integrated a project to study mites behaviour under the supervision of Thierry Hance and Anne Catherine Mailleux (EDIC-ELIB-UCL) and in collaboration with the lab of Claire Detrain and Jean-Louis Deneubourg (Unité d'écologie sociale-ULB). He obtained his "DEA" in 2006 (High distinction) and in June 2011 he defended his PhD thesis in science at the UCL.



UCL

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Guillaume Le Goff

229 | 2011

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GUILLAUME LE GOFF

JUIN 2011

Thèse présentée en vue de l'obtention
du grade de docteur en sciences

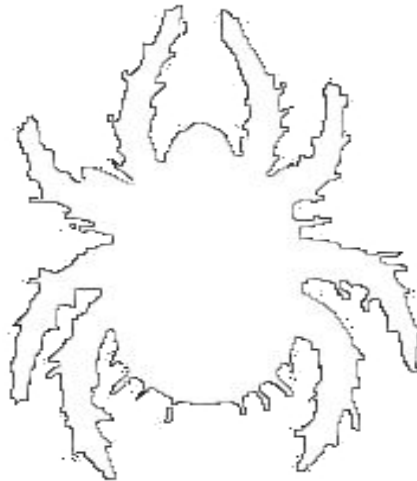
Faculté des sciences
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Faculté des sciences
Biodiversity research centre
Earth and life institute

Benefits of aggregation in *Tetranychus urticae*



Thèse présentée par Guillaume Le Goff en vue de l'obtention du grade de docteur en science

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Juin 2011

*Il vaut mieux mobiliser son
intelligence sur des conneries,
que mobiliser sa connerie sur des
choses intelligentes...*

Devise Shadok

Remerciements

Voici sans aucun doute la partie la plus difficile à rédiger (en tout cas pour moi) de cette thèse. Déjà parce que ce sera probablement la partie la plus lue de cette thèse, mais aussi parce que après cinq années de dur labeur passées sur ce magnifique sujet qu'est l'étude de la vie sociale chez les acaréens, ça fait quand même pas mal de monde. Donc quand faut y aller, faut y aller.

Mes premiers remerciements vont évidemment à mes deux promoteurs de thèse: Thierry Hance et Anne-Catherine Mailleux. Merci à tous les deux, tout d'abord pour votre accueil dans ce nouveau pays qu'était pour moi la Belgique, pour vos nombreux conseils, votre écoute et vos corrections. J'aurais appris au cours de ces cinq années quelques menues choses (la rédaction, les statistiques, etc.) qui me serviront (ou pas), au cours des prochaines années. Vous aurez tous les deux contribué grandement à ma formation de chercheur. Un grand merci également à Claire Detrain et Jean-Louis Deneubourg, les deux promoteurs officiels de cette thèse qui avec leurs précieux conseils et leurs point de vue avisés, auront apporté un oeil différent sur mes recherches.

Merci également à Audrey Dussutour, Maurice Sabelis et Philippe Baret pour avoir accepté de faire partie de mon jury de thèse

Je souhaite aussi remercier toute l'équipe du laboratoire d'Ecologie et de biogéographie rebaptisé earth and life institute-Biodiversity research center (c'était probablement plus facile pour revendre à l'exportation), à savoir :

- La mite-be team, composé de Aina (métisse ULB/UCL), Gwendoline, Sabine et Anne Catherine (que de filles, que de filles...) qui m'auront aidé à apporter un souffle acarologique dans ce laboratoire envahi de pucerons, parasitoïdes et autres maudits insectes.
- Les trois autres geeks de service: Alain, David, et Marc qui auront alimenté moult discussions sur divers sujets comme les jeux de cartes, de rôles, vidéos, de société (oui le geek est très joueur) ou encore le cinéma qui m'auront permis de me sentir moins seul dans ce monde de gens "normaux".
- Le gang des sportifs: Julie (come on Julie!!!), Franck et Thomas, parce qu'on peut transpirer ailleurs que dans une pièce à 26°C et qu'il faut aussi pouvoir se défouler entre deux

manips.

- Gilles, pour tout ses conseils “R”iens, linuxiens et freesoftwariens qui m'auront grandement aidés durant ma thèse mais aussi pour ses magnifiques photos qui illustrent ce document.
- Tous les autres membres du labo pour l'ambiance amicale qu'ils auront contribué à créer: Brigitte, Dominique, Valerianne, Delphine, Séverin, Christophe M, Fred, Hervé, Thomas, DJ Isa, Christophe S, Mélanie B, Gauthier, Sébastien, Audrey, Ellyn, Paul, Chris, Jérôme, Sofie, Julie S, Camille, Frank, Lesley, Victoria, Stéphane, Mélanie, Laurent, Augustin et Michel.
- Et bien sûr ceux qui nous ont quittés, parfois trop tôt... et qui sont partis vers d'autres horizons: Jean-Pierre, Benoît, Claire, Stéphane, Mohanat, Géraldine, JF, Geoffrey, Hubert et les peasty boys Baptiste et Alexis.

Une petite pensée également pour l'équipe du laboratoire d'écobiologie des insectes parasitoïdes de l'Université de Rennes 1 dans lequel j'ai fait mon stage de master 1 qui m'a donné envie de poursuivre dans le monde de la recherche: Anne-Marie, Liliane, Denis, Anne, Antonin, Alex et Cicile.

Un grand merci également à l'équipe du laboratoire d'écologie sociale de l'ULB où j'ai quand même passé quelques journées: Greg, Pablo, José, Aurélie, Bertrand, Lise, Etienne et Zac.

Je voudrais également remercier tous ceux qui sont fondamentalement extérieurs au monde de la recherche et de la biologie de m'avoir supporté, soutenu, aidé et rappelé qu'il pouvait y avoir autre chose que les acariens dans la vie:

- Les Bretonniens qui me supportent (dans tous les sens du terme), pour quelques-uns d'entre eux depuis l'époque du bac à sable: Tony, Nico, Vincent, Yann, Gueno, Céline, Brendan, Gurvan et Morgane.
- Les psycho's girl et pièces rapportées qui m'auront aidé à passer mes week end et quelques soirées à penser à autre chose qu'à mes petites bêtes à huit pattes: Flo, Amandine, Astrid, Steph, Gwen, Jen, Amandine, Paco, Christian et Gauthier.

- Ptite miemielle et Ptit gwigwi qui m'auront permis d'avoir quelques bons délires et surtout quelques bons fous rires.
- Mes parents et ma famille bien sûr, sans qui je ne serais pas celui que je suis aujourd'hui et qui m'ont aidés et supportés tout au long de ce long accouchement qu'est la thèse.
- Enfin merci à ma ptite Mielle parce que tout...

Bonne lecture!!!

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Résumé

Dans cette thèse de doctorat, nous nous sommes intéressés au comportement agrégatif de l'acarien tisserand *Tetranychus urticae* et plus particulièrement à comment ils ajustent leur organisation sociale en fonction des conditions environnementales et de leurs besoins. Ce type de comportement est généralement étudié d'un point de vue fonctionnel tandis que les mécanismes qui en sont à l'origine ne sont en revanche pas bien compris. C'est pourquoi il est nécessaire d'étudier à la fois les relations comportementales inter-individuelles et la valeur adaptative des productions coopératives en résultant, pour décrypter les mécanismes à l'origine de l'émergence de la socialité. *T. urticae* est avant tout considéré comme un ravageur de culture, les études le concernant se sont dès lors concentrées sur les moyens permettant de le contrôler. Cependant, *T. urticae* présente plusieurs prérequis intéressants et nécessaires à une organisation de type sociale (vie en groupe, construction d'une toile commune, distribution hétérogène).

La première étape de cette étude a consisté à comparer la valeur adaptative d'individus vivant en groupe à celle d'individus isolés. Nous avons observés que des individus groupés produisaient significativement plus d'oeufs et de fèces (indicateur de production de soie) par individu et par jour. De plus, les individus isolés ont un taux de mortalité plus important que les individus vivant en groupe. Ces derniers ont probablement bénéficié de la production de soie des autres acariens et ont alors pu investir leurs ressources dans d'autres activités, telle que la production d'oeufs ou de fèces/soie. Par ailleurs, nous

avons observé un impact de l'origine génétique des individus sur cet effet de groupe avec une diminution de la production en fécès/soie en présence d'individus non apparentés.

Dans la deuxième partie, nous avons étudié les mécanismes contrôlant le comportement agrégatif de *T. urticae*. Nous avons observé une influence de la composition génétique du groupe sur la distribution spatiale des individus. En effet, la distance inter-individuelle augmente avec la consanguinité. De plus, nous avons observé un procédé de recrutement lors de l'infestation des plants, ce qui permettrait aux acariens de former des agrégats dans des parties bien précises de la plante et de bénéficier ainsi des avantages à vivre en groupe (Effet Allee, effet de dilution, toile commune...).

La dernière étape de cette étude concerne les mécanismes de communication entre individus. Nous avons étudié l'influence de la présence de soie sur la distribution spatiale et le comportement des individus. L'attractivité de la soie est modulée en fonction de son âge et du niveau de satiété des individus: les femelles nourries sont attirées par la soie fraîche tandis que les femelles affamées sont attirées par les zones sans soie, quel que soit l'âge de cette soie. *T. urticae* se servirait donc de la soie comme d'un indicateur de la présence de conspécifiques, ce qui influencerait la distribution spatiale des individus. Un individu nourri va rechercher la présence de soie et donc de conspécifique, tandis que des individus affamés va fuir la soie pour diminuer les risques de compétition.

Ce travail est une contribution à la définition du statut social de *T. urticae* ainsi qu'à la compréhension des mécanismes régissant leur

vie en groupe. Cette étude souligne également le rôle que semble jouer l'environnement génétique des individus sur leur distribution spatiale ainsi que sur leurs traits d'histoire de vie, laissant ainsi supposer une capacité de reconnaissance de parenté chez cette espèce.

Abstract

In this PhD thesis, we investigate the aggregation and cooperation mechanisms in the phytophagous mite *Tetranychus urticae* and more particularly to how they adjust their social organization in function of the environmental conditions and their needs. This type of behavior is usually studied from a functional point of view while the mechanisms that are at the origin are however not well understood. Therefore, it is necessary to study both the behavioural relationships between individuals and the fitness of resulting collective productions, to understand the mechanisms behind the emergence of sociality. *T. urticae* is considered as a major pest and most of research done was on its control whereas its social behaviours is poorly understood. However, *T. urticae* has several interesting and necessary prerequisites to a social organization (group living, building of a common web, heterogeneous distribution).

Our first step was to analyse the impact of group living compared to solitary individuals on fitness. We observed that grouped mites produced significantly more eggs and faecal pellets (silk production indicator) per mite per day. Moreover, single mites had a higher death rate than mites living in groups. We assumed that mites could benefit from the silk production of other individuals to invest resources in other activities such as eggs or faecal pellets production. Furthermore, we showed an impact of the genetic origin of individuals on this group; the productions of feces decreased in the presence of unrelated individuals.

In the second part, we studied the mechanisms controlling the aggregate behaviour of *T. urticae*. Our work showed an influence of the genetic composition of the group on the spatial distribution of individuals. For instance, the interindividual distances increases with inbreeding. Furthermore, we observed a recruitment process for the whole plant infestation, that allows the mites to form large aggregates in a same part of the host-plant and to benefit from group living advantages (e.g. Allee effect, dilution effect, common web building).

The last step of this study concerned the communication mechanisms between individual. We studied the influence of the presence of silk on the spatial distribution and behaviour of individuals. Our results showed a modulation of the attraction to silk according to its age and the level of starvation. Indeed, fed females were more attracted by fresh silk, while starved females were more attracted by zones without silk. This showed that the silk could be an indicator of the presence of conspecifics which would therefore, influence the spatial distribution. Fed individuals would look for the presence of silk/conspecific whereas starved individuals would avoid silk to decrease the risk of competition.

This work contributed to define the social status of *T. urticae* and to understand the group-living mechanisms present in this species. It underlines also the key role played by the genetic background of individuals on their spatial distribution and their life history traits, which let us suppose a kin discrimination ability in that species.

General introduction

Aggregation and group living

Aggregation, which is the most basic social phenomenon, is defined as an assemblage of individuals that results in a higher density than in the surrounding area (Camazine et al. 2001). It occurs in a wide range of taxa, including bacteria, arthropods, fish, birds and mammals (Parrish and Hamner 1997; Parrish and Edelstein-Keshet 1999; Parrish et al. 2002), and allows many activities such as carrying large prey, exchanging supplies, building nests, reproducing, maintenance of body temperatures and coordinated movements (Camazine et al., 2001) (Deneubourg et al., 2002). It can therefore be considered as a prerequisite in the evolutionary pathway to more complex social ways of life. This kind of interaction is observed between individuals of the same sex and therefore, is not due to a sexual attraction. Cockroaches, for example, live in groups under the same shelter, and individuals are attracted to each other thanks to pheromone production (Rivault et al., 1998). To understand how this behavior could have evolved, we will start by discussing the pros and cons.

Why live together?

In the literature, four main non-exclusive benefits are widely accepted for group living: 1) protection against predators, 2) reproductive success, 3) probability of food finding and finally 4) economy of energy.

1) Protection against predators

It has been proposed that a major factor underlying the evolution of gregarious behaviour is a reduction in the risk of being attacked by predators. Five main mechanisms may occur in predator avoidance/protection in aggregative species.

- Cooperative defence

Living in a group would give group members the opportunity to coordinate defence behaviours and collectively fight with any out-group invader. This strategy resembles the ‘mobbing’ behaviour that many species of small birds, such as starlings, use to drive away large predators (Alcock, 1998).

- Vigilance

In 1973, Pulliam advanced the hypothesis that animals benefit from flocking because the vigilance of flock-mates leads to an increase in the probability of detecting a predator within the time it takes to attack (detection effect or many eyes hypothesis). A reduction in individual vigilance with an increase in group size is one of the most frequently reported relationships in the study of animal behaviour. By taking advantage of the vigilance of other group members, individuals can reduce their own vigilance and allocate more time to foraging and other fitness rewarding activities such as in Columbian ground squirrels (Fairbanks and Dobson, 2007).

- The dilution effect

One way in which individuals may gain protection from predators by joining a group is through a simple 'dilution' effect: for any one predator attack, the larger the group of prey animals, the smaller the chance that any particular individual will be the victim. For example, in the interaction between the aphid *Aphis varians* and its predator *Hippodamia convergens* (Turchin and Kareiva, 1989): the more conspecifics there are surrounding an aphid, the less is that aphid's risk of being killed by a predator. Therefore, the risk of predation to individual prey was "diluted" by prey numbers. However, large colonies of aphids were attacked by more predators than were small colonies. In addition, the ladybird feeding rate increased with increasing colony size.

- The confusion

The "confusion effect" describes the reduced attack-to-kill ratio experienced by a predator resulting from an inability to single out and attack an individual prey in a group (Krakauer, 1995). For example in shoals of fish the predators success decreases with an increase in the size of the prey group (Landeau and Terborgh, 1986).

- The individuals position within the group:
« the selfish herd hypothesis »

The selfish herd hypothesis (Hamilton, 1971) addresses the issue of

spatial dynamics within a group whereby an animal could pass on to group mates some of the risk of being attacked by moving closer to other animals. His model showed that individuals in peripheral group positions would be at higher predation/parasitism risk than those in the group centre (Bumann et al., 1997). An essential component of Hamilton's hypothesis is the estimation of each individual group member's 'domain of danger' (DOD). The domain of danger is the area closer to the focal individual than to any other group member. Because the predator attacks the nearest target, if it first appears inside an individual's DOD, the predator will attack that individual (Viscido and Wethey, 2002). Therefore, the DOD would decrease with the distance to the nearest neighbour. For example, in crab flocks, the level of aggregation increases and the DOD decreases after panic initiation (Viscido and Wethey, 2002).

2) Reproductive success

Aggregation may influence the reproductive success of individuals in two ways. The first is that it increases their probability of finding a mate. The second is that it also increases offspring survival. Therefore, as population sizes become high, every individual within the population will be able to find a mate of the opposite sex (Stephen et al., 1999). In the sea urchin *Strongylocentrotus franciscanus*, an individual in the centre of a large spawning aggregation has been shown to have greater reproductive success compared to other individuals at more distant positions or involved in smaller spawning

events (Levitan et al., 1992).

Aggregation may also increase offspring survival, as is the case when cooperation for brood care occurs within a group. Parental behaviour that may increase offspring survival has two major components: provisioning and guarding. An outstanding example is found in burying beetles, *Nicrophorus* (Coleoptera: Silphidae). Males and females cooperate to bury carrion, which becomes the food source for their larvae, and to rear young (Scott, 1990).

3) The probability of finding food

The third advantage of group living concerns feeding. Benefits occur at three different level: 1) finding, 2) capture and 3) consumption.

- Food finding

Food finding is enhanced in aggregate which may act as an information centre. Grouped individuals gather and analyse more information about a system than a single individual would achieve (Parrish and Edelstein-Keshet 1999). Such a sensory integration system could transduce physical signals into social cues, which amplify the group response (Parrish and Hammer, 1997). For example, colonies of cliff swallows (Aves: *Hirundo pyrrhonota*) appear to be information centres in which colony residents acquire information on the location of food sources (Brown, 1986). Individuals that have been unsuccessful on a foraging trip return to the colony, locate a successful forager, and follow that individual to a food source.

Food finding may also come through a recruitment process. It corresponds to trail laying behaviour, amplified by a positive feedback mechanism. The snail *Terebralia palustris* uses olfaction to locate suitable food resources and more particularly chemical cues released by broken mangrove leaves. This means that this snail is able to locate a mangrove leaf feeder thanks to the cues released by the leaves when grazed, while, conversely, it is not attracted to snails that are not feeding nor to food items that have not been scraped (Fratini et al., 2008).

- Food capture

In group living, species hunting tactics allow for catching larger prey than the individual would alone. This is the case of the African wild dogs *Lycaon pictus* that are 20-25 kg social carnivores whose major prey are ungulates ranging from 15 to 200 kg (Creel and Creel, 1995). This range of prey would be unattainable for alone individual. This behaviour can also be observed in other groups such as social spiders, frogs or crabs.

- Food consumption

Group food consumption gives the advantage of better defending a food resource against other competitors. It also leads to better food intake thanks to a decrease of vigilance (Podolsky, 1990) and finally it improves access to food for individuals that would not get food if they were alone such as offspring (Waser, 1984).

4) Economy of energy

The fourth advantage of aggregation concerns the economy of energy. Indeed, aggregation may create a microclimate within the aggregate and give the opportunity for individuals to regulate their body temperature (Brown, 1999) and to regulate water loss by evaporation (Dambach and Goehlen, 1999). Furthermore, during collective displacements, being in a group would decrease energy loss induced by movement (Hummel, 1983).

There are several benefits from group living but two costs have been recognised as inevitable (Alexander 1974): (1) increased competition for nest sites, nest materials, mates or other resources; and (2) an increased probability of transmitting parasites and diseases. Therefore, considering advantages and drawbacks of group living, explaining why a species evolved to a social way of life is not so obvious.

Proximal causes of aggregation

Aggregation is quite a common phenomenon in the animal kingdom and has been relatively well studied from an evolutionary point of view. However few works have studied the mechanisms of aggregation: what is the dynamic of aggregation? What kinds of cues or active behaviours lead to aggregate formation?

To answer these questions we will described three kinds of aggregation: 1) passive aggregation, 2) active aggregation due to

environmental cues and 3) active aggregation due to attraction to conspecifics.

1) Passive aggregation

Passive aggregation is a swarming of individuals by physical forces. This phenomenon can be observed in species that present low mobility. One example is the dense aggregation of plankton in open water, in which plankton organisms are not actively attracted to each other but are transported passively in the same place via physical forces such as water currents (Parrish and Hammer, 1997; He et al., 2004).

2) Active aggregation

Active aggregation is the results of the convergence towards an attractive cue, such as food, space or conspecifics. It may lead to active recruitment. Active aggregation can be due to responses to environmental heterogeneities and/or to a variety of inter-attractions.

- Environmental heterogeneity

Usually, experimental studies on aggregation behaviour work in a homogeneous environments with constant external factors (Camazine et al. 2001, Sempo et al. 2006, Depickère et al. 2008). However, environmental heterogeneities, like temperature or humidity may influence the patterns of spatial distribution and cause the emergence of new structures adapted to the environment (Fraenkel and Gunn, 1961; Southwood 1966; for ants see, e.g., Ceuster, 1977). Environmental heterogeneity can therefore increase aggregation and

decrease segregation, depending on the strength of competition between individuals whatever their species.

- Inter-attractions

This kind of aggregation is observed when numerous interactions among individuals lead to aggregation. The term congregation is also used to describe groups formed and maintained by mutual attraction (Parrish & Hamner 1997). The role of interactions in group formation can be studied only under laboratory conditions. Indeed, it would be difficult in the field to separate the impact of environmental heterogeneity to that of inter-attraction.

Contrary to aggregation caused by environmental heterogeneity, aggregation caused by inter-attraction is mediated by social signals that will induce aggregation and reinforce individual cohesion. These signals can be visual, tactile, acoustic or chemical and their efficiency will depend to a great extent on their range. Visual and acoustic communications are widely spread among birds and mammals and are effective at large distances compared to their size (Forbes and Kaiser, 1994). On the contrary, tactile communication is approximately reduced to the size of the individuals. Chemical communication is commonly observed in arthropods and several examples can be observed in the wild such as chemical trails in ants or caterpillars (Hölldober and Wilson, 1990) or volatile compounds from the faeces (Weiss, 2006). Aggregation pheromones act as a recognition signal allowing proximity tolerance between individuals of the same group (Pasteels and Deneubourg, 1987). For example, in the

cockroach *Blatella germanica*, cuticular hydrocarbons are chemical compounds that induce aggregation and can therefore be qualified as aggregation pheromones (Rivault *et al.*, 1998).

Furthermore, the presence of related individuals (the genetic environment) has to be taken into account when studying the spatial distribution of a species. Indeed, when the benefits of group living are shared by closely related individuals, they potentially increase indirect fitness for group members (Hamilton 1964) and therefore also increase the probability of aggregation. On the contrary, when drawbacks of group living are higher than the benefits (even with kin), an avoidance of other individuals should be the rule.

In many aggregation processes, heterogeneity of environment and inter-attraction play both a significant role. For example, a group of individuals can present the same level of aggregation in both heterogeneous and homogeneous environments, but the place of the aggregate will be influenced by environmental heterogeneities and, on the contrary, will be placed at random in a homogeneous environment. Furthermore, a synergy between heterogeneities and inter-attractions can induce a higher level of aggregation than with only one of the two driving forces. For example, in the case of a food patches, the first individuals that discover them will aggregate around these patches. Then thanks to inter-attraction, this aggregation could be amplified and lead to the growth of the aggregate.

In conclusion, individuals have to perceive the information coming from biotic and the abiotic environment before deciding how they should be distributed in the environment.

What is sociality?

Sociality is quite a difficult concept to define. The simplest way would be to consider all species living in group as social species. However, groups of individuals may present a panoply of body sizes (from bacteria to whales), demographic traits (variations in lifespan and living places) and compositions (overlapping generations, kin or non kin, even clones). Groups may also present labour division and various communication systems between individuals through visual, physical or chemical stimulation. Furthermore, living close to other conspecifics does not necessarily mean social interactions. Indeed, crowds and some aggregates may be due to a common attraction to the same environmental stimulus such as light for nocturnal insects. Therefore, with such a variety of possibilities in social interactions, sum up sociality as “living together” is just not possible.

Wilson (1971) underlined that the base of sociality is the communication among individuals. Furthermore, Goldberg (1998) defined social interactions as the sum of all intraspecific interactions, except sexual interactions. Indeed, even if sexual attraction is important in social organisation, it is not sufficient to explain it. A society exists as soon as an individual is able to influence the society and when this society is able to react specifically to each individual (Goldberg 1998).

Table 1: different level of sociality (from Aron and Passera, 2002)

Level of sociality	Social inter- actions	Parental care	Breeding site	Cooperation for parental care	Common breeding/Task allocation	Individuals specialized in reproduction
Solitary						
Gregarious	+					
Subsocial	+	+				
Colonial	+	+	+			
Communal	+	+	+	+		
Primitive eusociality	+	+	+	+	+	
Advanced eusociality	+	+	+	+	+	+

Degrees of sociality

Aggregation can be considered as a first step towards a social life. However it is not always synonymous with social interactions and solitary species can group only because of environmental heterogeneities. Different levels of sociality may be characterised by intraspecific interactions and social behaviours expressed by individuals within the society (Table 1) (Aron and Passera 2000, Costa 2006). Groups where all individuals have a similar opportunity to reproduce and groups where a single individual completely monopolises reproduction represent the two end points of a continuum of variation in the reproductive investment among group members (Keller and Perrin, 1995). The level zero of sociality corresponds to a solitary way of life, whereas the highest level of sociality is called eusociality. Between those two levels, there is a wide range of possibilities:

Solitary species

Members of solitary species do not interact with each other except during courtship and mating (or territorial interactions).

Gregarism

In many species, individuals form aggregates, increasing local population density much more than in the surrounding environment (Rivault et al., 1998; Amé, et al., 2004). Forming such aggregates has many advantages both for the individual and the group (access to food

and mates, protection against predators) (Ranta et al., 1993; Wertheim, 2004). It also presents disadvantages (sharing food, mates and living space) and may result in inter-individual conflicts (Ranta et al., 1993).

This kind of interaction is observed between individuals of the same sex and therefore, is not due to a sexual attraction.

Subsociality

The subsocial level differs from gregarism because in this case, adults care for their offspring for some period. This kind of behaviour corresponds to an energy investment of the parents to increase the survival rate of their offspring (Aron and Passera 2000). It represents therefore, a cost compensated by the benefit of being selected. For example, this cost can be a defence of their offspring against predators. Brood care is observed in all animal societies and is a prerequisite for the evolution of the society. Many examples of this kind of behaviour may be found among crustaceans, spiders, mites, scorpions, millipedes, centipedes, cockroaches, crickets, bugs and beetles, birds and mammals.

Colonial level

In this case, there is a common breeding site in the animal community and thus a better defence and socialisation of the offspring. Furthermore, although the offspring of different females are bred in the same place, there is no cooperation between the mothers. For example, in social spiders, the colonial level may occur because individuals may live in the same place, but generally forage and feed

alone and there is no maternal care beyond the egg stage (Lubin and Bilde, 2007).

Communal level

In this case, grouped females of the same generation cooperate on the same breeding site for the brood care without labour division. For instance, *Necrophorus coleopterans* cooperates to gather and bury animal bodies. Then, the decomposition of the dead body is used to feed the young (Trumbo et al., 1994, Trumbo, 1995, Aron and Passera, 2000).

Eusociality

Eusociality is considered to be the highest level on the social scale (Michener 1969, Wilson 1975) and is based on three fundamental criteria:

- overlap of generations
- task allocation
- cooperation for brood care

Ants, bees, wasps (Order: Hymenoptera) and termites (Order: Isoptera) are typical examples of eusociality. However, a few more groups also present eusociality: in galling aphids (Homoptera) there are sterile soldiers that would sacrifice their lives in case of attack by a predator to save their clonal sisters that can then reproduce (Itô, 1989). This is also the case for social thrips that are gall-forming

(Thysanoptera) (Crespi, 1992). In non-insects, eusociality appeared in a mammal (Clarke and Faulkes, 1999): naked mole rats (*Heterocephalus glaber*) live in complex underground tunnel systems in Africa and animals in the same nest are closely related. Only one female (the queen) reproduces, although workers, normally sterile, can ovulate when removed from the nest, presumably due to a lack of inhibition from the queen.

Eusociality is divided in two types: primitive and high eusociality:

- Primitive eusociality refers to cases such as in many wasps and bees where there is little or no morphological caste differentiation, and as a consequence, there is often considerable flexibility in social roles that a given animal may adopt. Reversal of roles from queen to worker and vice-versa is also sometimes seen. Caste differentiation takes place at least partly after hatching and usually involves simple behavioural mechanisms. The primitive eusocial species are very interesting for understanding the evolutionary pathways of eusociality.

- High eusociality refers to the most advanced societies where there is clear cut morphological caste differentiation and little, if any, flexibility in the social roles that adult insects may adopt. Caste determination occurs almost exclusively before hatching and involves

rather complicated nutritional and hormonal mechanisms. All termites, most ants and many bees and wasps such as the honey-bees and vespine wasps, respectively, are highly eusocial. Without exception, the highly eusocial state is restricted to the Hymenoptera and Isoptera orders in the entire animal kingdom.

Origin of sociality

Social individuals have a special place in evolutionary biology. The question is, as some individuals do not reproduce, how can they do to transmit their altruistic traits to the next generation? (Darwin, 1859). Why does selection lead to the abandonment or a decrease of the reproductive success of some individuals, if it is through reproduction that the genes for such behaviours are passed on? This problem, if we agree that the evolution of sociality has been adaptative, leads immediately to further questions such as what are the elements triggering and controlling the emergence, the spreading, and the persistence of social interactions among a population.

Kin selection

During the 1960s, Hamilton made his contribution to natural selection, with the kin selection theory (1964). This theory suggests that the genetic proximity of individuals would decrease aggression and would favour altruistic and cooperative behaviours. The basic

logic of kin selection is that a gene can produce copies of itself either by the familiar route of increasing the fitness of its bearer (direct fitness) or by increasing the fitness of relatives who share copies of the gene (indirect fitness) (Queller and Strassmann, 1998). The sum of these effects weighted by relatedness is called inclusive fitness. Kin selection theory formalises the obvious point that helping relatives is advantageous, whereas harming them is not.

It can be expressed as an equation:

$$W_i = W_x + W_y \cdot (R_{xy})$$

where:

W_i = inclusive fitness of individual x

W_x = direct fitness of individual x

W_y = direct fitness of individual y

R_{xy} = relatedness coefficient between individual x and y

Altruistic behaviour will be favoured when the fitness gain to the beneficiary (B) times the actor relatedness to the beneficiary (R_b) exceeds the fitness loss to the actor experiencing the cost (C) times the actor relatedness to this individual (R_c). If the actor suffers the cost and the relevant R_c is 1, aid to a relative is termed altruistic (Queller and Strassmann, 1998).

$$R_b \cdot B > R_c \cdot C$$

Therefore, if there is a sufficiently high relatedness coefficient between interacting individuals, such an interaction will evolve under kin selection.

This mechanism has to be divided in two steps. The first one is the recognition of the degree of relatedness and the second one is the discrimination between kin and non-kin (Sherman 1997). Therefore, we can observe recognition without discrimination but not the opposite situation (Reeve 1989). The observation of kin discrimination is a useful way to prove the ability of kin recognition in a species.

In social biology, genetic relatedness plays a key role in the organisation and functioning of social groups of many species (Lihoreau et al., 2007). Hamilton (1964) predicted that individuals behaving altruistically toward their closest kin increase their own fitness as closely related individuals, sharing at least a part of a common gene, can reproduce and transmit their genes to the next generation. The fact that individuals of social species associate with conspecifics for different activities (foraging, resting, or mating) shows that the choice of preferred partners is related to the associated costs and benefits in many contexts (Fellowes 1998).

Group selection

During the 1970s, Wilson (1975) developed a new form of group selection based on a different conception of the group. The idea here was that at a certain stages of an organism's life cycle, interactions take place between only a small number of individuals.

Under these conditions, cooperative behaviour can be favoured (West et al., 2007). A study by Wilson and Hölldobler (2005) argued that group selection is more important than kin selection for the evolution of social insects (see also Reeve and Hölldobler, 2007). Wilson (2007) further questions the importance of kin selection for eusociality. A recent paper from Nowak *et al.* (2010) shows the limitations of kin selection theory and argues that standard natural selection theory in the context of precise models of population structure represents a simpler and superior approach to explain the evolution to sociality. Indeed, termites and other diplo-diploid social species (platypodid ambrosia beetles, *Synalpheus* sponge dwelling shrimp and bathyergid mole rats) had never fit into the inclusive fitness theory (Nowak et al., 2010). According to these authors, evolution to sociality, and more especially to eusociality, can be explained by a series of selection processes:

- 1) Group formation
- 2) Combination of pre-adaptive traits, causing the group to be tightly formed. For example, to a valuable/defensive nest or kin associations
- 3) Mutations that prescribe the persistence of the group
- 4) Emergent traits caused by the interactions of group members are shaped through natural selection by environmental forces
- 5) Multi-level selection drives changes in the

colony life cycle and social structures, often to elaborate extremes, such as reproductive and non reproductive individuals in a same group.

Therefore, relatedness among individuals within a society would be a consequence rather than a cause of group living.

Group selection is also referred to in the literature as ‘trait-group selection’ or ‘demic selection’ or ‘intrademic selection’.

Kin selection vs. group selection

The relative merits of kin and group selection in the evolution of sociality is an old debate in evolutionary biology. Kin and group selection are just different ways of conceptualising the same evolutionary process. Group selection does not necessarily depend on genetic relatedness or genetic reproduction (Traulsen et al., 2008) and argues that sociality can occur between non-kin. Furthermore, models show that for group selection, cooperation is favoured when there is competition between groups and when the response to group selection outweighs the response to within-group selection (Traulsen, 2008), but it can be applied only to group structured populations (Lion *et al.*, 2011). On the contrary, kin selection depends on genetic relatedness between individuals. This kind of selection works for populations that present a more fluid spatial structure in which simply occasional local clustering occurs but no clearly predefined groups exist (Lion et al., 2011).



Figure 1: web trap of social spider (© Joe Lapp)



Figure 2: aggregate of social spiders on their common web

Sociality in spider mites

Sociality has been discovered in mites and more particularly in the Tetranychidae family which presents the most extensively social organization in mites (Costa, 2006). Various communal behaviours such as aggregation or deposition of faeces at certain places are commonly observed in all subsocial species (Saito, 1997). Spider mites exhibit two examples of complex sociality: *Schizotetranychus longus* Saito and *S. miscanthi* Saito. They live in the same nesting habitat, a nest built with silk over a depression along leaves (nerves of host plants (Saito, 1997). Their sociality is characterised by four traits: (1) spatiotemporal overlap of generations, (2) cooperative nest building, repairing and enlarging, (3) cooperative use of nest space and resources and (4) cooperative brood defence by adults against predators (Saito, 1997).

Silk production

In the animal kingdom, only arthropods produce silk. Numerous uses of this material are known. Silk may provide shelter, is used to catch preys, helps with reproduction and partner encounter, plays a role in dispersal or serves as a support for chemical communication. Spider webs correspond to the most famous example of silk use for trapping prey (figure 1), but also as a means of communication to maintain group cohesion in social spiders (figure 2)



Figure 3: social caterpillar's nest (© Will Richards)



Figure 4: social caterpillar's trail (© Brewbooks)

(Bernard 2002). In the case of social caterpillars, silk provides shelter against external aggressions such as predators, rain or wind (figure 3). It is also used as a trail for communication between individuals (figure 4). Considering all species producing silk, only a few present some degrees of social organisation (about fifty spider species and some caterpillars and mites) like brood care, common web or silk trail following. All these social behaviours can be explained by a mechanism of group cohesion that decreases the probability of being isolated (Bernard and Krafft, 2002). Many studies on social spiders show that silk maintains group cohesion, especially for group displacement, playing the same role as pheromones in ant societies.

« If one can consider that 'the road to insect sociality is paved with pheromones' (Blum, 1974), one must grant that the society of the spiders hangs by a thread » (Krafft, 1982b).

Concerning mites, silk production also plays a key role in the evolution to a social way of life of some Tetranychidae species. Indeed, a single individual cannot produce enough silk to build a protective web. On the contrary, a common web built by many individuals offer many advantages to each member of the group. Among these advantages are protections against the biotic agents like predators (Helle and Sabelis, 1985; Hoy and Smilanick, 1981) or competitors (Van Impe, 1985) and a shelter against abiotic agents like humidity (Hazan et al. 1975), wind (Davis 1952), rain (Linke 1953) or acaricides (Davis 1952, Linke 1953). For example, in the subsocial

spider mite *S. longus*, the nest web has an important function in the survival of young. It prevents the entry of pedestrian predators (generalist); therefore, females may play a role in defending the brood against specialized predators that can intrude into the nests (Mori et al., 1999). This silk is also a substrate for sexual pheromones (Cone *et al.* 1971; Penman and Cone 1972, 1974) or can be used as a vector of locomotion and dispersal (Saito 1977, 1979; Yano 2008). Indeed, Yano (2008) experimentally investigated the role of *T. urticae* silk trails in collective and solitary behaviours. He found that dispersing females are able to follow the trails left by preceding ones. This behaviour seems to induce an amplification process, resulting in aggregation at a new colony site.

The genetic system

Parthenogenesis is a form of asexual reproduction, where the growth and development of eggs may occur without fertilisation by a male. More than 15% of all animal species are haplodiploid (Pamilo et al. 1997) and has been observed in seven animal orders: Monogonata, Acarina, Oxyurida, Hymenoptera, Thysanoptera, Homoptera and Coleoptera (Adamson 1989). In the case of the arrhenotokous parthenogenesis, unfertilised eggs produce haploid males, while fertilized eggs produce diploid females. Therefore, the offspring of a non-mated female will be exclusively composed of males. Such a way of reproduction is considered to derive from the “normal” sexual way system of sex determination. It has a great impact on the genetic

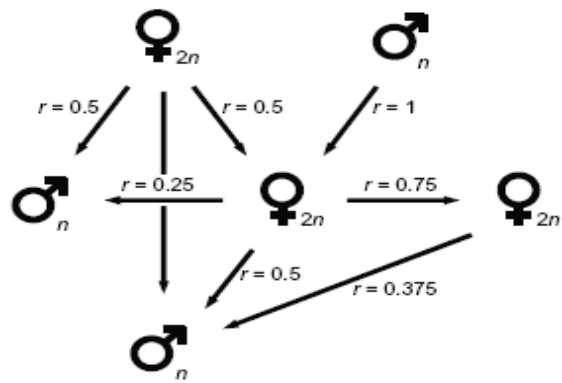


Figure 5: kin coefficient in a haplodiploid family (from Pearcy 2005)

relatedness of individuals in a group. Indeed, sisters share a greater part of their genome between themselves ($R=0.75$) than with their own offspring ($R=0.5$) or their brothers ($R=0.25$) (figure 5). Males transmit all their genes to their daughters ($R=1$), but it is impossible for them to have sons ($R=0$). In haplodiploid communities, females are genetically closer to their sisters ($R=0.75$) than to their brothers ($R=0.25$). The kin selection theory of Hamilton (1964) foresaw that the probability of cooperation between two individuals would be high when the kin coefficient K between them was also high. So, arrhenotokous parthenogenetic reproduction would induce a higher probability of cooperation between sisters than between a female and her offspring/parents. Such a system of reproduction would also influence the evolution towards a social way of life of a species. The ultimate example is the task allocation in hymenopteran eusocieties with on one side the queen(s) reproductive female and on the other side the workers that do not reproduce.

Tetranychidae presents a haplodiploid reproduction system. This combined with a low mobility favour departures toward heterozygote deficiencies (Saito 1997). Therefore, examples of highly inbred populations are known for spider mites (Hinomoto and Takafuji 1994). On the contrary, mite populations can also be created by migrants from different origins. This process tends to assemble mites carrying different genetic pools (Carbonnelle *et al.* 2007). Indeed, in the field, various types of genetic structures can be observed in Tetranychidae, from monomorphic to polymorphic populations (Carbonnelle *et al.* 2007). Therefore, within its population, an

individual is confronted with a gradient of relatedness from unrelated to highly related individuals. The genetic distances between individual may thus influence their interactions and behaviours. For example, Roeder *et al.* (1996) suspected a kin recognition ability in *T. urticae* as they experimentally showed sex ratio shifts in response to the relatedness between founders while their number remained equal.

Sex ratio

A biased sex ratio is a very important prerequisite of kin selection, because it sometimes results in unbalanced relatedness between siblings (Hamilton, 1972). It is well known that the sex ratio of mites varies considerably (Saito, 1999). Several important models have been proposed to explain the « evolutionary » factors capable of biasing the sex ratio of mites (Saito, 2009):

-*The local mate competition model*: as an explanation for such female-biased sex ratios, Hamilton (1967) proposed the local mate competition (LMC) model: female-biased sex ratios are favoured in species where a small number of fertilised females find a patch, and their offspring mate only within that patch before the females disperse. Hamilton showed the generality of the LMC model by listing several animals, including mites, as an important group, all of which have female-biased sex ratios and whose life histories are expected to fit to the assumptions of his model.

-*The haystack model*: the population structure influences both within- and between-group selection (Sato and Saito, 2007). Therefore, the influence of population structure must be considered in relation to LMC and the relatedness between founders (Sato and Saito, 2007). Nagelkerke and Sabelis (1996) developed the *haystack* model by subdividing each group into one-generation mating groups, in which each group lasts several generations. This model succeeded in explaining the surplus female-biased sex ratio in mites and some small arthropods.

Moreover, the extent of the optimal sex allocation bias resulting from LMC is predicted to vary according to the relatedness among offspring (Frank, 1987). For example, Roeder *et al.* (1996) suspected a kin recognition ability in *T. urticae* as they experimentally showed that the sex ratio shifts in response to the relatedness between founders while their number remains equal.

An example of cooperation: nest sanitation

Living organisms produce waste products that can handicap their fitness if not eliminated by attracting predators or promoting diseases (Jackson and Hart, 2009). Living in a group may increase parasitism and disease in animals (Poiani, 1992), but successful animal societies have solved the problem of waste control. In 14 group-living (under web nests) Tetranychidae mite species of the genera *Eotetranychus*, *Oligonychus* and *Schizotetranychus*, waste

management behaviours occur (Saito, 1995). They appear to avoid soiling their living space or nests by defecating only at specific sites (Saito, 1983). Sato *et al.* (2003) investigated the mechanisms by which *S. miscanthi* selects the site of defecation. Their results show that nest members defecate at only one site inside the nest, and that waste management is maintained by two simple rules. First, mites defecate near the nest entrances if no volatile chemical cues are available, and second, when chemical cues are available from deposited previously faeces, they defecate at this site.

Parental care has also been reported in spider mites. In *S. tenuinidus*, both male and female parents provide care for their offspring. Females build nests and lay eggs in the nest and males guard the eggs until they hatch (Zhanh *et al.* 2001).

There is no doubt that several factors are important for the evolution of sociality in mites. Silk production, life history, the haplo-diploid reproductive system, the sex ratio and gregarious behaviour may be related to Tetranychidae sociality (Saito, 2009).

Context of the research

T. urticae is considered to be a major pest of many cultivated plants (more than 900 listed host-plants, Van Impe 1985). Consequently, research has mainly focused on the management and control of this species while its social behaviour has been disregarded. In the two spotted spider mite *T. urticae*, no evidence of a task allocation has been observed (Saito 1997). However, *T. urticae*

presents many necessary prerequisites to the emergence of social behaviours: its arrhenotoky, which induces high kin relation among sisters, or the construction of a common web that favours spatio-temporal overlap of generations (Saito, 1983; Clotuche et al., 2009). Furthermore, mites are small organisms with low mobility which may increase the spatial proximity and the probability of interaction among kin (Saito, 1997). Consequently, mites might exhibit complex social behaviours and examples of their ability to discriminate individuals differing by their original environments and genetics (Saito 1997). Influence of the genetic background has been observed on *T. urticae* behaviour and biology. For example, Roeder *et al.* (1996) showed that in *T. urticae*, females (sisters or non-sisters) coming from inbred and outbred populations are able to modify their offspring sex-ratio according to the genetic relatedness of the group. Therefore, *T. urticae* mites have the ability to discriminate individuals of different genetic pools. Furthermore, we know that this mite is able to express aggregative behaviour (Strong et al. 1997) which is essential for the development of other components of social behaviours. As previously mentioned, *T. urticae* produces silk continuously when walking. This silk can be a key factor favouring the emergence of social interactions; an isolated individual cannot produce enough silk to build its own protective web. On the contrary, common web building by many individuals gives many advantages to the individual and its colony such as protection against biotic (predators) and abiotic agents (rain, wind and humidity).

Hypotheses and main questions

The present study focuses on the tetranychid mite *T. urticae*. This mite is known to live in groups (Strong *et al.* 1997). Our assumption is that *T. urticae* is a species that presents social characteristics. Furthermore, we hypothesise that the environment and the relatedness between individuals play a key role in social interactions among individuals.

We tested this hypothesis through three main questions:

- 1) Is there an impact of living in a group compared to being alone on individual fitness?
- 2) Does *T. urticae* present aggregative behaviour?
- 3) Does the silk production of an individual influence the spatial distribution and the locomotor activity of its conspecifics?

These main questions have been subdivided in nine subquestions as presented in the flowchart (figure 6).

T. urticae often exhausts its host plant resources; adult females then disperse to new hosts, primarily by walking (e.g., Kondo and Takafuji, 1985). Over the course of such an invasion–exploitation–dispersal cycle, *T. urticae* may experience different ecological and

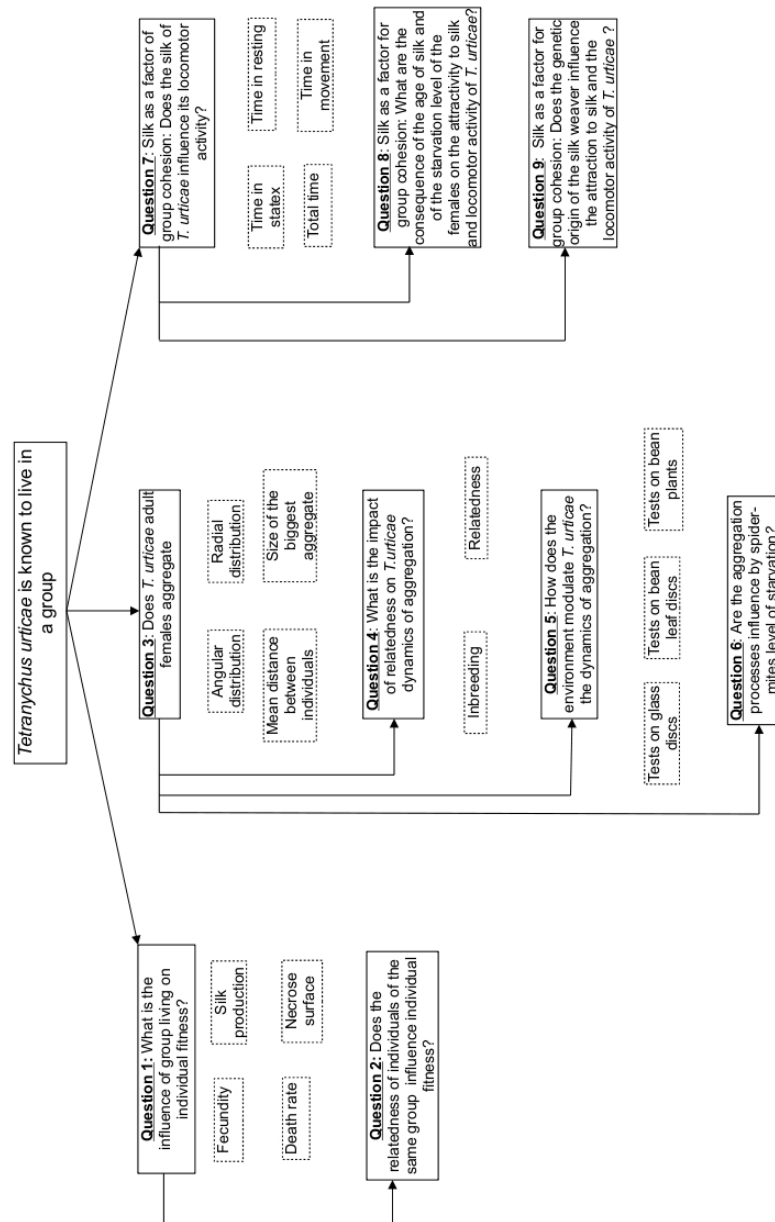


Figure 6: Flow chart of the PhD thesis, showing the different steps of the study

physiological conditions and also meet individuals from different genetic origins. All these factors may affect the interactions between individuals and therefore, the social organization of the group. Therefore, for each of these questions, we tested different parameters that would modulate their aggregation behaviour, the influence of group living or the communication among individuals (figure 6):

a) the genetic proximity between individuals: we expect to observe an increase in the social interactions between kin or at least a decrease of the social interactions between non-kin.

b) the environmental conditions: we expect an influence of the tested environmental conditions on the aggregation behaviour of *T. urticae*.

c) the physiological state: we tested the influence of being starved or not on the behaviour of *T. urticae*. By using starved individuals in our experimental set-up, we expect to observe a decrease in social interactions. Indeed, it would be reasonable to expect settlers to avoid overcrowded areas.

Materials and methods



Figure 7: female of the red form of *T. urticae* (© Gille San Martin y Gomez)



Figure 8: female of the green form of *T. urticae* (© Gille San Martin y Gomez)

The biological model: the two spotted spider mite *Tetranychus urticae*

Classification

Our biological model is the mite *Tetranychus urticae*. This arthropod belongs to the class of Arachnida, the order of Acari, the sub-order of Prostigmata and the family of Tetranychidae. This family includes many phytophagous mites also called “spider-mites.”

T. urticae is a worldwide distributed species with several forms of which the specific status is not really well defined and is still debated. Two colour forms of *T. urticae* can be found in the nature: the red form (also called *T. cinnabarinus*) (figure 7) and the green form (figure 8). The two forms of *T. urticae* are morphologically very similar, Dupont (1979) and Navajas (1998) concluded that both forms should be considered as belonging to the same species, whereas other works suggest a differentiation (Goka *et al.* 1996; Hance *et al.* 1998; Zhang and Jacobson 2000; Sugawara *et al.* 2002).

Despite many controversies about how species are defined and how they evolve, reproductive isolation remains central in the biological species concept (Navajas *et al.* 2000). Preliminary experiments of cross mating between the two populations have been done in the lab: green males*red females (n=30) and red males*green females (n=30). Mating occurs between the two populations but it seems that there were very few fecundations. Indeed, we obtained

females in the offspring in only one replicate (green males*red females). Furthermore in another replicate, all eggs died before hatching. These results would indicate a genetic incompatibility between the two populations or the presence of *Wolbachia* in one or both populations that prevent cross mating (Breeuwer, 1997).

Partial reproductive incompatibility between conspecific populations originating from different localities or from different plants is a common phenomenon in spider mites (Helle and Pieterse, 1965; de Boer, 1985). Despite such genetic barriers among local populations, *T. urticae* appears as a relatively homogeneous species and no long-standing differentiation emerges as suggested by the wide survey of ITS2 sequences (Navajas *et al.*, 1998). During the last International Congress of Acarology (XIII ICA, Recife Brazil, 2010) some presentations talked about this subject and most acarologists (based on morphological, and genetic data) agreed that these two forms belong to the same species: *T. urticae*. Furthermore, the most accepted taxonomical position does not recognize *T. cinnabarinus* as a valid species as in the World Catalogue of the Spider Mite Family (Bolland *et al.* 1998), which cites it as asynonym of *T. urticae*. Finally, a recent paper from de Mendonça *et al* (2011) provides additional evidence of the invalidity of the *T. cinnabarinus* taxon.

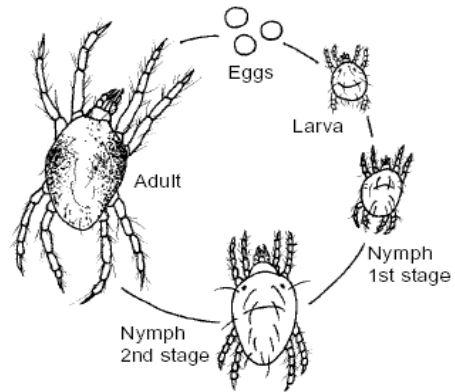


Figure 9: life cycle of *T. urticae*

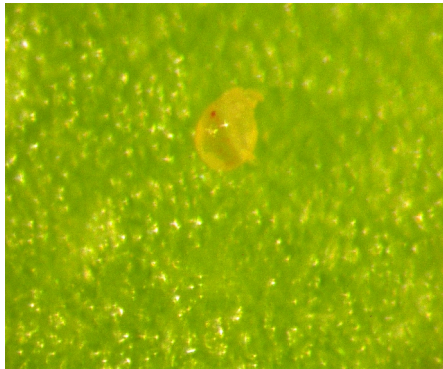


Figure 10: *T. urticae* hatching egg



Figure 11: *T. urticae* larvae



Figure 12: *T. urticae* male (© Gille San Martin y Gomez)

Biological development

The development time varies according to environmental conditions such as temperature (Mitchel, 1973) and humidity (Hazan *et al.*, 1974). For both male and female there are eight stages (active or passive) in their biological development: egg, larvae, protochrysalis, protonymph, deutochrysalis, deutonymph, teleochrysalis and adult (figure 9), with a shorter development time for males than for females. Nymph and adult stages have four pairs of legs, whereas the larval stage has only three pairs.

Adult females lay eggs on the underside of leaves (Ashley 2003) and just before hatching, it is possible to see their red eye through the egg chorion (figure 10). The larvae become green after food ingestion (figure 11). After a first metamorphosis (protochrysalis), they develop a new pair of legs and reach the protonymph stage. Then, they undergo a second metamorphosis (deutochrysalis) and reach the deutonymph stage. At this step of development, it is possible to distinguish males and females as males are smaller than females (2-3 times) and have a conical abdomen (figure 12), whereas females have a spherical abdomen (figure 6-7). The last step is the third metamorphosis (teleochrysalis) that transforms them into adults.

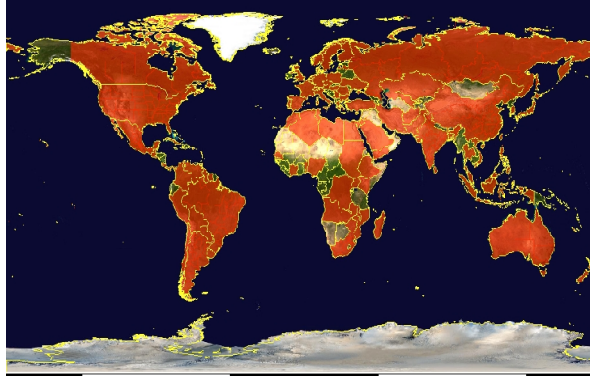


Figure 13: global distribution of *T. urticae*. The countries where *T. urticae* has been observed are in red (© 2006-2010 INRA – CBGP)



Figure 14: white necroses caused by *T. urticae* infestation (© MAPAQ)



Figure 15: infested plant surrounded by *T. urticae* silk (© D. Blancard (INRA))

Pest status

The phytophagous two-spotted spider mite *Tetranychus urticae* Koch is a ubiquitous species, present worldwide in a wide variety of contrasting environments (figure 13). This mite feeds on a large variety of plant families and displays much variation in its morphology, physiology (tolerance to pesticides, response to photoperiod) and sex ratio (Helle and Sabelis 1985). It is considered a major pest of many cultivated plants (more than 900 host-plants listed (van Impe, 1985)). Consequently, research has focused mainly on its management, whereas its social behaviour has often been disregarded. Spider mites can undoubtedly cause severe crop losses. From larvae to adults, most individuals feed on the underside of leaves. They use their pair of stylets to pierce the leaf and suck the parenchyma cell content (Brandenburg and Kennedy, 1987) (figure 14).

This way of feeding has two main consequences for the plant. The first one is physiological. Indeed, by feeding on the plant, mites damage protective leaf surfaces, the stomata, and the palisade and spongy parenchyma, which decreases plant photosynthetic activity (Sance *et al.*, 1979). Furthermore, they may inject toxic substances into the leaf while feeding and interfere with vital (Avery and Briggs, 1968; Huffaker *et al.*, 1969). The second impact of *T. urticae* on the plant concerns the accumulation of silk and faecal pellets which can cause many problems, especially for ornamental plants (figure 15). Therefore, the impact of a *T. urticae* colony on a plant can be huge and may cause economic losses.

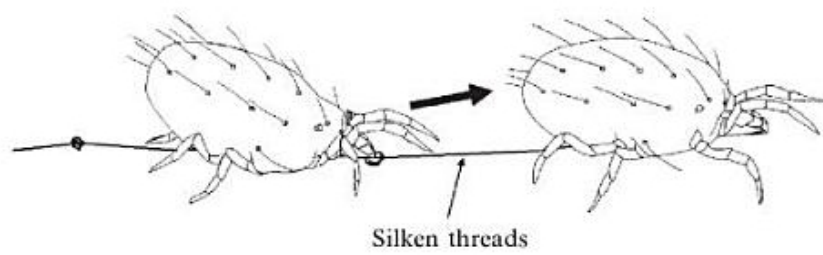


Figure 16: how a spider mite draws out its silken threads (from Saito, 2009)

Silk production

T. urticae mites produce silk. This silk comes from the modified salivary glands and is produced by all stages when walking. Spider mites produce two thread lines from a pair of organs (called terminal eupathids) on the pedipalpi (Saito 2009). They never eject these silken threads but rather use ambulatory movement to pull silk out from the organs (Saito 2009). They stick the threads from the silk production organs onto the leaf surface and then silken threads are pulled out as they walk (Figure 16: from Saito 2009) or sometimes by falling down (Saito 2009). *T. urticae* (and probably other spider mites) is able to produce a pair of silk threads simultaneously (Saito 1977), with a closely located pair of pedipalpi and silk producing organs. Spinning silk helps females to protect their eggs from abiotic and biotic agents (Davis, 1952, McMurtry *et al.*, 1970; Brandenburg and Kennedy, 1987; Hazan, 1974).

Reproduction

In a population of *T. urticae*, females are approximately three times more numerous than males (Helle and Pijnacker 1985). Sex determination of individuals is due to an arrhenotokous parthenogenesis reproductive system (Brandenburg and Kennedy 1987). Furthermore, a recent paper by Macke *et al.* (2010) showed that egg size significantly determines the fertilization probability and, that female eggs (fertilised) are significantly larger than male eggs (unfertilised). The sex ratio can also vary according to environmental

factors such as the density of individuals and host plant quality (Boudreau 1963, Wrench and Young 1978), abiotic factors (temperature, humidity, photoperiod, light conditions) (Wrench 1993), or genetic factors like the level of inbreeding in the population (Roeder *et al.*, 1996).

Thanks to its arrhenotokous reproductive system, *T. urticae* has four important evolutionary advantages. First, females may control the offspring sex-ratio by controlling the fertilisation process of each egg (Bull, 1983; Young *et al.*, 1986; Roeder *et al.*, 1996; Sabelis *et al.*, 1999). Second, virgin females can disperse and found colonies on a host plant where they can mate with their sons (Yano, 2008). Third, there is a two-fold representation of maternal genes in the gametes of haploid sons in comparison to diploid sons of biparental origin (Carbonnelle, 2004). The probability of gene identity-by-descent between grandmother and grandchild through uniparental sons is, therefore, double the probability that is shown through biparental sons (Bull, 1983). Finally, the fourth advantage of this kind of reproduction is that males can only have daughters, whereas females can have both daughters and sons. Therefore, females will have much more offsprings than males. This induces, that a population presenting a high quantity of females will present also a high demographic rate.

Dispersal behaviour

The dispersal ability of *T. urticae* is an important factor to take



Figure 17: massive aggregate at the top of an infested plant (Picture: Gwendoline Clotuche)

into account in its management. When local resources are exhausted, females move in the same direction, following other females (Yano 2008). *T. urticae* may reach potential host plants by walking, a primary form of dispersal (e.g., Kondo and Takafuji, 1985), or by anemochory by raising their forelegs upright to assume a dispersal posture or inside a web structure enclosing many individuals (Brandenburg and Kennedy 1982) (figure 17). In the third case of aerial dispersal, the mites reach the apex of the plant and form massive aggregates surrounded by silk, which can sometimes spin down on silk thread. These massive aggregates may contain more than 1500 individuals and are mainly composed of immature individuals (Clotuche *et al.*, 2011), and may be dispersed through the landscape by anemochory or zoochory.

Methods

1) Impact of group living on individuals fitness (papers 1 and 2)

In this first section, the aim was to investigate the impact of group living on group on individual's fitness. To do so, at the beginning of each experiment, females (single or in groups of two, four or six) were put onto one new bean leaf disc (*Phaseolus vulgaris*) placed on damp cotton in a Petri dish (92 mm in diameter). The density of mites (0.02 individuals/mm² in article 1 and 0.06 individuals/mm² in article 2) was kept constant between compared groups. Indeed, the aim was to test the influence of conspecific presence independently of any density effect which corresponds to a different amount of food and living space per individual. The following parameters were measured every day for five days (before the first eggs hatched) per alive individual:

1) The mean number of white faecal pellets on the web per live individual per day. White faecal pellets were considered as a silk production indicator by Hazan *et al.*, 1974.

2) The mean number of eggs laid per alive individual per day for five days.

3) The cumulative death rate after five days

4) The cumulative necrotic area of the leaf per live individual (Candolfi *et al.*, 1991) which is an indicator of the intensity of the feeding process.

2) Study of aggregation dynamics (articles 3, 4 and 5)

In this section, we studied the aggregation dynamic of *T. urticae* under different conditions. We tested three different environments, from the simplest and artificial one to a natural substrate (the whole plant). With this method, we progressively investigated what the factors that mediate the spatial distribution of *T. urticae* were. (1) Glass discs, a homogeneous environment, were used in order to investigate the influence of conspecifics on spatial distribution. (2) Then, bean leaf discs, an heterogeneous environment (presence of food and leaf heterogeneity (nerves)), that allows to take into account the micro-environment on *T. urticae* micro-spatial distribution. (3) Finally, whole bean plants, a common host of *T. urticae*.

To analyse the aggregation dynamics of *T. urticae* in the cases of glass and bean leaf experiments, an analogue camera was focused on the set-up (leaf disc or glass disc) and recorded the behaviour of females. One picture was taken every minute for 30 minutes. These pictures were analysed using a data processing program called Fourmilier V3.3 (software creator, Jean- Michel Hudry (jm.hudry@ifrance.com)) (Sempo *et al.*, 2006; Le Goff *et al.*, 2009),

which attributed spatial coordinates to each individual and thus allowed further analyses of the spatial distribution of individuals. By computing these locations, we characterised the spatial distribution of each group with three different indicators:

1. The angular distribution “*s*” is the angular position of individuals on the disc (Zar, 1999). It was measured for each mite group at the end of each session.
2. The radial distribution is the radial position of mites on the leaf. Quantification of the radial distribution allows for analysing the trend to follow the leaf border as a kind of thigmotaxis (also called the border effect). The leaf was divided into five rings of 1.5 mm (0-1.5, 1.5-3, 3-4.5, 4.5-6 and 6-7.5), the external ring corresponding to the edge of the area. We took into account the differences between the areas of all rings by comparing spider mite density per ring.
3. The mean distance between individuals which is an indicator of inter-individual spatial proximity (Dambach and Goehlen, 1999).
4. The mean size of the largest aggregate, which is a commonly used indicator to characterise the spatial distribution of eusocial (Depickere *et al.*, 2004, Sempo *et al.*, 2006) and non-eusocial insects (Jeanson *et al.*, 2005).

Concerning aggregation on plant we infested a standardized bean plant initially infested with a population of sixty females of less than 72h. The plant architecture consisted of two first levels with two leaves each and a third level with only one leaf. Two days after infestation, the leaves of each plant were collected and placed in a freezer (-20°C) for 1 hour. Then, we took a picture of the upper- and underside of each leaf. Finally, we analysed each picture with Image J software (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, MD, USA, <http://rsb.info.nih.gov/ij/>, 1997–2008), and counted the number of individuals on the upper-and underside of each leaf. Based on the number of recovered individuals on each plant we calculated the mean percentage of recovered individuals that stopped at the first, the second and the third level.

3) Influence of silk on individuals spatial distribution and locomotor activity (papers 6 and 7)

A microscope cover glass (ø15 mm) was placed on a wet paper, itself deposited on damp cotton to avoid individuals from leaving the experimental design. One virgin female of *T. urticae* aged less than 24h was allowed to walk freely on one half of the cover glass whereas the other half was protected with a wet paper. For 30 minutes, the individual was allowed to deposit silk (and possibly pheromones and excrements) by walking on the free half of the cover glass (Saito

1977). Therefore, we obtained a silk-covered part (SP) and a clean part (CP) over the same cover glass. The female was removed and a new virgin female less than 48h (fed or starved for 24h) was placed at the centre of this cover glass at the SP/CP frontier by using a single-haired brush and its behaviour was video-recorded for 30 minutes. Four behavioural parameters were recorded on each part of the set-up with the software Jwatcher (Copyright © 2000–Daniel T. Blumstein, Christopher S. Evans & Janice C. Daniel): (1) the total time spent on each part of the glass plate, (2) the time in movement, (3) the resting time and (4) the time in static exploration (“statex”). Statex is a behaviour characterised by individuals staying at the same place but turning on themselves.

4) *Influence of the genetic proximity*

We modulated the genetic proximity of *T. urticae* by two methods. The first consisted of creating inbred lines and therefore, testing the impact of inbreeding for each subsequent generation on *T. urticae*. For the second method, we used two different and well recognised populations of *T. urticae*: a red form and a green form. The behaviour of groups composed of individuals from the same population or from a mix of both was recorded. Using the red and the green form allowed us to investigate whether interactions between individuals from different populations/genetic pools influenced the behaviours involved in social organisation of *T. urticae*.

5) Influence of starvation

Females were isolated on bean leaf discs at the teleochrysalid stage and then transferred on a cover glass just after molting. This starvation period lasted 24H and did not induce any abnormal mortality.

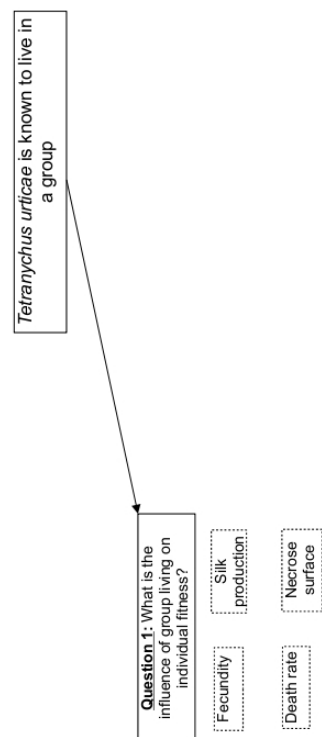


Figure 6: Flow chart of the PhD thesis, showing the different steps of the study

Question 1 – paper 1: What is the influence of group living on individual fitness?

Living in group can provide several kind of benefits, for instance in terms of foraging success, protection against predator, competitive ability, or some combination of the foregoing (Depickère, 2003). However these advantages have also drawbacks. Indeed group living have inevitable costs within the group: individuals may compete to resource acquisition and an aggregate of several individuals is more easy to locate by a predator than isolated preys.

T. urticae is known to live in groups. In this study, we investigated the consequence of group living on some life history traits without significant external selective pressure such as the predation. The indicators we used were namely, the production of white faecal pellets (presented as a indicator of web production), fecundity, death rate and the leaf area undergoing necrosis consequently to feeding activities.

Group effect on fertility, survival and silk production in the web spinner *Tetranychus urticae* (Acari: Tetranychidae) during colony foundation

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(Accepted: 10 May 2010)

Summary

In many vertebrates and invertebrates, individuals reared in isolation show biological modifications compared with those reared in groups of two or more. The spider mite *Tetranychus urticae* is characterised by a communal organization and displays some forms of cooperative behaviour (aggregation and common web spinning). To evaluate the potential fitness cost or gain of group living, we investigated the effect of being in group on life history parameters (silk production, fecundity, death rate, feeding rate). In this respect, virgin single females and grouped females (two to six individuals) were compared every day for 5 days. Grouped mites produced significantly more web/mite per day (since the second day of experiment) and more eggs/mite per day (since the fourth day of experiment) than single mites. Moreover, single mites had a higher death rate than mites living in groups. However, no difference was found concerning feeding rate. We assume that mites could benefit from the web production of other individuals and invested resources in other activities such as egg production. We showed that these group effects already exist in small groups. In natural conditions with a higher group size, the impact of group living might be stronger for the dynamics of *T. urticae* populations and the colony foundation.

Keywords: *Tetranychus urticae*, group effect, Allee effect, fitness indicators.

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Introduction

The social environment of an individual may have a great impact on its behaviour (Wilson, 1975; Prokopy & Duan, 1998; Prokopy & Reynolds, 1998; Krams et al., 2009) or on its physiology (Chauvin, 1946, 1958; Grassé, 1946; Long, 1953; Wharton et al., 1967, 1968; Yoder & Grojean, 1997; Holbrook et al., 2000; Rotem & Agrawal, 2003; Lihoreau & Rivault, 2008), for both vertebrates and invertebrates. Such alterations of individuals living in a group have been termed 'group effects' (Grassé, 1946; Holbrook & Schal, 1998; Boulay et al., 1999; Holbrook et al., 2000; Lihoreau & Rivault, 2008) and imply the costs and benefits of so living (Barbault, 1977). These group effects might be derived from simple numerical effects and/or synergistic inter-individual effects. For example, in vertebrates, Krams et al. (2009) showed the importance of group size for communal defence against predators in the pied flycatchers *Ficedula hypoleuca*. In invertebrates, Lihoreau & Rivault (2008) showed that the presence of conspecifics increased rates of both nymphal development and oothecae production in *Blatella germanica*. Furthermore, Avilés & Tufiño (1998) and Salomon & Lubin (2007) observed that in social spiders, the average number of eggs per female and the probability of offspring survival to maturity increased with colony size.

Tetranychus urticae is considered as a major pest of many cultivated plants (more than 900 listed host-plants; Van Impe, 1985). Consequently, research has mainly focused on the management and control of this species whereas its behaviour, especially its social behaviour, has often been disregarded. *T. urticae* was chosen as a biological model for studying group effects for several reasons. Firstly, the individuals of this species can survive on their own and/or in groups of two or more. Indeed, thanks to its arrhenotokous parthenogenetic reproductive system, *T. urticae* females can disperse on their own and found colonies on a host plant by mating with her own sons (Yano, 2008). This feature enables experimentation with a continuum in the group sizes. Secondly, *T. urticae* exhibit various forms of social behaviours such as aggregation (Le Goff et al., 2009) or construction of a common web (Saito, 1983; Clotuche et al., 2009). These studies suggest that there is a higher level of sociality than just being gregarious. However, according to Costa (2006), the next level of sociality corresponds to subsocial with parental care behaviours. Parental care behaviours have never been described for *T. urticae*, but if we consider the web as a common living space for individuals of the same generation, *T. urticae* could be so a communal species.

From an evolutionary point of view, group living has many advantages for both individuals and groups because it helps access to food and mates and protects against predators (Ranta et al., 1993; Dyer, 2000; Wertheim, 2005; Costa, 2006; Mori & Saito, 2006; Sato & Saito, 2006). Indeed group formation confers on individuals a net benefit from prédation (Wertheim et al., 2005). However, it also presents disadvantages such as having to share food, mates and living space and can result in inter-individual conflicts (Ranta et al., 1993). The construction of a common web also confers a selective advantage over the group. A group of spider mites produce a denser web than an single individual. The resulting web offers better protection from the rain (Davis, 1952), pesticides (Ashley, 2003) and predator attacks (McMurtry et al., 1970). One group effect has already been evidenced in *T. urticae*. Van Impe (1984, 1985) found that male development times decreased when the density of individuals increased. However, besides this work and despite indicators of a social organization (aggregation and common web), group effects in *T. urticae* have been poorly investigated.

In this study, we wanted to explore the importance of group living in this species without any important selective pressure like predation. In this respect, we continued the work of Van Impe (1984, 1985) one step further as we focused on the individual consequences of being associated to conspecifics in *T. urticae*. In this respect, we studied the impact of group living on some major biological features by assessing the variations of different indicators, i.e., white faecal pellet production as a web production indicator (Hazan et al., 1971), fecundity, cumulative death rate and the necrotic area of the leaf as a feeding indicator (Candolfi et al., 1991).

Material and methods

The mites used in this study belonged to the red form of *T. urticae* (also called *T. cinnabarinus*) and were provided by the Institut National Agronomique, Tunisia (Tunis, Dr K. Lebdi-Grissa). The breeding stocks were maintained at 26°C, 50–60% relative humidity, 16:8 (L/D). The experiments were undertaken in the same environmental conditions as breeding. Only virgin females less than 24 h old were used to standardize the experiments as mated and virgin females have different oviposition rates (Van Impe, 1985) and furthermore, mated females have a greater variable ovoposition behaviour than virgin females (Van Impe, 1985).

At the beginning of each experiment, females (single or in groups of 2, 4 or 6) were put onto one new bean leaf disc (*Phaseolus vulgaris*) and this disc was placed onto damp cotton in a Petri dish (92 mm diameter). Thirty replications were done for each group size.

For experiments with one, 2, 4 and 6 individuals, the diameters of the leaf discs were 7.5 mm, 10 mm, 15 mm and 18 mm, respectively, to insure the same density of mites (0.02 individuals/mm²). Indeed, the aim was to test the influence of conspecific presence independently of any density effect (different amount of food and living space per individual). The following parameters were measured every day for five days (before the first eggs hatched) per live individual:

(1) The silk production indicator, which was the mean number of white faecal pellets on the web per live individual per day (Hazan et al., 1971) for 5 days. We also measured the volume (μm^3) of white faecal pellets as a function of the number of individuals. According to Wiesmann (1968), the pellets were roughly spherical. This approximation was supported by preliminary experiments. White faecal pellets produced by single individuals and individuals in groups of 2, 4 and 6 were chosen at random ($N = 30$). The areas were photographed from the top and side points of view (Mailleux et al., 2000) and measured with the ImageJ software (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, MD, USA, <http://rsb.info.nih.gov/ij/>, 1997–2008). The side and top areas were correlated when pellets were laid by single individuals ($N = 30$) and groups of 2 ($N = 30$), 4 ($N = 30$) and 6 ($N = 30$) individuals using the Spearman's rank correlation (R_s) coefficient (1: $R_s = 0.71$, $p < 0.001$, 2: $R_s = 0.80$, $p < 0.001$, 4: $R_s = 0.57$, $p < 0.001$, 6: $R_s = 0.86$, $p < 0.001$). The average top:side ratio was 1.01 ± 0.01 (mean $\pm$ 95% confidence interval (CI)), with ratios of 1.13 ± 0.09 , 1.16 ± 0.01 and 1.14 ± 0.09 for single individuals and individuals in groups of 2, 4 and 6, respectively. This confirmed that the pellets were spherical and we only measured the top area of the pellets to calculate and compare their volumes for single individuals and individuals in groups of 2, 4 and 6. The focal distance between the white faecal pellets and the lens was carefully kept constant.

(2) The mean number of eggs laid per live individual per day for 5 days. We also measured the volume (μm^3) of 30 eggs of less than 24 h old, chosen at random in each group size. Eggs of *T. urticae* are spherical (Crooker, 1985). The eggs were photographed (the focal distance between the eggs and

the lens was carefully kept constant) and measured with the ImageJ software (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, MD, USA, <http://rsb.info.nih.gov/ij/>, 1997–2008).

(3) The mortality indicator, which was the cumulative death rate after 5 days.

(4) The feeding indicator, which was the cumulative necrotic area of the leaf per live individual (Candolfi et al., 1991) after 5 days. It was measured with the software ImageJ. This area was measured only for single females and females in groups of six individuals at the fifth day.

Statistical tests

As all data were normally distributed, parametric statistics were used. Repeated measures ANOVA, one-way ANOVA, *t*-tests and linear regressions were performed using GraphPad Prism version 5.01 for Mac OS (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Linear regressions, which are represented on all graph were calculated on 120 points (4 group sizes and 30 replicates) and analysed using an *F* test to understand the influence of group size on these parameters and whether the slope was significantly non-zero. All tests were applied under two-tailed hypotheses and the significance level *p* was set at 0.05.

Results

Silk production

Repeated measures ANOVA showed that whether a female was kept single or in group had a significant effect ($F = 22.20$, $df_n = 3$, $df_d = 435$, $p < 0.001$, Figure 1) on silk production per individual. This finding was, however, rendered ambiguous by a significant interaction ($F = 2.10$, $df_n = 12$, $df_d = 435$, $p < 0.05$) between the day of experiment and the group size. We, therefore, used the Bonferroni procedure to detect differences in silk production for similarly aged single and grouped females (Table 1). Grouped females did not produced more silk than single ones on the first day (Table 1). For each of the next four days of experiments, significant differences were observed between grouped females and isolated ones (Table 1). At the fifth day, single individuals produced 2.94 ± 1.24 pellets/live individuals per day,

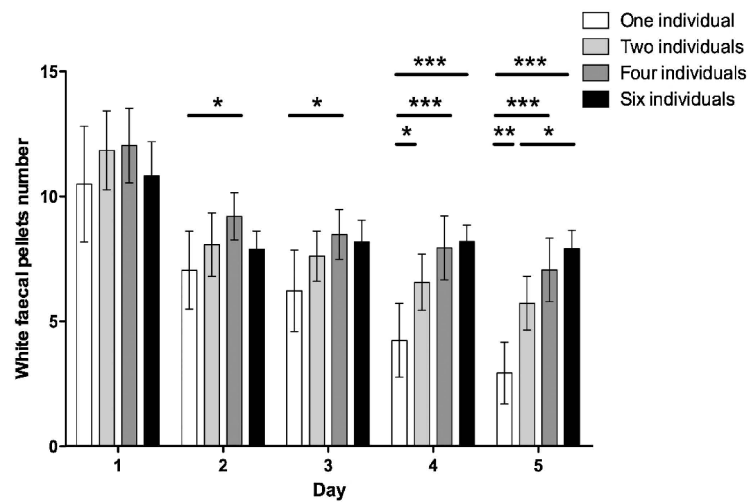


Figure 1. Mean daily white faecal pellet production per live individual for five days of experiment and each group size ($N = 30$). Error bars indicate 95% CI.

Table 1. Student's t -values of the Bonferroni's multiple comparison test between each group sizes for the silk production, the egg production and the cumulative death rate (CDR).

	Silk production					Egg production					CDR
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5	Day 5
1 vs 2	1.64	1.25	1.68	2.83*	3.40**	0.49	1.34	1.04	1.56	2.10	2.58
1 vs 4	1.87	2.62*	2.75*	4.49***	5.01***	0.64	0.56	1.62	3.75***	3.61**	2.58
1 vs 6	0.40	1.02	2.39	4.80***	6.03***	0.29	0.44	1.46	2.83*	4.00***	3.19*
2 vs 4	0.23	1.38	1.06	1.67	1.61	0.15	0.78	0.58	2.20	1.50	0.00
2 vs 6	1.24	0.22	0.70	1.98	2.64*	0.20	0.89	0.41	1.28	1.90	0.61
4 vs 6	1.47	1.60	0.36	0.31	1.02	0.34	0.12	0.16	1.67	0.39	0.61

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from control.

whereas individuals in groups of 2, 4 and 6 produced 5.74 ± 1.08 , 7.06 ± 1.28 and 7.91 ± 0.74 pellets/live individual per day, respectively.

The volume of white faecal pellets increased with the size of the group ($R^2 = 0.06$, $p < 0.01$, $N = 120$, slope $\pm$ CI = $16\,270 \pm 11\,613$, y-intercept $\pm$ CI = $222\,800 \pm 43\,800$) ($F = 7.54$, $p < 0.01$) (Figure 2). The volume of pellets laid by single individuals was $253\,505 \pm 48\,585 \mu\text{m}^3$,

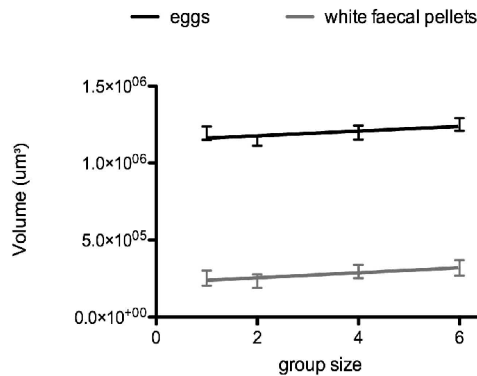


Figure 2. Linear regression of eggs and white faecal pellets of less than 24 h versus group size ($N = 30$). Error bars indicate 95% CI.

an area similar ($232\,923 \pm 43\,667 \mu\text{m}^3$) to that of the pellets laid by groups of 2 mites. For white faecal pellets laid by groups of 4 and 6 mites, the volumes were $296\,645 \pm 43\,252 \mu\text{m}^3$ and $319\,644 \pm 50\,094 \mu\text{m}^3$ respectively.

Fecundity

Repeated measures ANOVA showed that being in group did not significantly influence the fecundity/female per day ($F = 2.64$, $\text{df}_n = 3$, $\text{df}_d = 464$, $p > 0.05$, Figure 3). However, the significant interaction between the day of experiment and the group size rendered difficult to interpret this result ($F = 2.91$, $\text{df}_n = 12$, $\text{df}_d = 464$, $p < 0.001$). We, therefore, used the Bonferroni procedure to detect differences in fecundity for similarly aged single and grouped females (Table 1). Grouped females did not lay more eggs than single ones on the three first days (Table 1). For the fourth and the fifth day of experiments, significant differences were observed between grouped females and isolated ones (Table 1). At the fifth day, a single individual produced 0.97 ± 0.41 eggs/live individual per day, whereas individuals in groups of 2, 4 and 6 produced 1.33 ± 0.38 , 1.58 ± 0.25 and 1.65 ± 0.21 eggs/live individual per day, respectively.

The volume of eggs laid also increased with the size of the group ($R^2 = 0.07$, $p < 0.01$, $N = 120$, slope $\pm$ CI = $15\,150 \pm 10\,385$, y-intercept $\pm$ CI = $1\,147\,000 \pm 39\,000$) ($F = 8.35$, $p < 0.01$) (Figure 2). Regarding individuals in groups of 2 mites, the egg volume was $1\,143\,000 \pm 31\,000 \mu\text{m}^3$. Larger egg volumes were measured for single individuals ($1\,194\,000 \pm 43\,000$

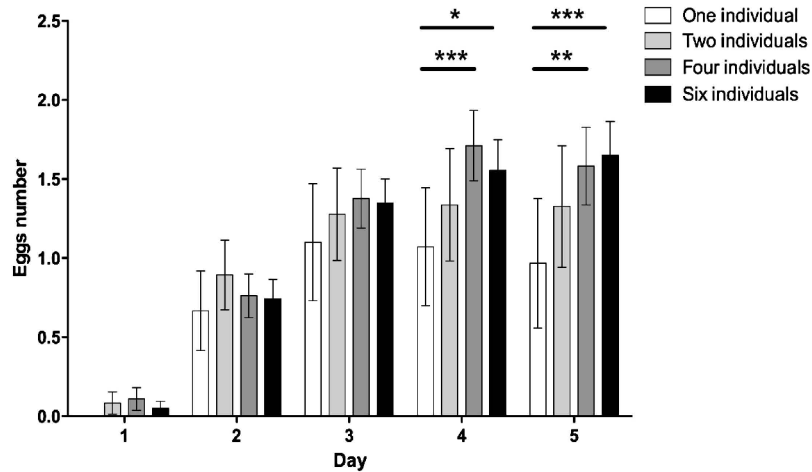


Figure 3. Mean daily egg production per live individual for 5 days of experiment and each group size ($N = 30$). Error bars indicate 95% CI.

μm^3) and individuals in groups of 4 ($1\,197\,000 \pm 45\,000 \mu\text{m}^3$) and 6 mites ($1\,251\,000 \pm 41\,000 \mu\text{m}^3$).

Cumulative death rate

In all treatments, a percentage of individuals died at the beginning of the experiment (death rate approx. 15%), probably because of possible injuries during mite transfers. However, because the same mortality bias was observed on day one for each tested condition, the comparison of our data remained valid (ANOVA $F = 0.04$; $df = 3$; residual error = 116; $p > 0.05$). A one-way ANOVA followed by a Bonferroni's multiple comparison test showed a decrease in cumulative death rate as the size of the group increased. We observed a large decrease of the cumulative death rate between single individuals and individuals in groups of 6 (ANOVA $F = 4.05$; $df = 3$; residual error = 116; $p < 0.001$, Bonferroni; Table 1) (Figure 4). With single individuals, on the fifth day the cumulative death rate reached $56.67 \pm 18.82\%$, whereas it decreased to approx. 30% in the other groups.

Feeding

The leaf necrotic area/individual did not change with the size of the group ($t = 0.07$, $df = 58$, $p > 0.5$). On the fifth day, the leaf necrotic area of the

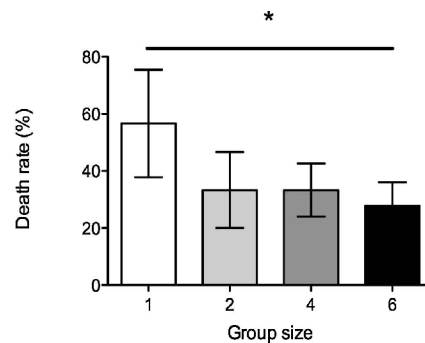


Figure 4. Percentage of death rate after 5 days of experiment and each group size ($N = 30$). Error bars indicate 95% CI.

leaf eaten by single individuals was $0.92 \pm 0.28 \text{ mm}^2$. A similar surface of leaf necrotic area/individual ($0.93 \pm 0.14 \text{ mm}^2$) was eaten by groups of 6 mites.

Discussion

In nature, single individuals of *T. urticae* can disperse to found a colony, or they can disperse in groups of two or more (Yano, 2008). The presence of conspecifics in this species can induce life history traits modifications in individuals compared to single individuals.

Our results showed that in this species (under experimental conditions) living in groups increased silk production, fecundity and survival. This implies that individuals can perceive the presence of their conspecifics through direct and/or indirect interactions. (In)direct perception through chemicals (pheromones) or tactile perception of conspecifics might constitute indicators of group size (Prokopy & Duan, 1998; Prokopy & Reynolds, 1998). Chemical or tactile perception of eggs, faecal pellets, the web, necrotic leaf areas and compounds emitted by the leaf could also play indirect roles in other individuals' presence perception.

Our work showed that white faecal pellet production increased with the size of the group (more than one pellet more per individual for each added individual). This increase in pellet production per individual indicates an increase in silk production per individual (Hazan et al., 1971), leading to a denser and more protective web against external aggressions (rain, wind

and predators). This increase in pellet production can also be explained at the ecological level. We assume that being in a group reduces the ecological stress that mites might face. For instance, being in an aggregate reduce the evaporating surface and avoid desiccation (Joosse, 1970; Wertheim et al., 2005). When confronted with a reduction in ecological stress, individuals can allocate their time differently when they are in groups compared to when they are single. Indeed, the same necrotic surface area does not imply the same necrotic depth and so the same amount of food. Therefore, individuals in groups may spend more time feeding at the same place and, therefore, cause the same necrotic surface with a greater depth and a larger amount of white faecal pellets than when they are alone. Furthermore, there might be one (or more) ecological advantages in producing numerous bigger white faecal pellets when the group size increases. A previous work showed that white faecal pellet volume increases with relative humidity, probably through water absorbency (Hazan et al., 1971). White faecal pellets might buffer variations in relative humidity and stabilise the humidity conditions in the web. It has also been observed that white faecal pellets are frequently the node connecting many silk threads (Figure 5). Hence, we assume that white faecal pellets might also be a component of the web structure; they could be cohesive points or 'tension adjusters' and could affect the web's mechanical properties. White faecal pellets might also have a repellent effect on predators. Oku (2008) showed that white faecal pellets produced by *T. kanzawai* repelled the predator *Neoseiulus womersleyi*. How the predators of *T. urticae* might be influenced by their pellets would be an interesting future study.

Our results showed a positive group effect on the fecundity of *T. urticae*. Living in groups increases egg production by around a third of an egg per individual for each added individual. The next step of this work will be to compare the individuals hatching from eggs laid by single individuals and individuals in groups (hatching rate, fitness of the newly hatched mites). *T. urticae* seems to benefit from the presence of other individuals as this induces life history trait changes which allow an investment in egg production (number and volume). Indeed, as producing a web is likely to entail a cost, mites that arrive on a leaf with a web are expected to refrain from producing it because they will benefit from the protection of the existing web (Oku et al., 2009). Mites that spend less energy producing a web may then allocate resources not spent on web construction to other fitness-enhancing activities, such as laying eggs (Oku et al., 2009). Holbrook et al. (2000) showed

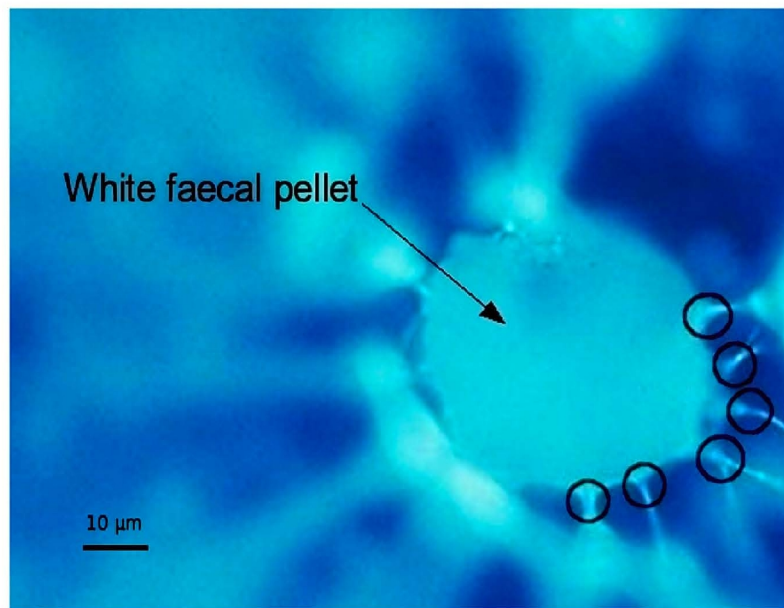


Figure 5. A white faecal pellet penetrated by many silk threads. Silk penetrations are encircled by black rings. The pellet was coloured using the technique described in Clotuche et al. (2009). Photo by G. Clotuche. This figure is published in colour in the online edition of this journal, that can be accessed via <http://www.brill.nl/beh>

in *Blattella germanica* that group rearing accelerates oocyte development by diminishing the brain's inhibition of the synthesis of juvenile hormones. In *T. urticae*, such a similar physiological change might also be at work (Regev & Cone, 1976; Thind & Edwards, 1990) and further biochemical measurements can be envisaged, such as the measurement of biochemical messages (hormones, pheromones), as a function of group size. However, it must be noted that our experiments were performed using virgin females and that group effects on egg production could differ with mated females (Van Impe, 1985).

We observed a positive group effect on the survival of *T. urticae*. It has to be noted that this group effect became limited as a plateau of death rate (approx. 30%) was reached, since these individuals were in a group. The death rate of single individuals was higher than for individuals in groups of 6 after the fifth day of the experiment (56.67% versus 27.78%). This supports the view that individuals benefit from environmental modifications

created by other individuals, such as increases in web and white faecal pellet production.

T. urticae is a well-known worldwide pest of many agricultural crops, orchards, nurseries, greenhouses and field crops (Margolies & Kennedy, 1985; Eastbrook et al., 2001; Cilli et al., 2005). To control such a pest, a prime necessity is an understanding of the impacts of group living (Myllymäki, 1975). Indeed, social behaviours are behind spatial distribution, dispersal movement and invasions by the pest and, more generally, rule most of their life history traits. Social behaviours usually influence the immediate result of a control measure as individuals living in group may initiate the compensative responses to induced mortality more rapidly. In turn, upsetting normal behavioural mechanisms of a social pest species (e.g., lure with aggregative/dispersal pheromone) might be used as means of control. Therefore, studying social behaviour has great relevance to control a group living pest (Myllymäki, 1975). The control of mites in greenhouses and fields can be complicated. Their nests are covered with dense webs that protect them from acaricides and mites are also difficult to eradicate because of the large number of eggs produced. One proposed technique might be the use of traps or baits loaded with mite pheromones that could attract adults, nymphs and larvae and to hold them in a place without a protecting web. Our study strongly suggests that this method of control would fail because increasing the local density would increase the rate of fecundity, resulting in larger populations which would be much more difficult to control. From a biological control point of view, the use of dispersing pheromones might be more efficient (Dicke et al., 2008). The Allee effect observed in *T. urticae* strongly suggests that regular treatments of the culture in such a way as to prevent its arrival in the cultures, or the maintenance of populations under economically acceptable thresholds might help to control these mites. These might be the next steps of our study.

This paper is a thorough study that opens several doors in an unexplored field of social biology. Indeed, the effects of group size have been poorly studied in species characterised by a communal organization such as spider mites. In this paper, we showed a possible Allee effect in *T. urticae*. Indeed, the Allee effect describes a scenario in which populations at low numbers are affected by a positive relationship between population growth rate and density, which increases their likelihood of extinction (Courchamp et al., 1999). Species in which fitness is enhanced by any type of conspecific facilitation

might suffer from reduced density when intraspecific competitive processes are of secondary importance (Courchamp et al., 1999). Despite our experiments was conducted at a constant density, the fact that *T. urticae* is an aggregative species (Le Goff et al., 2009) caused an increase in density at a local scale which increased the encounter rate. The presence of conspecifics seems to facilitate an increase in the fitness of other individuals. In other words, better living conditions created by group living (more web production and humidity buffering), seem to induce a higher investment per mite in eggs and pellets/web production, which also increase the survival rate.

Moreover, we showed that these group effects already exist in small groups (only two to six individuals). We assume that in natural conditions with a higher group size, these Allee effects might have a stronger impact on the dynamics of *T. urticae* populations, especially for colony foundation. Indeed, a positive consequence of this group effect in *T. urticae* concerns the foundation of a new colony. Although a single female is able to found a new colony thanks to its parthenogenetic reproductive system (Yano, 2008), the success of a colony foundation would be much higher in groups than when alone (Ranta et al., 1993; Yano, 2004, 2008) as this would give a passive protection to the individual (Keough, 1983; Rivault et al., 1998; Ame et al., 2004) and also, as we have shown in this study, this would increase an individual's fitness.

However, in overcrowded conditions, the presence of conspecifics could have a negative impact on population dynamics (negative group effect). The physiological and/or behavioural mechanisms involved in these group effects remain unknown and deserve further study (Grassé, 1946; Prokopy & Duan, 1998; Prokopy & Reynolds, 1998; Holbrook et al., 2000).

Acknowledgements

We are very grateful to Prof. Kaoutar Lebdi who supplied the red form of *Tetranychus urticae*, which was used in our experiments, and to George Van Impe for the useful discussions about *Tetranychus urticae*. We would like to thank all the team of the Earth and Life Institute for their help in data analysis. The authors are also indebted to the National Fund for Scientific Research (FNRS, Belgium) for funding through the Fund for Fundamental and Collective Research (FRFC, convention 2.4622.06). A.C.M. was financially supported by IRSIB (Institut d'encouragement de la Recherche Scientifique et de l'Innovation de Bruxelles). This publication is number BRC173 of the Biodiversity Research Center.

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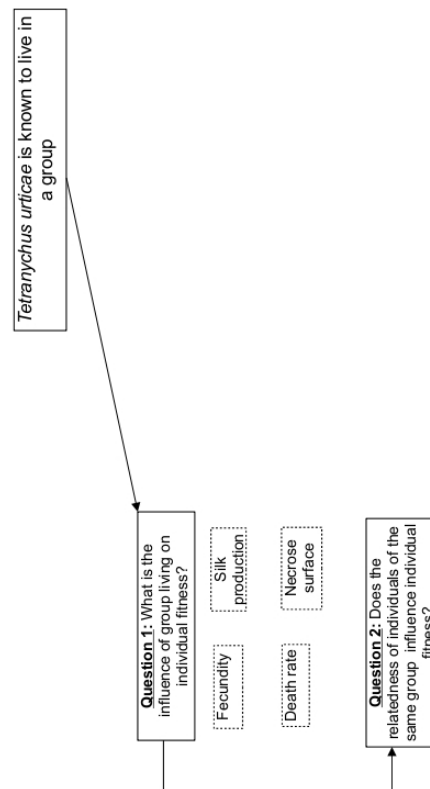


Figure 6: Flow chart of the PhD thesis, showing the different steps of the study

Question 2 – paper 2: Does the relatedness of individuals of the same group influence individual fitness?

For species that live in group, the ability to recognize and discriminate kin among conspecifics can constitute advantages and thus influence the choice of the partners during an aggregative process. In addition, one generally assume that the genetic relatedness plays a key role in the organization and functioning of societies. For instance, in *T. urticae* females (sisters or non-sisters) from inbred and outbred populations are able to modify the sex-ratio of their offspring according to the genetic relatedness of the group (Roeder *et al.*, 1996). In this part, the influence of the genetic background on some biological traits of *T. urticae* was studied. Indeed, we analysed the impact of living with related individual on some major traits of fitness such as fecundity rate, death rate and white faecal pellets an indicator of metabolism and silk production. According to literature, relatedness could promote cooperative behaviours (Queller and Strassmann, 1998; Queller, 2000). Consequently, we expected to measure a positive group effect with an increase in silk production, fecundity and survival in groups composed of related individuals.

Impact of living in group on the life history traits of pure and mixed populations of *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract

In many vertebrates and invertebrates, group living may influence the behaviour, physiology and all other life-history traits of individuals. This group effect of social interactions can be modulated by the genetic relatedness between individuals.

The spider mite *Tetranychus urticae* is characterised by communal organization. Its silk production probably plays a key role in its evolution to a social way of life. Indeed, it protects the colony against biotic and abiotic agents such as predators, competitors, humidity, wind, rain and acaricides. To evaluate the potential cost or gain of being associated with genetically distant individuals in *T. urticae* on different fitness indicators (silk production, fecundity, death rate), we compared two distinct populations of *T. urticae*: a red form population (Tunisia) and a green form population.

Here, we report that group living brings different benefits and costs to the two red and green populations. The red form population benefits from group living with a higher silk production whereas the fecundity (number and volume of eggs) and the death rate are not changed in big groups. For the green populations, living in group has a cost as in big groups, eggs are produced in smaller quantity while silk production and the death rate are not changed. In a mixed group composed of green and red individuals, egg production, silk production and the death rate were statistically similar to the values obtained in the pure group of green individuals.

Our study allows us suppose that strain recognition ability may

occur in *T. urticae* and proves that the genetic background of an individual may have a great impact on several of its life history traits.

Key words: Life history traits, kin discrimination, group living, *Tetranychus urticae*

Introduction

In vertebrates and invertebrates, living in a group may have a great impact on the behaviour of individuals (Wilson, 1975; Prokopy and Duan, 1998; Prokopy and Reynolds, 1998; Krams *et al.*, 2009) or on their physiology (Grassé, 1946; Chauvin, 1958; Wharton *et al.*, 1967, 1968; Yoder and Grojean, 1997; Holbrook *et al.*, 2000; Rotem and Agrawal, 2003; Lihoreau and Rivault, 2008). From an evolutionary point of view, group living gives advantages and drawbacks to both the individual and the group as it provides, for instance, protection against predators (Ranta *et al.*, 1993; Wertheim, 2005). Indeed, individuals living in larger groups have a higher probability to detect predators and a lower probability of being selected as prey namely due to the confusing effect on predators during their attack (Molvar and Bowyer 1994, Krams *et al.* 2009). Group living also provides easier access to food and mates (Ranta *et al.* 1993). For instance, Wertheim *et al.* (2002) showed that, in the fruit fly *Drosophila melanogaster*, a high density of adults enhanced the quality of the larval substrate. It can also lower energetic costs by decreasing temperature loss or water

evaporation (Krause and Ruxton 2002). As a result, living in a group can be beneficial for individual fitness. This has been shown in social spiders in which the average number of eggs per female and the probability of offspring survival to maturity increases with colony size (Avilés and Tufiño, 1998; Salomon and Lubin 2007).

On the other hand, living in a group brings drawbacks including intensified competition, increased conspicuousness to predators and increased chances of misdirected parental care (Rubenstein 1978). Moreover, since diseases and parasite transmission is often density-dependent, group living might lead to increased exposure to parasitism. Consequently, animals living in groups invest more resources (energy, time) in parasite defence than those living solitarily (Alexander 1974.) These costs and benefits of group living compared to living alone are termed ‘group effects’ (Grassé, 1946; Holbrook *et al.*, 1998, 2000; Lihoreau and Rivault, 2009; Le Goff *et al.*, 2010) and might be derived from simple density effects and/or synergistic interindividual effects.

Group effects may be influenced by the relatedness between individuals. In group-living species and whatever the degree of complexity of the social organisation, association with related conspecifics for different activities (foraging, resting, or mating) involves that the choice of these preferred partners is based on kin recognition. This choice also implies that individuals evaluate the associated costs and benefits of group living with closed individuals in many contexts (Fellows, 1998). When benefits are shared by close individuals, group living potentially increases indirect fitness gain of group members (Hamilton, 1964). Attraction for kin probably plays a key role in the

formation and maintenance of social groups of many species, namely by lowering the level of intra-group competition (Fellowes, 1998; Lihoreau and Rivault, 2009). On the other hand, kin avoidance allows for avoiding sibmating and therefore maintains a higher level of genetic diversity in the population (Banks *et al.*, 2005; Stow and Beattie, 2008). Most of the studies exploring the evolutionary causes of group living have been done on birds (Griesser and Ekman, 2005; Krams *et al.*, 2009), mammals, fish (Kirkwood and Dickie, 2005; Solorzano-Filho, 2006), and in some social invertebrates (Sharov, 1993; Davies, 1998; Ken *et al.* 2005; Mori and Saito 2005).

Tetranychus urticae is considered a major pest of many cultivated plants (more than 200 listed host plants, Van Impe, 1985). Consequently, research has mainly been focused on the management and control of this species, whereas its social behaviour has often been disregarded. However, *T. urticae* exhibit various forms of social behaviours such as aggregation (Le Goff *et al.*, 2009) or construction of a common web (Saito, 1983; Clotuche *et al.*, 2009). Moreover, spider mites are small organisms with low mobility, which may increase the number of interactions among kin (Saito, 1997). The group effect has already been evidenced in *T. urticae*. Indeed, Van Impe (1984, 1985) found that male development times decreased when the density of individuals increased. Furthermore, Le Goff *et al.* (2010) showed that grouped mites live longer and produce significantly more eggs and web per mite per day than single mites.

The influence of the genetic background has been observed on *T. urticae* behaviour and biology. Roeder *et al.* (1996) showed that in *T.*

urticae, females (sisters or non-sisters) coming from inbred and outbred population are able to modify their offspring sex-ratio according to the genetic relatedness of the group. The spatial distribution of *T. urticae* is also influenced by the level of inbreeding in the group; the inter-individual distance increases with inbreeding (Le Goff *et al.* 2009). Therefore, *T. urticae* mites have the ability to discriminate individuals differing by their original populations.

Besides this work and despite indicators of a simple social organisation (aggregation and common web), group effects and the influence of the relatedness have been poorly investigated in *T. urticae* and deserve further investigation. In this respect, we continued the work of Van Impe (1984, 1985) and Le Goff *et al.* (2010) one step further as we focused on the individual consequences of living in a group with kin or non-kin in *T. urticae*. Therefore, we studied the impact of living with related individuals or not on some major fitness traits by assessing the variations in fecundity rate, death rate and silk production, which was evaluated by the amount of white faecal pellets on the web per live individual per day (Hazan *et al.*, 1974). Our assumption here is that relatedness is expected to promote cooperative behaviour. Consequentially, we expect to observe a positive group effect with an increase of silk production, fecundity and survival in groups composed of related individuals.

Materials and methods

Two populations of *T. urticae* were used for the experiments: a red form population (also called *T. cinnabarinus*) provided by the Institut National Agronomique, Tunis, Tunisia (Dr K. Lebdi-Grissa) and a green form population, which was collected in the green houses of our laboratory in Louvain-la-Neuve, Belgium. The most accepted taxonomical position does not recognize *T. cinnabarinus* as a valid species as in the World Catalogue of the Spider Mite Family (Bolland *et al.* 1998), which cites it as synonym of *T. urticae*. The two populations were reared on bean leaves placed on damp cotton (*Phaseolus vulgaris*) in Petri dishes (28). Breeding stocks were maintained in a climate room at 26° C, 50–60% RH, 16:8 (L:D).

Genetic distance evaluation

DNA extraction and microsatellite amplification

For each population the DNA of 30 living adult females was individually extracted using a CTAB protocol described in Navajas *et al.* (1998) and Carbonnelle *et al.* (2007). The PCR procedures used in this paper were described in Carbonnelle *et al.* (2007) for the microsatellites Tu35b and Tu1 and in Uesugi and Osakabe (2007) for the microsatellites TuCT18, TuCT09 and TuCA02. Negative controls without DNA were included in each PCR run.

Data analysis

PCR products were then separated using an ABI 3730 automated DNA sequencer (Applied Biosystems) and allele size was determined by comparison with reference control alleles (GS400HD). For each form, the frequency of each allele per locus (N_a), the observed heterozygosity (H_o) and the unbiased expected heterozygosity (H_e) were computed using GENETIX version 4.05 software. An exact test was performed to test deviation from Hardy-Weinberg equilibrium (HWE) across loci in each population. When the hypothesis of HWE was rejected, tests were performed using genepop to find whether deviations were due to a deficit or an excess of heterozygosity.

The F_{ST} and the Nei distance values between the two populations were calculated and an exact test for genetic structure (differentiation pairwise test for population differentiation using allelic distribution in the various populations) was used as implemented in GENEPOP.

Group living experiments

Experiments were performed under the same conditions as breeding. At the beginning of each experiment, virgin females of less than 24Hh old were placed on a bean leaf disc (*Phaseolus vulgaris*) in groups of 5 or 10 individuals belonging to the same form (red: R5, R10 or green: G5, G10). We also mixed the two forms (M10), by

placing five red and five green virgin females less than 24h old on the bean leaf disc. Individuals were placed one by one in the centre of the bean leaf disc (red and green individuals alternately for the mixed experiments; number of replicates: red 10: $n = 33$; red 5: $n = 32$; mix: $n = 32$; green 10: $n = 31$; green 5: $n = 31$). To keep the density constant in all kinds of experiments (0.06 ind/mm^2), the leaf diameter of the bean leaf discs was 15mm for groups of ten and 10 mm for groups of five individuals. Three indicators were measured:

1. The mean cumulative number of white faecal pellets on the web per live individual which was considered as a silk production indicator, (Hazan *et al.*, 1974). The white faecal pellet production was measured and divided by the number of individuals that were still alive every day for five days (before the first eggs hatched). The results were then presented as the mean cumulative numbers of white faecal pellets per live individual per day for five days (before the first eggs hatched). We also measured the mean volume (mm^3) of white faecal pellets according to the method described in Le Goff *et al.*, 2010. The pellets are roughly spherical (Wiesmann 1968, Le Goff *et al.* 2010). The areas of the pellets were photographed from the top and we measured the top area of the pellets with the ImageJ software (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, MD, USA, <http://rsb.info.nih.gov/ij/>, 1997–2008). Their volumes (mm^3) were then calculated.

2. Fecundity, which was represented by the mean cumulative number of eggs. The egg production was measured and divided by the number of individuals that were still alive every day, for five days (before the first eggs hatched). The results were then presented as the mean cumulative egg production per live individual per day for five days (before the first eggs hatched). We also measured the volume (mm³) of thirty eggs less than 24 hours old, chosen at random in each kind of group with the ImageJ software.

3. The mortality indicator, which was the mean cumulative death rate after five days.

Statistical tests

Cumulative number of white faecal pellets, cumulative number of eggs, cumulative death rate after days, white faecal pellet volume and eggs volume were compared between groups with a one-way ANOVA followed by Bonferonni post hoc tests. All tests were applied under two-tailed hypotheses and the significance level *p* was set at 0.05. All tests were performed using GraphPad Prism version 5.01 for Mac OS (GraphPad Software, San Diego California USA, www.graphpad.com).

Results

Genetic distance evaluation

The number of alleles per locus were for the red and the green

form populations 2.60 and 4.40, respectively. The expected heterozygosity was 0.29 and 0.57, while the observed heterozygosity was 0.16 and 0.44 and the FIS (confidence interval (CI)): 0.47 (0.32-0.58) and 0.26 (0.15-0.30) for the red and green form populations, respectively. No linkage disequilibrium between loci was detected.

A Hardy-Weinberg test across loci indicated that our two forms showed an overall significant departure from the Hardy-Weinberg genotypic proportions (green form: $\chi^2=124.81$, $df=10$, $P<0.001$; red form: $\chi^2=116.09$, $df=6$, $P<0.001$) and displayed a significant heterozygote deficiency (green form: $P<0.001$; red form: $P<0.001$).

The F_{st} and the Nei distance value between our two populations were 0.46 and 0.95, respectively. The exact test for genetic structure revealed that our two forms differ genetically significantly across all loci ($\chi^2=132.85$, $df=10$, $P<0.001$).

Silk production

The numbers of white faecal pellets

This indicator of silk production differed according to the composition of the group (one-way ANOVA, $F=44.03$, $dfn=4$, $dfd=177$, $P<0.001$) (Figure 1). The red 10 had the highest mean number of white faecal pellets per individual and is higher than red 5 (Bonferroni: red 10 vs. red 5: $t=4.33$, $P<0.001$) (Figure 1). The white faecal pellet production of individuals in mix, green 10 and green 5 were not statistically different (Bonferroni: green 10 vs. green 5: $t=1.44$, $P>0.05$; green 10 vs. mix: $t=0.52$, $P>0.05$; green 5 vs. mix:

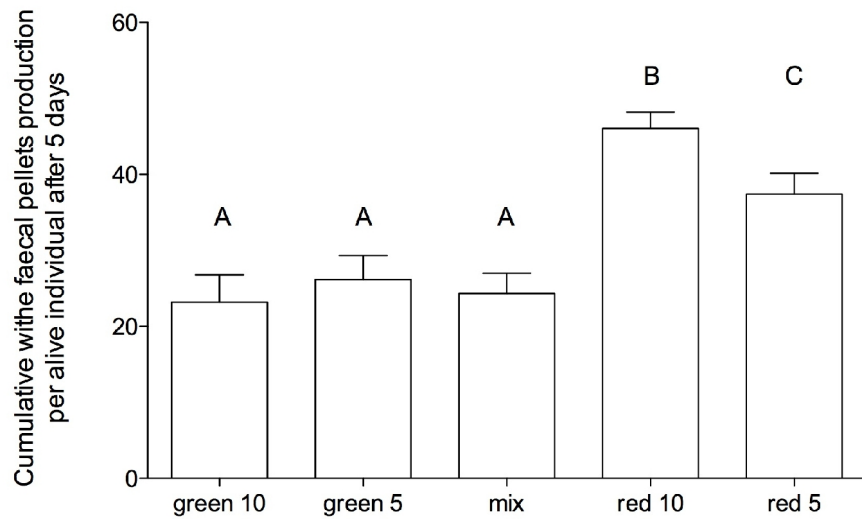


Figure 1: Mean cumulative numbers of white faecal pellets per live individual after five days for each kind of group (R5, N=32; R10, N=33; M, N= 32; G5, N=32; G10, N= 31). Different letters represent statistical differences according to the Bonferroni test ($P<0.05$). Error bars indicate the 95% CI

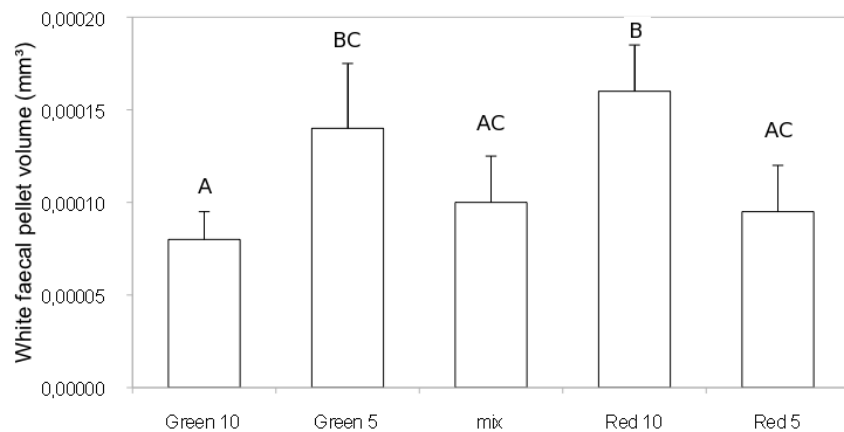


Figure 2: Mean white faecal pellets volumes for each kind of group (R5, N=32; R10, N=33; M, N= 32; G5, N=32; G10, N= 31). Different letters represent statistical differences according to the Bonferroni test ($P<0.05$). Error bars indicate the 95% CI.

$t=0.88$, $P>0.05$) (Figure 1) and were the lowest of all tested group (Bonferroni: red 5 vs. green 10: $t=7.00$, $P<0.001$; red 5 vs. mix: $t=6.43$, $P<0.001$; red 5 vs. green 5: $t=5.99$, $P<0.001$) (Figure 1).

The white faecal pellet volumes

The group composition had an influence on the white faecal pellet volume (One-way ANOVA $F=7.76$, $dfn=4$, $dfd=145$, $P<0.001$) (Figure 2). The volume increased with the group size for the red form (Bonferroni: red 5 vs. red 10: $t=3.89$, $P<0.01$) (Figure 2) but decreased for the green form (Bonferroni: green 5 vs. green 10: $t=3.47$, $P<0.01$) (Figure 2). We also observed a difference of volume between the two forms when individuals were in group of ten (Bonferroni: green 10 vs. red 10: $t=4.77$, $P<0.001$) (Figure 2), but not when they were in groups of five individuals (Bonferroni: green 5 vs. red 5: $t=2.59$, $P>0.05$) (Figure 2). Concerning the mix experiments, only the individuals in the red 10 group produce larger white faecal pellets than individuals in mix groups (Bonferroni: mix vs. red 10: $t=3.46$, $P<0.01$), the other groups were statistically equal to the mix group (Bonferroni: mix vs. red 5: $t=0.43$, $P>0.05$; mix vs. green 10: $t=1.31$, $P>0.05$; mix vs. green 5: $t=2.66$, $P>0.05$) (Figure 2).

Fecundity

The number of eggs

Concerning the mean cumulative number of eggs laid per live individual after five days, one-way ANOVA followed by Bonferroni

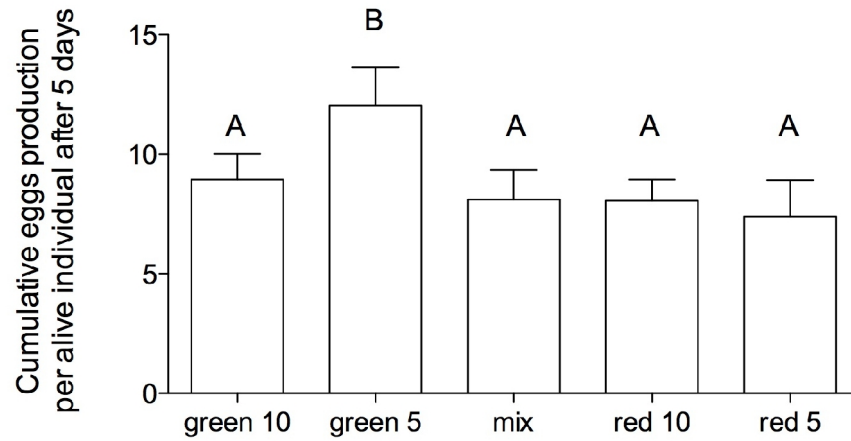


Figure 3: Mean cumulative numbers of eggs per live individual after five days and each kind of group (R5, N=32; R10, N=33; M, N= 32; G5, N=32; G10, N= 31). Different letters represent statistical differences according to the Bonferroni test ($P<0.05$). Error bars indicate the 95% CI

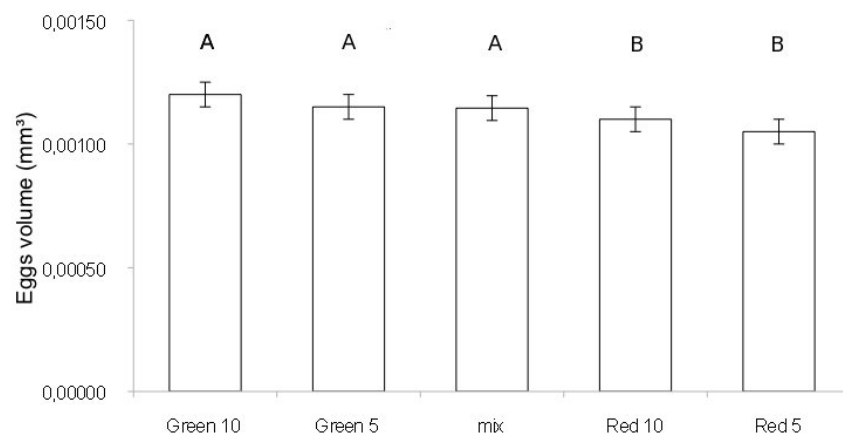


Figure 4: Mean egg volumes for each kind of group (R5, N=32; R10, N=33; M, N= 32; G5, N=32; G10, N= 31). Different letters represent statistical differences according to Bonferroni test ($P<0.05$). Error bars indicate 95% CI

tests showed that individuals in the green 5 group had the highest egg production per individual (ANOVA: $F=7.76$, $dfn=4$, $dfd=145$, $P<0.001$; Bonferroni: green 5 vs green 10: $t=3.18$, $P<0.05$; green 5 vs. mix: $t=4.56$, $P<0.01$; green 5 vs. red 10: $t=4.14$, $P<0.001$; green 5 vs. red 5: $t=5.24$, $P<0.001$) (Figure 3). Egg production was statistically the same in all other groups, green 10, mix, red 10 and red 5 (Bonferroni: green 10 vs. mix: $t=1.44$, $P>0.05$; green 10 vs. red 10: $t=0.87$, $P>0.05$; green 10 vs. red 5: $t=1.67$, $P>0.05$; mix vs. red 10: $t=0.04$, $P>0.05$; mix vs. red 5: $t=0.78$, $P>0.05$; red 10 vs. red 5: $t=0.74$, $P>0.05$) (Figure 3).

Egg volume

Concerning egg volume, individuals of the green form population in groups of ten, produced larger eggs than individuals of the red form population in groups of five and ten (Bonferroni green 10 vs. red 5: $t=3.25$, $P<0.05$; green 10 vs. red 10: $t=2.98$, $P<0.05$) (Figure 4). The egg volumes of other groups were not statistically different (Bonferroni: green 10 vs. green 5: $t=1.48$, $P>0.05$; green 10 vs. mix: $t=1.71$, $P>0.05$; green 5 vs. mix: $t=0.23$, $P>0.05$; green 5 vs. red 10: $t=1.50$, $P>0.05$; green 5 vs. red 5: $t=1.50$, $P>0.05$; mix vs. red 10: $t=1.28$, $P>0.05$; mix vs. red 5: $t=1.55$, $P>0.05$; red 10 vs. red 5: $t=0.27$, $P>0.05$) (Figure 4).

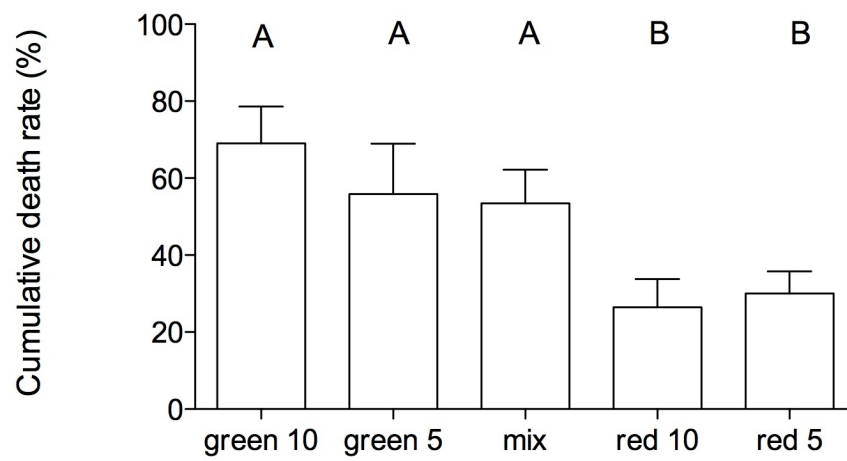


Figure 5: Mean cumulative death rates after five days of experiment for each kind of group (R5, N=32; R10, N=33; M, N= 32; G5, N=32; G10, N= 31). Different letters represent statistical differences according to the Bonferroni test ($P<0.05$). Error bars indicate 95% CI.

Mortality

The population and group composition influenced the cumulative death rate ($F=15.93$, $dfn=4$, $dfd=160$, $P<0.001$) (Figure 5). Individuals in the red 10 and red 5 groups had the same cumulative death rate, which is the lowest of all groups (Bonferroni post-hoc tests: green 10 vs. red 10: $t=6.59$, $P<0.001$; green 10 vs. red 5: $t=6.04$, $P<0.001$; green 5 vs. red 10: $t=4.66$, $P<0.001$; green 5 vs. red 5: $t=4.10$, $P<0.001$; mix vs. red 10: $t=4.21$, $P<0.001$; mix vs. red 5: $t=3.66$, $P<0.01$) (Figure 5). The mean cumulative death rates of other groups (green 10, green 5, mix) were not statistically different (Bonferroni: green 10 vs. green 5: $t=2.03$, $P>0.05$; green 10 vs. mix: $t=2.38$, $P>0.05$; green 5 vs. mix: $t=0.38$, $P>0.05$) (Figure 5).

Discussion

Whatever the level of social organisation, from basic aggregation to sophisticated eusociality, group living is widespread and provides fitness benefits and costs to individuals (Ranta *et al.*, 1993, Krause and Ruxton, 2002). Here, we report that group living brings different benefits and costs to the two tested red and green populations. The red population benefits from a higher silk production and a lower death rate while the fecundity (number and volume of eggs) is not changed in bigger groups. For the green populations, living in group has a cost as their eggs are produced in smaller quantities while silk production and the death rate are not changed.

The preference for forming associations with conspecifics for

many activities such as foraging, resting, mating and so on, implies the capability to recognise kin and to prefer related individuals as a function of associated costs and benefits (Fellowes 1998; Lihoreau and Rivault, 2009). Our study confirms the previous findings of Roeder *et al.* (1996) and Le Goff *et al.* (2009) that *T. urticae* is affected by the genetic environment. Indeed, if genetic origin had no influence, the values of the number of eggs and white faecal pellets in mixed groups composed of green and red individuals would be intermediate between those of the full green or red groups. In our case, in mixed groups, silk production and the death rate were statistically similar to the values obtained in the pure group of green individuals. These results are evidence for probable kin recognition ability in *T. urticae*, which results here in a cost to the fitness of the mixed group or at least an absence of a positive group effect. Another possible explanation for this result is that the coexistence of two different strategies for silk/white faecal pellet production between individuals coming from red and green form populations could influence the group and lead to a level of silk/white faecal pellet production similar to the one observed in green groups. In our results, the strategy of the green form to invest more in reproduction than in survival is imposed on the other red mites.

In 2010, Le Goff *et al.* reported a group effect in the red form of *T. urticae* on egg and silk production. Here, our results show a group effect but only for the silk production (indicated by the number and volume of white faecal pellets) and not for egg production in the red form. Concerning the green form, no such group effect has been

observed for eggs and white faecal pellets. On the contrary, the increase in the number of conspecifics (from five to ten) significantly decreased the egg (number) and the white faecal pellet (volume) production per live individual. The difference observed between these results and our previous study (Le Goff *et al.* 2010) can be explained by the fact that the experiments were not made at the same density of individuals: 0.02 ind/mm² in Le Goff *et al.* (2010) against 0.06 ind/mm² in the present study, i.e. three times more. Therefore, this group effect observed in the red form population on egg production (Le Goff *et al.*, 2010) was probably determined by a threshold of density. However, at higher mite density, the presence of conspecifics (related or not) could have a negative impact on population dynamics (negative group effect). The physiological and/or behavioural mechanisms involved in these group effects remain unknown and deserve further study (Grassé, 1946; Prokopy and Duan, 1998; Prokopy and Reynolds, 1998; Holbrook *et al.*, 2000).

From an evolutionary/adaptive point of view, these differences in the impact of group living between the two forms can be explained by a different level of genetic diversity within the groups. Both populations in this study can be considered inbred. This can be due to the haplodiploidy reproduction system of *T. urticae* combined to a high density of population (Hinomoto and Takafuji 1994), which sometimes results in monomorphic and highly inbred populations (Carbonnelle *et al.*, 2007). The red form population has a higher FIS than the green form population, therefore, individuals in pure red groups are closer relatives than in green groups. According to

Hamilton (1964), living with closely related individuals increases the indirect fitness of group members by sharing the benefits of group living. Therefore, the fact that the red form has a lower level of genetic diversity can induce a higher level of sociality in this population. On the contrary, the green form presents a higher level of genetic diversity and so a lower interest in group living. This should be confirmed by comparing several populations of the two forms.

Our study reveals some differences in the life history traits of these two populations. As egg and silk production are two costly mechanisms, there is probably a trade-off for females between their egg production (reproductive success) and their silk production (structure of the protective web). If we compare the number of eggs in groups of similar number of individuals, we note that the green form population always produces bigger eggs than the red form population. In *T. urticae*, larger eggs are more likely to reach adulthood than smaller eggs (Macke *et al.*, 2010) as egg size affects juvenile survival. In addition, the egg size in *T. urticae* influences the probability of an egg being fertilised, and thus bigger eggs will produce statistically more females (Macke *et al.*, 2010). Given all these factors, the bigger eggs produced by the green form population will produce a higher quantity of females. This higher quantity of females should improve reproductive success. On the other hand, in similar groups, individuals of the red form produce significantly more and bigger white faecal pellets than the green form. Greater white faecal pellet production can be an advantage for the group as it might stabilise the humidity conditions in the web (Hazan *et al.*, 1974, Le Goff *et al.* 2010) and

might stabilise the web structure (as suggested by results in le Goff *et al.* 2010). Greater white faecal pellet production is also an indicator of a higher silk production (Hazan *et al.*, 1974). Individuals of the red form population may therefore benefit from environmental modifications created by conspecifics and therefore survive longer than individuals of the green form. This should be confirmed by comparing several populations of the two forms.

To conclude, we observed that the individuals of the green form population produce more and bigger eggs and fewer and smaller white faecal pellets than the red form population. Furthermore, they seemed to be affected by the presence of other conspecifics as they died sooner than the individuals of the red form, probably due to a combination of higher investment in egg production and weaker protection of silk and white faecal pellets. Individuals of the red form produced more and bigger white faecal pellets and fewer and smaller eggs than individuals of the green form. Moreover, their environmental conditions were improved by the presence of conspecifics, which induced a higher survival rate than for individuals of the green form. We can therefore conclude from these results that these two populations present two different life-history strategies. Indeed, the strategy of the green form to invest more in reproduction than in survival looks like an « r » strategy, whereas for individuals of the red form, investing more in group living and survival than in reproduction looks like a « k » strategy. To explain such differences between the two forms, we can refer to local selection theory. Indeed, as natural selection should favour the genotypes that are best able to use the information in their

environment (Price *et al.* 1980; Dicke and Sabelis 1988), it follows that individuals of different local populations may have been subject to different local selection regimes (Maeda 2005). The green form is found in temperate zones (Belgium in our case) and diapause, whereas the red form is sub-tropical and tropical (Tunisia in our case) and does not diapause. In a temperate climate the populations can only develop during a defined period, from spring to summer, and therefore a k strategy appears inappropriate from an evolutionary point of view. Conversely, in the tropical zone populations are not subject to climatic uncertainties and can reproduce all year round (Bonato and Gutierrez, 1999), which could favour an evolution to a social/k strategy. Therefore, different local populations of *T. urticae* submitted to different local selection pressure could exhibit between-population variation in their life history strategies.

Our study allows us suppose that a recognition ability of kin proximity may occur in *T. urticae* and describes the influence of the « genetic background » on the life history traits of *T. urticae*. The genetic mechanisms leading to differences in life history traits are still unknown. Measuring social behaviours (aggregation, group living, recruitment processes, etc.) on different generations of isofemale lines coming from different geographical places could be an interesting next step in this study. It could allow comparison between the behaviour of different populations with the same genetic diversity and conversely, individuals from the same population with different levels of genetic diversity.

Acknowledgements

We are very grateful to Pr. Kaouthar Lebdi who supplied the red form of *Tetranychus urticae*, which was used in our experiments and to George van Impe for the useful discussions about *Tetranychus urticae*. We would like to thank all the team of the “Laboratoire d’écologie et biogéographie” for their help in data analysis. G.J. Le Goff is supported by a Grant from the FRIA (Fonds pour la Formation de la Recherche dans Industrie et l’Agriculture). The authors are also indebted to the National Fund for Scientific Research (FNRS, Belgium) for funding through the Fund for Fundamental and Collective Research (FRFC, convention 2.4622.06). AC Mailleux is financially supported by IRSIB (Institut d’encouragement de la Recherche Scientifique et de l’Innovation de Bruxelles).

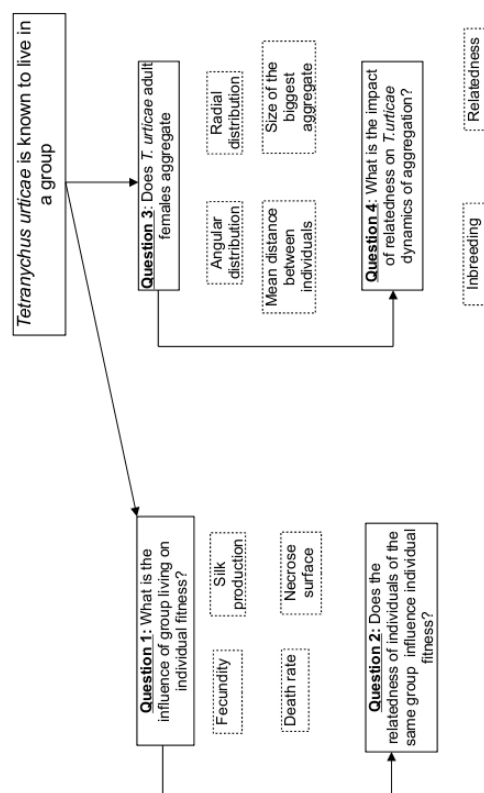


Figure 6: Flow chart of the PhD thesis, showing the different steps of the study

Question 3 and 4 paper 3, 4 and 5: “Does *T. urticae* adult females aggregate?” and “What is the impact of relatedness on *T.urticae* dynamics of aggregation?”

Aggregation is one of the most basic social phenomena, and many activities of social arthropods are linked to this behaviour. Spatial and temporal cohesion of aggregates can be explained by individual attraction to the same environmental stimulus and/or by mutual attraction between group members.. In nature, *T. urticae* show a strong aggregation (Strong, 1997).

In the two following papers, we quantified the aggregation dynamics of this spider-mite under controlled conditions and studied the impact of relatedness on this process. Two different methods were used to modulate the relatedness

1. The first method consisted of creating inbred lines during three successive generations. We and, therefore, testinged the impact of inbreeding on aggregation for each subsequent generation on *T. urticae*. The experimental conditions mimicked the evolution of an isolated population becoming more and more inbred (**paper 3**).
2. The second method consisted of mixing individuals coming from two different populations carrying two different genetic pools. The experimental conditions mimicked the evolution of two populations gathering to form one. This, tested the impact of forming aggregates in function of relatedness (**paper 4 and 5**).



Ethology / Éthologie

Spatial distribution and inbreeding in *Tetranychus urticae*

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Received 13 February 2009; accepted after revision 29 June 2009

Available online 19 August 2009

Presented by Pierre Buser

Abstract

In group living, species spatial distribution results from responses to environmental heterogeneity and/or mutual interactions between individuals. These mutual interactions can be regulated by genetic and/or epigenetic factors. In this study, we focus on genetic factors and investigate how the spatial distribution of some individuals colonizing a new environment is influenced by inbreeding.

Our biological model is *Tetranychus urticae*, a phytophagous mite considered as a major pest of many cultivated plants. Groups of *T. urticae* were composed of individuals from successive inbreeding (sister–brother sib-mating). Our results show that the inter-individual distances increase with inbreeding. Indeed, inbreeding level seems to be an important factor affecting the intra-plant spatial distribution of mites. These results confirm that mites have the capability to discriminate their kin and, moreover, that they are able to accurately perceive differences between close relatives from sib-mating lines.

To cite this article: G. Le Goff et al., *C. R. Biologies* 332 (2009).

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Résumé

Distribution spatiale et consanguinité chez *Tetranychus urticae*. La distribution spatiale d'individus peut être le résultat de réponses à des hétérogénéités environnementales et/ou à des interactions interindividuelles. Dans cette étude, nous nous intéressons aux facteurs génétiques pouvant influencer ces interactions et étudions comment la distribution spatiale d'individus colonisant un nouvel environnement peut être influencée par leur proximité génétique.

Notre modèle biologique est *Tetranychus urticae*, c'est un acarien phytophage considéré comme un ravageur majeur dans beaucoup de cultures. Les groupes d'acariens testés sont composés d'individus issus de générations consanguines successives (accouplement frère–sœur).

Les résultats montrent une distribution spatiale influencée par la consanguinité du groupe ainsi qu'une augmentation de la distance inter-individuelle avec le taux de consanguinité. Il apparaît donc que le lien génétique entre individus est un facteur capable d'influencer la distribution spatiale de *T. urticae*. Ce qui signifie également que ces acariens sont capables de discriminer des individus issus de lignées consanguines et donc très proches. **Pour citer cet article :** G. Le Goff et al., *C. R. Biologies* 332 (2009).

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Keywords: *Tetranychus urticae*; Spatial distribution; Sib-mating line

Mots-clés: *Tetranychus urticae*; Distribution spatiale; Consanguinité; Lignées consanguines

1. Introduction

In social biology, the spatial distribution of individuals is an important issue that determines the organization of groups. In many species, individuals form aggregates, increasing local population density much more than in the surrounding environment [1,2]. Forming such aggregates has many advantages both for the individual and the group as it provides easier access to food and mates as well as protection against predators [3,4]. It also presents disadvantages as forming aggregates means sharing food, mates and living space, and therefore may result in inter-individual conflicts [3]. Although group formation is of critical importance for the survival of many species, few studies have been done on the parameters modulating spatial distribution, especially in non-eusocial arthropods such as *Tetranychus urticae* [5,6].

T. urticae is considered as a major pest of many cultivated plants (more than 200 host-plants listed [7]). Consequently, research has focused mainly on the management and control of this species, whereas its behaviour, especially its social behaviour, has often been disregarded. However, *T. urticae* is a very good biological model with which to study group formation, as this species builds a collective web by weaving silk threads, cooperating in its enlargement and repair [8]. The web is used like a shield protecting mites from rain [9], pesticides [10] and predators [11], and it seems to be a prevailing factor inducing the formation of mite aggregates observed in the field from leaf to leaf or plant to plant [12,13].

Another crucial factor that modulates spatial distribution is the individual genetic background [1,2,14–18]. Aggregate with kin may help individuals avoid predators by diluting each individuals' risk of being attacked (dilution hypothesis [19]) [1,2,16], whereas kin avoidance can be a mechanism to avoid kin mating and so maintain a high level of genetic diversity in the population [15,17]. In insects [1,18,20–22] and arachnids [23], chemical cues such as specific cuticular hydrocarbon may mediate kin recognition. Many arthropod species have the ability to discriminate between kin and non-kin odors and often choose to form an aggregate with close relatives [1,18,21,24]. In *T. urticae*, females (sisters or non-sisters) coming from inbred and outbred population

are able to modify their offspring sex-ratio according to the genetic relatedness of the group (Roeder [25]). Therefore, *T. urticae* mites have the ability to discriminate individuals differing by their original populations and environments. Do mites still display such discriminative abilities when they face very close relatives? In other words, are they able to perceive tiny genetic differences between close relatives coming from sib-mating lines? Such questions are very important for the study of *T. urticae* socio-ecology. Indeed, in the field, the probability of inbreeding is very high in groups living on the same plant due to the low mobility exhibited by mites (increasing the likelihood of interactions among kin) and to their mating systems (parthenogenesis and haplo-diploidy) [26,27].

How inbreeding influences the spatial distribution of mites is also unknown. Previous studies on this topic have mainly focused on insects [1,2,18,20,22] while mites have been disregarded. In order to address this question, we studied the spatial distribution within small groups of mites during the colonization of a new leaf through successive generations of inbred individuals. Our results will be discussed in the context of *T. urticae* ecology.

2. Materials and methods

We tested the relation between inbreeding and spatial distribution by using a red form of *T. urticae* provided by Dr. Lebdi Grissa (Institut National Agronomique, Tunis). The strain was reared on bean leaves placed on damp cotton (*Phaseolus vulgaris*) in Petri dishes [28]. Breeding stocks were maintained in a climate room at 26 °C, 50–60% RH, 16:8 (L:D).

2.1. Establishment of sib-mating lines

Founding sib-mating lines is a basic and essential tool for investigating the influence of inbreeding [29]. Three sib-mating lines were established; each was initiated from a virgin female isolated at the deutonymph stage and placed with three unrelated males on a bean leaf for 48 h. Mated females were then isolated on a new bean leaf. Their offspring was subsequently used for sib-mating for four generations to form a highly related line. In each line, three females of the first generation

(G1) were fertilized by their brothers. The other females of this first generation were used in aggregation experiments and were called G1. Three females of the second generation were also fertilized by their brothers. Their sisters were also used for the test and were called G2. The above manipulations were repeated in order to obtain many individuals of known age for four generations (G1 to G4). Generation after generation, inbreeding increased. Data obtained using G3 are not presented in this paper because of a lack of replication.

2.2. Spatial distribution tests

Experiments were performed in a climate room at 26 °C, 50–60% RH, 16:8 (L:D). Ten virgin females belonging to the same sib-mating line, of the same generation and less than 48 hours old (number of replicates: G1: $n = 14$; G2: $n = 13$; G4: $n = 25$) were placed one by one in the centre of a bean leaf disc ($\varnothing$ 5 mm). The time taken to place the individuals never exceeded 8 min. Bean leaf discs were placed on damp cotton, to prevent mite escape. These bean leaves were freshly cut before the experiment and were chosen to minimize heterogeneity due to veins. A numeric camera (Panasonic WV-CP450/G) with a macroscopic lens (1:1.2/12.5–75) and a magnifying lens (1.2 $\times$) was focused on the leaf disc and recorded female behaviour.

We started recording 15 minutes after the last individual was placed on the leaf to avoid stress behaviour. One picture was taken every 30 seconds for 15 minutes. These pictures were analysed using a data processing program called Fourmilier V3.3 (software creator, Jean-Michel Hudry (jm.hudry@ifrance.com)) [30], which attributed spatial coordinates to each individual and thus allowed further analyses and quantification of the spatial distribution of individuals.

In order to link the inbreeding and the spatial distribution, three parameters were measured:

- (1) *The angular distribution* “ s ” is the angular position of individuals on the leaf (Fig. 1) [31,32]. It was measured for each mite group at the end of each session. The angular distribution determined whether individuals were closer than expected by chance alone. We compared the s experimental values to the reference value obtained for randomly distributed individuals (reference value $H = 68.3$ degrees for ten individuals). The reference value H was obtained from 50 000 simulations of ten individuals randomly distributed on a circular area. A high value of s corresponds to a homogeneous distribution whereas a low value of this parameter

would mean an aggregated distribution of individuals. This indicator is not a precise measure of the spatial distribution, as several individuals can stay in the same angle and go away from each other. That is why this indicator is used as a qualitative indicator of the spatial distribution to answer the question whether individuals are randomly distributed or not and so if they are attracted to a particular stimulus in its environment (individual, place, heterogeneity) or not.

- (2) *The radial distribution* is the radial position of mites on the leaf. Quantification of the radial distribution allows one to analyse the trend to follow the leaf border a kind of thigmotaxis (also called the border effect). In other words, this parameter determined the position at which mites were numerous. The leaf was divided into five rings of 1.5 mm (0–1.5, 1.5–3, 3–4.5, 4.5–6 and 6–7.5), the external ring corresponds to the edge of the arena. We took into account the differences between the areas of all rings by comparing spider mite density per ring.
- (3) *The mean distance between individuals* is an indicator of the inter-individual spatial proximity [33]. The individual distance is the length between individuals at a given moment. This was drawn from the spatial coordinates assigned to each individual. The mean distance between individuals is calculated by adding all distances between an individual and another one and by dividing the result by 45, the total number of such distances [34]. This certainly reflected the attraction to other individuals and/or to a same place.

These three parameters were measured for each individual, for each generation and each sib-mating line.

2.3. Statistical tests

Friedman tests were used to test whether the three measured indicators (the angular distribution, the radial distribution, the mean distance between individuals) changed according to time. Kruskal Wallis tests of variance (KW) and Dunn post test were also used to compare changes in angular distribution, radial distribution and distances between individuals and between generations. Tests were performed using GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego, California, USA (<http://www.graphpad.com>). All tests were applied under two-tailed hypotheses and the significance level p was set at 0.05.

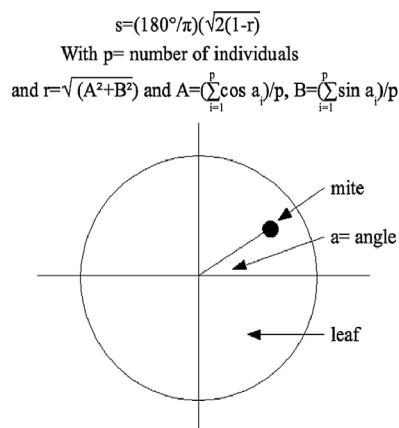


Fig. 1. Calculation of the mean angular deviation.

3. Results

The three measured indicators (the angular distribution, the radial distribution, the mean distance between individuals) did not change according to time since the beginning of the recording in any tested generation (Friedman test for the angular distribution: G1: $Fr = 40.32$, $P > 0.05$; G2: $Fr = 32.14$, $P > 0.05$; G4: $Fr = 28.49$, $P > 0.05$; for the radial distribution, G1: $Fr = 25.77$, $P > 0.05$; G2: $Fr = 42.82$, $P > 0.05$; G4: $Fr = 28.49$, $P > 0.05$; the mean distances between individuals: G1: $Fr = 38.23$, $P > 0.05$; G2: $Fr = 37.18$, $P > 0.05$; G4: $Fr = 26.86$, $P > 0.05$). That is why the results presented here focused on the final state of the mite group (31st picture).

3.1. The mean angular distribution “s”

The mean s ($^\circ$) values for G1, G2, and G4 were not statistically different but ($G1 \pm \text{Standard Error (S.E.)} = 51.91 \pm 3.46$, $G2 \pm \text{S.E.} = 54.54 \pm 3.15$, $G4 \pm \text{S.E.} = 54.15 \pm 3.58$; $KW = 30.41$, $df = 3$; $P > 0.05$; Dunn: G1 vs. G2: $P > 0.05$, G1 vs. G4: $P > 0.05$, G2 vs. G4: $P > 0.05$) (Fig. 2) for each generation, s was statistically different from the reference value i.e. the expected H values of randomly distributed individuals ($KW = 30.41$, $df = 3$, $P < 0.001$; Dunn: G1 vs. H: $P < 0.001$, G2 vs. H: $P < 0.001$, G4 vs. H: $P < 0.001$) (Fig. 2). This result showed that mites were non-randomly distributed at the end of the experiments.

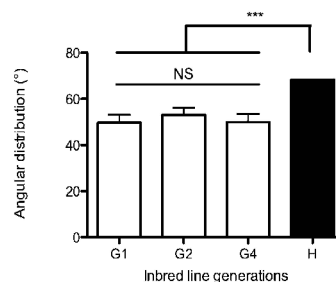


Fig. 2. Mean angular distribution “s” ($^\circ$) of individuals for each generation (G1, $n = 14$; G2, $n = 13$; G4, $n = 25$) $\pm$ S.E. at the end of experiments (30 min). H is the reference for ten randomly distributed individuals, and was obtained from 50 000 simulations of ten individuals that were randomly distributed in a circular area.

3.2. The radial distribution of the mites

The mean values of the radial distribution differed between successive generations: In the central ring, the highest density of mites was observed at G1 (density $\pm$ S.E. = 0.07 ± 0.02 individual/ mm^2) and G2 (density $\pm$ S.E. = 0.08 ± 0.04 individual/ mm^2), whereas the lowest density was observed at G4 (density $\pm$ S.E. = 0.006 ± 0.006 individual/ mm^2). The difference was statistically significant ($KW = 8.23$, $df = 2$; $P < 0.05$; Dunn: G1 vs. G2: $P > 0.05$, G1 vs. G4: $P < 0.05$, G2 vs. G4: $P > 0.05$) (Fig. 3). In terms of mite population fraction, 7% of G1 individuals and 0.4% of G4 individuals were found in the central ring.

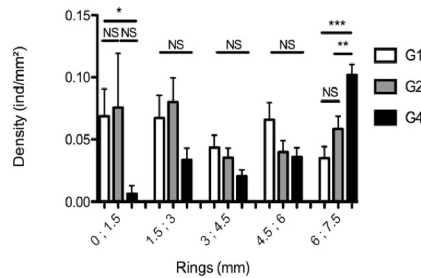


Fig. 3. Mean radial distribution of individuals; density of individuals for each generation (G1, $n = 14$; G2, $n = 13$; G4, $n = 25$) (mean number of ind/mm² ± S.E.) in the five rings (0 represents the centre of the leaf disc) at the end of the experiment (30 min).

On the other hand, in the external ring, the highest mite density was observed at G4 (density ± S.E. = 0.10 ± 0.009 individual/mm²), whereas the lowest density was observed at G1 (density ± S.E. = 0.04 ± 0.009 individual/mm²). The difference was statistically significant (KW = 19.37, $df = 2$; $P < 0.001$; Dunn: G1 vs. G2: $P > 0.05$, G1 vs. G4: $P < 0.001$, G2 vs. G4: $P < 0.01$) (Fig. 3). In other words, 23% of G1 individuals and 68% of G4 individuals were found in the external ring.

No difference across generations was found in the other rings (1.5; 3: KW = 5.46, $df = 2$, $P > 0.05$; 3; 4.5: KW = 5.52, $df = 2$, $P > 0.05$; 4.5; 6: KW = 3.81, $df = 2$, $P > 0.05$).

3.3. The mean distance between individuals

The mean distance between individuals (mm) increased significantly across generations from $4.53 \text{ mm} \pm 0.13$ (S.E.) in G1 to 6.14 ± 0.13 (S.E.) in G4 (KW = 44.72, $df = 2$; $P < 0.001$; Dunn: G1 vs. G2: $P < 0.001$, G1 vs. G4: $P < 0.001$, G2 vs. G4: $P > 0.05$) (Fig. 4). After four inbred generations, the mean distance between individuals increased by about 1.6 mm. This result showed that inbreeding lowered mutual attraction among spider mites. The higher the inbreeding level, the higher the inter-individual distances.

4. Discussion

In group-living species spatial distribution results from responses to environmental heterogeneity [35–37] and/or to mutual interactions between individuals [38]. During this study, we showed that the level of inbreeding and environmental heterogeneity influence mite distribution during the colonization of a new leaf.

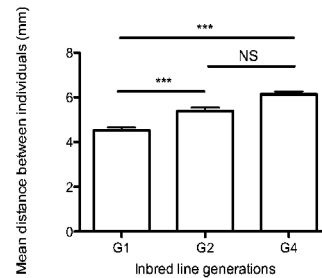


Fig. 4. Mean distance (mm) between individuals for each generation (G1, $n = 14$; G2, $n = 13$; G4, $n = 25$) ± S.E. at the end of experiments (30 min).

Indeed the results of mean angular distribution are about 50° for all generation of sib-mating and each of them is different from the reference value $H = 68.3^\circ$ for ten individuals randomly distributed. Therefore individuals are not randomly distributed in our experiments, they are so attracted each other or/and to a same place. For each generation our results showed a difference of about 27% between empirical and theoretical distribution of s . In our experimental set up, leaf disc has been chosen to minimize heterogeneities due to vein. We can thus imagine that in more natural condition there would be more unevenness like veins to initiate web spinning (personal observation) and aggregation, and so we would have a lower level of angular distribution, and a higher level of aggregation.

We also observed that after 4 generations of sib-mating, the mean distance between *T. urticae* mites increased of about 1.6 mm which means three times the size of an individual. These results confirm that mites have the capability to discriminate their kin [25,39,40] and to perceive tiny differences between very close relatives.

These differences between generations suggest that inbreeding should increase the tendency to leave the patch or, at least, increase the inter-individual distance. What could be the benefit of such avoidance of close relatives? Increasing inter-individual distance may decrease competition for food, mates and space between relatives [41,42]. This may also decrease the probability of mating with relatives [17,43] and thereby may prevent, at least partly, inbreeding depression and its negative physiological consequences [44].

In addition, the spatial distribution of mites is influenced by environmental heterogeneity such as the borders of the experimental set-up. The trend to follow the leaf border increases with inbred generations. This sug-

gests that individuals has a higher probability of leaving the leaf disc but are limited to it. That demonstrated link between radial distribution and inbreeding emphasizes the role of inbreeding in the spatial distribution of *T. urticae*.

Previous papers showed that spatial distribution results from social interactions mediated by visual, auditory and tactile stimuli [1,4]. The web in combination with social interactions mediated by pheromones could favor the initiation of clustering in a particular site or reinforce gregariousness [45]. Like many species of gregarious arthropods, spider mites are probably able to produce aggregative pheromones [4,46] that may be contact pheromones that are adsorbed on the cuticle [47,48], deposited on the leaf surface and/or on the silk [49,50]. They might also exert a remote aggregating effect as volatile pheromones attracting at short or long distances [1,51]. No aggregation pheromone has yet been found in *T. urticae* but such a pheromone has clearly been identified in families other than Tetranychidae [52,53]. These studies suggest the possible existence of an aggregation pheromone in this mite.

Our work contributes to the understanding of spatial organization in spider mites during the colonization of a new leaf. This study shows that with 10 individuals, the spatial distribution is influenced by the genetic background of the group. The following step in this study should be to test with different numbers and density of individuals, different stages, with a higher level of inbreeding. Identifying aggregative pheromones and studying their interplay with the web structure could also help to draw a coherent picture of the mechanisms involved in the spatial distribution of mites and to open new applied perspectives in the control of this worldwide pest.

Acknowledgements

We are very grateful to Dr. Lebdi Grissa Kaouthar who supplied the red form of *Tetranychus urticae* used in our experiments and to George van Impe for the useful discussions about *Tetranychus urticae*. We would like to thank Marie Pairon, Gregory Sempo and all the team of the “Unité d’écologie et biogéographie” for their help in data analysis. The authors are also indebted to the National Fund for Scientific Research (FNRS, Belgium) for funding through the Fund for Fundamental and Collective Research (FRFC, convention 2.4622.06). A.C. Mailleux is financially supported by IRSIB (Institut d’encouragement de la Recherche Scientifique et de l’Innovation de Bruxelles). This publication is number BRC140 of the Biodiversity Research Center.

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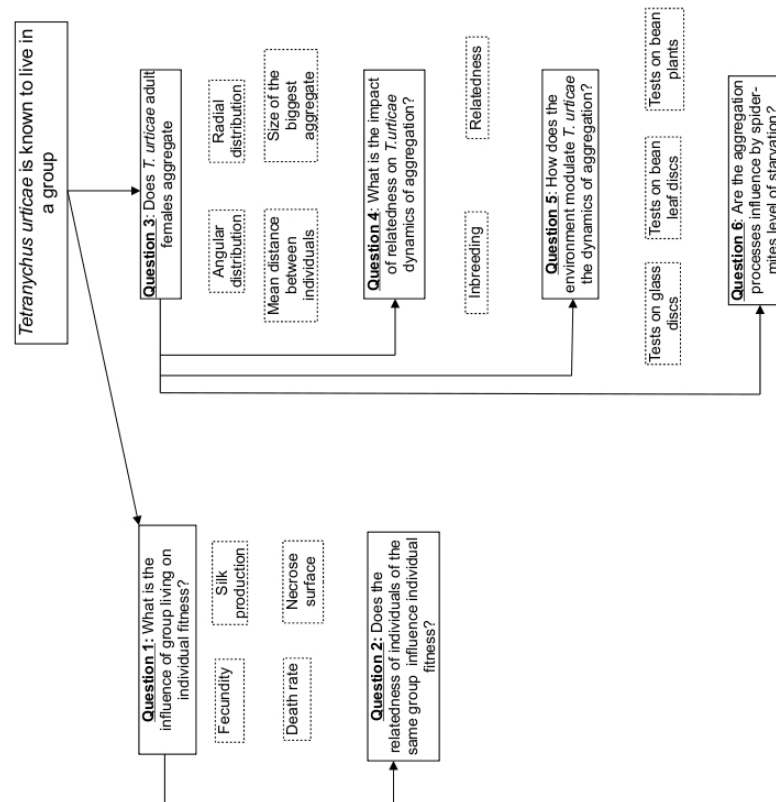


Figure 6: Flow chart of the PhD thesis, showing the different steps of the study

Question 5 and 6 – Paper 4 and 5: “How does the environment modulate *T. urticae* dynamics of aggregation?” and “Are the aggregation processes influence by spider-mites level of starvation?”

During its life cycle, *T. urticae* can experiment various ecological and physiological conditions. For instance, as leaves are not homogeneous surfaces and should influence the distribution of *T. urticae*. The spatial distribution of mites colonising a new environment was studied in relation with the nature of their substrate, from the simplest and more artificial one to the most complex and natural ones: (1) Glass disc was a homogeneous artificial substrate without feeding possibilities studied in order to evidence the influence of conspecifics on their spatial distribution (**paper 4**). (2) Bean leaf discs mimicked a natural environment offering a high quantity of food and possess irregularities. It correspond to a heterogeneous environment (presence of food and nerves) (**paper 4**). Finally (3) the whole bean plants the natural host of *T. urticae* add a new dimension in the complexity of the substrate (**paper 5**). With these set up, we investigated the consequence of an increase in environmental complexity on the spatial distribution of *T. urticae*.

In nature, a *T. urticae* population finally exhausts its host plant resources and then disperses to new hosts. According to literature, starvation should increase competition among individuals (Barrera, 1996) and therefore decrease the social cohesion of *T. urticae*. To test this assumption on *T. urticae*, individuals were starved and their aggregative behaviour was studied (**paper 5**).

Where and with whom should I aggregate? The dilemma of *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract

Spatial and temporal cohesion of aggregates can be explained by individual attraction to the same environmental stimulus or by mutual attraction between group members. As leaves are not homogeneous surfaces, the distribution of *T. urticae* individuals over the host plant leaves should not be uniform. In this paper, the spatial distribution of 10 adult females colonizing a new environment was studied in relation with the genetic proximity of individuals and the nature of the substrate: biotic environment (leaf disc) or abiotic environment (glass discs). Indeed, leaf discs mimic a natural environment offering a high quantity of food and possess irregularities, whereas a glass disc is a homogeneous artificial substrate without food. A red (Tunisia) and a green (Belgium) form of *T. urticae* were used to test the genetic compositions of small groups (10 red individuals, 5 green + 5 red individuals or 10 green individuals). Our results showed that the aggregation level increased with time for all groups. Mites from green groups were more aggregated than the red groups on leaf discs, whereas it was the opposite on glass discs. The aggregation dynamics of the mixed groups was closer to those observed for the green groups. The two groups presented different strategies of spatial distribution but no segregation occurred in our experimental devices between the two forms despite genetic differences.

Introduction

Social organisation is one of the fundamental features of animal behaviour: social groups have been studied for quite different group sizes from bacteria to mammals, including humans (Camazine *et al.*, 2001; Mogilner *et al.*, 2003). In social biology, the spatial distribution of individuals is an important issue that determines the level of interactions between individuals, and more generally the structuring as well of the organisation of populations (Okubo, 1980; Ame *et al.*, 2006; Le Goff *et al.*, 2009). Aggregation of individuals may result from attraction between individuals (Allee, 1931; Okubo, 1980; Parrish and Edelstein-Keshet, 1999; Camazine *et al.*, 2001). However, in most situations, patterns of aggregation, resulting from individual responses to conspecifics, are modulated by environmental heterogeneity (Fraenkel and Gunn, 1961; Ceuster, 1977, Dambach and Goehlen, 1999; Depickere, 2004). Therefore, the spatial distribution of individuals is highly sensitive to environmental and social factors

Kin selection is known to be a strong force in the evolution of social organisation in animals (Hamilton, 1964, Queller and Strassmann, 1998, Lihoreau *et al.*, 2007) and the individual genetic background is known to influence the spatial distribution of individuals in many species (Ame *et al.*, 2004; Banks *et al.*, 2005; Stow and Beattie, 2008; Lihoreau and Rivault, 2009). Aggregation gives both advantages and drawbacks to the individual and to the group as it decreases the probability of being attacked (dilution hypothesis (Pulliam and Caraco, 1984)), it provides easier access to

food and mates as well as protection against predators (Ranta, *et al.*, 1993; Wertheim, 2005) and it limits temperature loss or water evaporation (Krause and Ruxton, 2002). When these benefits are shared by closely related individuals, group living then potentially increases the indirect fitness of group members (Hamilton, 1964). Living with kin could thus be a strategy to increase inclusive fitness by favouring the development and survival of closely related individuals (Lihoreau and Rivault, 2009). Aggregation drawbacks consist of making prey location easier for predators and increasing competition for food and mates (Ranta *et al.*, 1993). Kin avoidance allows individuals to avoid kin competition. For example, increasing the inter-individual distance may decrease competition for food and mates between relatives (Charlesworth and Charlesworth, 1987; Lambin *et al.*, 2001, Le Goff *et al.*, 2009). This may also decrease the probability of mating with relatives (Stow and Beattie, 2008; Ridley and Sutherland, 2002) and thereby may prevent, at least partly, inbreeding depression and its negative consequences (Helle, 1965). Therefore, choosing « where and with whom should I aggregate? » is not so obvious.

The phytophagous two-spotted spider mite *Tetranychus urticae* Koch is an ubiquitous species, present worldwide. This mite is a very good biological model for the study of group formation, as this species exhibits some levels of social life, like collective web building (Saito, 1983) and a positive group effect (Le Goff *et al.*, 2010). Mites are small organisms with reduced mobility once settled on the plant that may increase their likelihood to interact among kin (Saito, 1997). One

may expect that kin discrimination occurs in mites and shapes their social organisation (Saito, 1997). Furthermore, the haplodiploidy reproduction system of *T. urticae* combined with low mobility increases the risks of heterozygote deficiencies and favours the occurrence of highly inbred populations (Hinomoto and Takafuji, 1994). On the contrary, *T. urticae* populations can also be initiated by migrants from different origins carrying different genetic pools (Carbonnelle *et al.*, 2007). Indeed, in a natural environment, various genetic structures can be observed for this species, from monomorphic to polymorphic populations (Carbonnelle *et al.*, 2007). Therefore, within its population an individual can be confronted by a gradient of relatedness from unrelated to highly related individuals.

Many arthropod species have the ability to discriminate between kin and non-kin and often choose to form an aggregate with close relatives (Rivault *et al.*, 1998; Rivault and Cloarec, 1998; Singer, 1998; Mailleux *et al.*, 2008). In *T. urticae*, females (sisters or non-sisters) coming from inbred and outbred populations are able to modify their offspring sex-ratio according to the genetic relatedness of the group (tufino *et al.*, 1996). Since *T. urticae* mites have the ability to assess their relatedness to other individuals, one may wonder how mites distribute themselves in the environment when they are interacting with conspecifics and/or with unrelated individuals.

Consequently, we studied the genetic composition of the group and its potential influence the spatial distribution of *T. urticae*. Previous studies on this topic have mainly focused on insects (Rivault *et al.*, 1998; Rivault and Cloarec, 1998; Van Dermeer *et al.*, 1998;

Ame *et al.*, 2004) while mites have been disregarded. To address this question, we studied the spatial distribution within small groups of mites composed of individuals presenting close or different genotype, during the colonisation of a new leaf (presence of food) or a glass disc (control area deprived of food) that mimic dispersal conditions.

Materials and methods

We tested the relationships between the level of genetic relatedness and spatial distribution by using the red (previously called *T. cinnabarinus*) and the green form of *T. urticae*. The red form population was provided by Dr. Kaoutar Lebdi Grissa (Institut National Agronomique, Tunis, Tunisia); the green form population was collected in the green-houses of our laboratory in Louvain-la-Neuve, Belgium. The most accepted taxonomical position does not recognize *T. cinnabarinus* as a valid species as in the World Catalogue of the Spider Mite Family (Bolland *et al.* 1998), which cites it as a synonym of *T. urticae*. The two populations were reared on bean leaves placed on damp cotton (*Phaseolus vulgaris*) in Petri dishes. Rearing stocks were maintained in a climate room at 26°C, 50–60% RH, 16:8 (L:D).

The genetic distance between the two populations used in this experiment has been evaluated in a previous study using microsatellites: both populations are considered inbred (FIS red form population: 0.47 confidence interval (CI): 0.32-0.58 and FIS green form population: 0.07 (CI): 0.15-0.30 but are genetically different for

all tested loci (FST=0.47, Nei distance=0.95) (Le Goff *et al.*, unpublished data). Experiments were undertaken under the same environmental conditions as for breeding. Only virgin females less than 24 hours old were used since mated and virgin females may express different behaviours, especially in egg laying (Van Impe, 1985), which may affect their spatial distribution. As only young virgin females were tested during our experiment, thus the influence of factors linked to oviposition behaviour was avoided.

Spatial distribution tests

Experiments were performed under the same conditions as for rearing. Ten virgin females of less than 24h old belonging to the same population (red or green groups) or five from each of the two populations (mixed group) were placed one by one in the centre of a bean leaf disc or a glass disc (Ø15 mm) within less than five minutes (number of replicates on leaf : red: $n = 33$; mixed: $n = 33$; green: $n = 31$; number of replicates on glass : red: $n = 24$; mixed: $n = 28$; green: $n = 30$). Glass discs correspond to a homogeneous environment; therefore, these experiments aimed to observe the influence of conspecifics on their spatial distribution. On the contrary, bean leaf discs correspond to a heterogeneous environment (presence of food patches and nerves) and will show the influence of the environment on *T. urticae* spatial distribution. An analogue camera was focused on the set-up (leaf disc or glass disc) and recorded females' behaviour. One picture was taken every minute for 30 minutes. These pictures were

analysed using a data processing program called Fourmilier V3.3 (software creator, Jean- Michel Hudry (jm.hudry@ifrance.com)) (Sempo *et al.*, 2006; Le Goff *et al.*, 2009), which attributed spatial coordinates to each individual and thus allowed further analyses of the spatial distribution of individuals. By computing these locations, we characterised the spatial distribution of each group with two indicators: 1) the mean size of the largest aggregate and the radial distribution. These two indicators are commonly used to characterise the spatial distribution of eusocial (Depickere *et al.*, 2004, Sempo *et al.*, 2006) and non eusocial insects (Jeanson *et al.*, 2005).

1. The mean size of the largest aggregate. Mites were considered as being aggregated when two or more individuals were at a distance (body to body) of less than 2 mm from each other.
2. The radial distribution, which was an indicator of the tendency to follow the leaf border (border effect). The leaf was divided into five rings 1.5 mm apart (R1 (central ring): 0-1.5, R2: 1.5-3, R3: 3-4.5, R4: 4.5-6 and R5: 6-7.5), the external ring corresponds to the edge of the arena. We took into account differences in the area size between rings by comparing spider mite density per ring.

We tested the segregation between the two forms by calculating the proportion of nearest-neighbours of the same colour at each time step. If these spider mites aggregated preferentially with kin, the proportion of same-colour nearest neighbours should increase with time. If they showed no preference for kin, the proportion of nearest neighbours of the same colour should be constant over time and equal to 4/9 (out of the 9 potential neighbours, 4 are of the same colour as the focal individual). If the latter case, this proportion was expected to follow a binomial distribution with parameters $n=10$ (10 pairs of nearest neighbours) and $p=4/9$ (probability of having a nearest neighbour of the same colour). From this binomial distribution, we calculated the confidence interval of 95% around the expected value of 4/9, as well as the p-value associated to the experimental results.

Statistical analysis

Semilog regression between the number of individuals in the largest aggregate and time were done for each kind of group (green, red and mixed) on each kind of substrate (leaf and glass). Extra sum of square F tests were used to compare the slope and the Y intercept of each regression to those of other regressions. Kruskal Wallis tests of variance (KW) and Dunn's post-tests were also used to compare changes in radial distribution between each kind of population (green, red and mixed) on each kind of substrate (leaf, glass). Tests were performed using GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego, California, USA

Table 1: parameter of the semi-log regressions (X is log, Y is linear) of the number of individuals in the biggest aggregate according to time for green, mixed and red groups on glass and bean leaf discs

	Leaf experiments			Glass experiments		
	Red	Mix	Green	Red	Mix	Green
R ²	0.05	0.11	0.11	0.23	0.28	0.24
Slope	1.02 ± 0.14	1.60 ± 0.15	1.79 ± 0.16	3.16 ± 0.20	3.69 ± 0.20	3.45 ± 0.20
Y-intercept	2.78 ± 0.15	2.75 ± 0.17	2.55 ± 0.19	2.58 ± 0.23	1.42 ± 0.23	1.17 ± 0.23

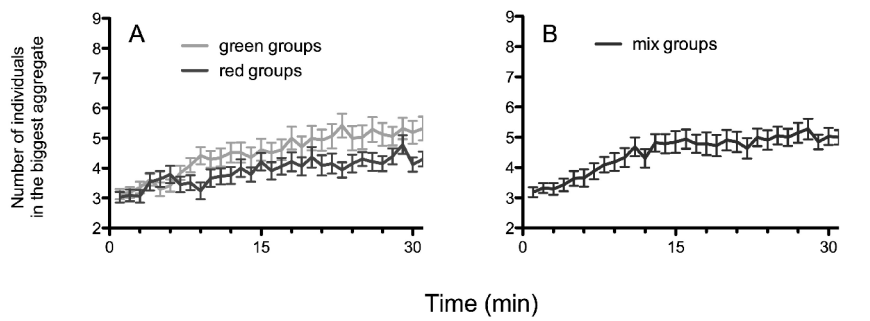


Figure 1: change with time of the biggest aggregate (mean number of individuals) for red, green (A) and mixed groups (B) for bean leaf discs experiments. Error bars indicate standard error (S.E.)

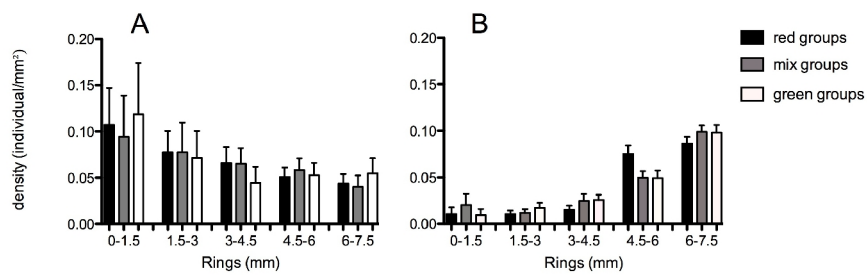


Figure 2: mean radial distribution of individuals: density of individuals for each red, mixed and green groups (mean number of ind/mm²+ S.E.) in the five rings (0 represents the centre of the leaf disc) at the end of the experiment (30 min) on bean leaf (A) and glass discs (B)

(<http://www.graphpad.com>). All tests were applied under two-tailed hypotheses and the significance level p was set at 0.05.

Results

Leaf experiments:

On leaf, the red groups aggregated more slowly than the green and the mixed groups, which aggregated at a similar speed. All semi log regressions (table 1) of the biggest aggregate according to time had the same intercept ($F= 0.55$, $DF=2$, $P>0.05$) but different slopes ($F= 7.50$, $DF=2$, $P<0.001$) (figure 1). The slopes of mixed and green groups did not statistically differ ($F=0.80$, $DF=1$, $P>0.05$), whereas the slope of red groups was the lowest (red vs. green: $F=13.58$, $DF=1$, $P<0.001$; red vs. mixed: $F=8.46$, $DF=1$, $P<0.01$) (figure 1).

On leaf, the mites did not seem to concentrate on the leaf margin. For each kind of group, we did not observe any significant difference of density between the rings: individuals were equally distributed in the different rings (red: $KW= 7.08$, $P>0.05$; mix: $KW= 5.67$, $P>0.05$; green: $KW= 3.46$, $P>0.05$) (figure 2). Furthermore, no difference was observed for the radial distribution at T_{30} between all kinds of groups whatever the ring (R1: $KW= 2.95$, $P>0.05$; R2: $KW= 0.64$, $P>0.05$; R3: $KW= 5.30$, $P>0.05$; R4: $KW= 0.73$, $P>0.05$; R5: $KW= 0.43$, $P>0.05$) (figure 2).

Glass experiments

On glass, the red groups were more gregarious than the two

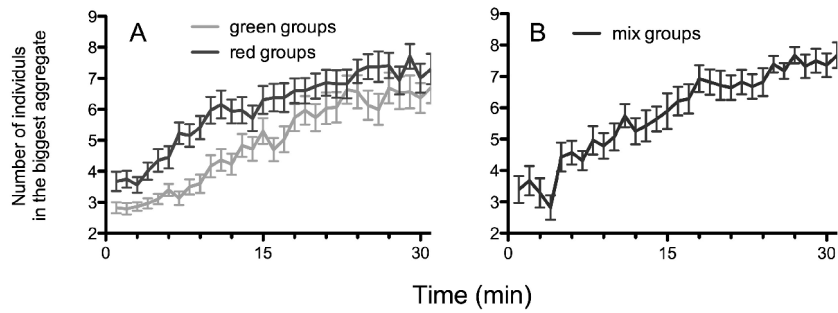


Figure 3: change with time of the biggest aggregate (mean number of individuals) for red, green (A) and mixed groups (B) for glass discs experiments. Error bars indicate S.E.

Table 2: Dunn's multiple comparison tests between the mean density of individuals of the different rings of the arena for red, mixed and green groups for glass discs experiments

	Red group	Mix group	Green group
R1 vs R2	P>0.05	P>0.05	P>0.05
R1 vs R3	P>0.05	P>0.05	P>0.05
R1 vs R4	P<0.001	P<0.001	P<0.001
R1 vs R5	P<0.001	P<0.001	P<0.001
R2 vs R3	P>0.05	P>0.05	P>0.05
R2 vs R4	P<0.001	P<0.01	P<0.05
R2 vs R5	P<0.001	P<0.001	P<0.001
R3 vs R4	P<0.001	P>0.05	P>0.05
R3 vs R5	P<0.001	P<0.001	P<0.001
R4 vs R5	P>0.05	P<0.05	P<0.05

other groups. Contrary to the semi log regressions of the leaf experiments, the semilog regressions made for the glass experiments (table 1) had the same slope ($F= 1.71, DF=2, P>0.05$) but different intercepts ($F= 10.36, DF=2, P<0.001$) (figure 3). The intercepts of mixed and green groups were statistically similar ($F=0.56, DF=1, P>0.05$), whereas the one for the red groups was the highest (red vs. green: $F=18.43, DF=1, P<0.001$; red vs. mixed: $F=12.61, DF=2, P<0.01$) (figure 3).

The mites were more concentrated on the border on glass than on the leaf, regardless of their group. For each kind of group, we observed significant differences in the density between the rings as in all cases the individuals were mainly situated in R4 and R5 (red: $KW= 77.35, P<0.001$; mix: $KW= 65.79, P<0.001$; green: $KW= 66.22, P<0.001$) (Dunn: Table 2) (figure 2). Furthermore, no difference was observed for the radial distribution at T_{30} between all kinds of groups whatever the ring (R1: $KW= 0.36, P>0.05$; R2: $KW= 0.34, P>0.05$; R3: $KW= 0.32, P>0.05$; R4: $KW= 5.16, P>0.05$; R5: $KW= 2.09, P>0.05$) (figure 2).

Leaf vs. glass

Concerning the evolution of the mean size of the largest aggregate according to time, we observed in each kind of group a systematically higher level of aggregation on the glass discs than on leaf discs (red: $F=356.30, DF=2, P<0.001$; mix: $F=97.01, DF=2, P<0.001$; green: $F=32.29, DF=2, P<0.001$).

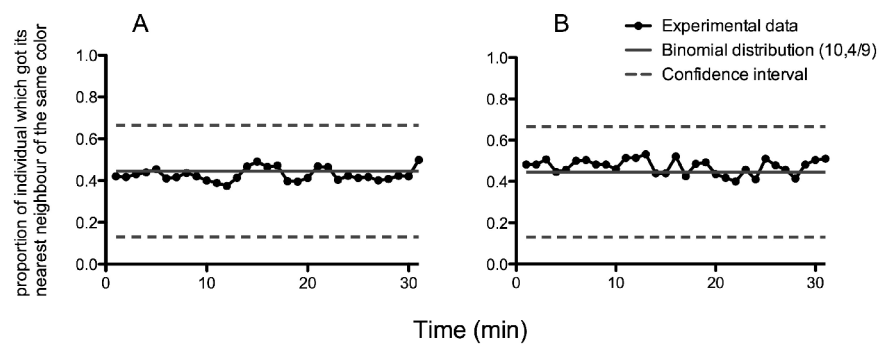


Figure 4: evolution of the mean proportion of individuals with its nearest neighbour of the same colour according to time for leaf (A) and glass (B) experiments

Segregation:

In mixed groups, no discrimination between the two forms was observed either on bean leaf or on glass discs. Indeed, in both cases, our experimental results did not differ from the binomial distribution described in the material and methods part (P-values of 0.48 on leaf and 0.40 on glass) (figure 4 a-b).

Discussion

In this paper, we investigated the spatial distribution within small groups of mites during the colonization of a new environment. Two types of environments were tested: a bean leaf considered as an heterogeneous environment with nerves and a glass disc considered as a homogeneous environment without any food. Groups of spider mites used in our experiments were composed of individuals coming from two genetic pools. In all tested situations, mites formed aggregates, even in a homogenous environment (on glass disc); this result strongly suggests that aggregation results from interactions between individuals, rather than or in addition to a common attraction to an environmental cue.

Our results highlight interesting behavioural differences between the two populations according to the kind of substrate. Indeed, the red form population seemed to have a higher level of aggregation without food than the green form population, whereas it was the opposite in the presence of food. Local selection theory can explain this difference between the two populations; individuals

coming from different local populations may have been subject to different local selection regimes (Maeda, 2005). The green form is found in temperate zones (Belgium), whereas the red form is subtropical, tropical and Mediterranean (Tunisia). Thus, different parameters of the mites' environment such as the plant nutrient content, or microclimate of certain regions of a host plant should have influenced mite aggregation dynamics differently (Barron and Margolies, 1991). Therefore, different local populations of *T. urticae* could exhibit between-population variations in their dynamics of spatial distribution. However, to test this hypothesis, different populations of each form are needed to be compared.

From an evolutionary point of view, different levels of aggregation between populations could also result from the difference in the level of genetic diversity within the populations. Red individuals are closer relatives than green ones. The lower tendency to aggregate of the red form individuals observed on leaf substrate (and compared to the green form) could result from its higher level of inbreeding. This avoidance of kin proximity could limit the competition for food, mates and space between relatives (Lambin *et al.*, 2001; Charlesworth and Charlesworth, 1987) and also decrease the probability of mating with relatives (Stow and beattie, 2008; Ridley and Sutherland, 2002). On the contrary, individuals of the green form population have a lower level of inbreeding and so a higher level of genetic diversity, which decreases the probability of inbred mating. This should explain their higher dynamics of aggregation on leaf substrate. On contrary, on glass substrate, the absence of food forces

individuals to disperse (Kondo and Takafuji, 1985). In that case, the lack of genetic diversity of the red form population should facilitate the formation of a migrating group composed of kin. Indeed, migrating with kin leads to founding a colony with kin and thus favours the development and survival of closely related individuals (increase of inclusive fitness).

It is noticeable that, for both forms, the level of aggregation was lower in the leaf experiments than in glass experiments. This may also correspond to an avoidance of other individuals and therefore to a mechanism that limits the competition for food, and space between relatives (Lambin *et al.*, 2001; Charlesworth and Charlesworth, 1987; Stow and beattie, 2008; Ridley and Sutherland, 2002) in the presence of food and leaf irregularities such as veins, which may be used to initiate web spinning (Le Goff *et al.*, 2009). This may prevent, at least partly, inbreeding depression and its negative physiological consequences (Helle, 1965).

Studying the fraction of aggregated individuals at a greater and more natural scale (more individuals and bigger leaf, on plant) should help us to determine if individuals want to leave the food patch or just move away from each other. We observed a higher level of aggregated individuals in the glass experiment on the border of the set up than in the leaf experiments. In our glass experiments, we put in evidence a border effect that indicate a propensity of *T. urticae* to follow the irregularity and increase the probability of meeting other individuals and to form aggregates. We can imagine the same kind of mechanisms on a plant completely eaten by a colony of *T. urticae*; individuals will

follow the irregularity of the leaves (nerves, borders, silk) and will form aggregate at the top of the plant or of a leaf. This may result in silk ball formation (Clotuche *et al.*, 2011), which seems to be involved in *T. urticae* dispersal through anemochory or zoochory.

Concerning the experiments where the red and green form populations were mixed, intermediate values of aggregation between green and red individuals should be obtained in the case of indifference to the genetic origin of other conspecifics. In fact, we observe that mixed groups aggregated similarly to green groups. This result might be linked to the genetic environment. One can think that, such as in many species, there is a trade-off between the degree of relatedness of individuals and the benefit it brings during the formation of aggregates in *T. urticae*. Being too close may have negative impacts on the fitness of relatives (inter-individual competition), whereas being distant may benefit each individual (Allee and dilution effect, cooperation). In our experiments the mix of the two forms might have created a group of individuals with a level of genetic relatedness similar to the green group's level. Thus the probability of encountering a non-relative increased, diluting the relatedness between red individuals. However, our experimental results followed the binomial law (described in the Materials and Methods section) and so no segregation between the two populations in our experimental design was observed, although this mite is capable of kin discrimination (Roeder *et al.*, 1996; Le Goff *et al.*, 2009). In the cockroach *Blattella germanica*, a weak attraction between individuals can lead to a mixed aggregation (Amé *et al.*, 2004). A

same mechanism could occur in *T. urticae*. This would mean that despite a clear genetic distance among the two populations, individuals are attracted to each other and that no trade-off is necessary between the degree of relatedness of individuals and the benefit it brings similar to what was observed in the green groups. Possible future experiments could be to study aggregation dynamics in a group with a controlled level of genetic diversity such as isofemale lines. We cannot reject the hypothesis that, under our experimental conditions (a low number of individuals in a restricted area), kin discrimination was hardly discernable. Therefore, testing the dynamics of aggregation under more natural conditions (on a plant) would help us to clarify the situation.

Acknowledgements

We are very grateful to Pr. Kaouthar Lebdi who supplied the red form of *Tetranychus urticae*, which was used in our experiments and to George van Impe for the useful discussions about *Tetranychus urticae*. We would like to thank all the team of the “Laboratoire d’écologie et biogéographie” for their help in data analysis. G.J. Le Goff is supported by a Grant from the FRIA (Fonds pour la Formation de la Recherche dans Industrie et l’Agriculture). The authors are also indebted to the National Fund for Scientific Research (FNRS, Belgium) for funding through the Fund for Fundamental and Collective Research (FRFC, convention 2.4622.06). AC Mailleux is financially supported by IRSIB (Institut d’encouragement de la Recherche Scientifique et de l’Innovation de Bruxelles).

Impact of starvation on the recruitment process during the invasion of a plant by pure and mixed populations of *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract

The two spotted spider mite, *Tetranychus urticae* is a generalist phytophagous pest present worldwide. This mite exhausts its host plant resources and then disperses to new hosts. During this period, individuals may meet conspecifics from different origins, carrying different genetic pools.

T. urticae, ambulatory behaviour was studied in the laboratory to evaluate within-plant displacement in relation to the level of starvation and the genetic diversity of the population. Groups composed of 60 females less than 78 hours old from the same population or from a mixed of two populations, starved or not for 24 hours, were placed at the base of a five leaved bean plant. Two days after infestation of this bean plant, all leaves were collected and the number of individuals on the upper and under side of each leaf was counted.

A recruitment process was observed during plant infestation that resulted in grouping together a high number of individuals on the same part of the plant. This grouping together enabled individuals to take advantage of group living (dilution effect, group effect, common web building, etc.) but also imposed drawbacks (competition, transmission diseases etc.).

Starvation decreased the recruitment process in the red population. This probably allowed individuals to avoid overcrowded area and therefore competition for food patches. This decrease in recruitment was not observed in the green population.

However, despite the fact that *T. urticae* is known to

discriminate their kin, we did not observe any influence of the genetic origin of conspecifics on the settlement strategy of *T. urticae*. As it is known that *T. urticae* is able to recognise their kin this result suggests that, during invasion, the recruitment process drives individual to form groups with non-related conspecifics. This might be a strategy to rapidly increase the population of the colony and to build more rapidly a protective web.

Introduction

The spatial distribution of individuals is an important subject in species biology (Mogilner *et al.*, 2003; Camazine *et al.*, 2001). It conditions the levels of interactions among individuals, and more generally, the structuring as well as the organisation of populations (Okubo, 1980; Ame *et al.*, 2006; Le Goff *et al.*, 2009). The challenge in the understanding of spatial patterns at the organism as well as at the population level (Camazine *et al.*, 2001) is the identification of the underlying mechanisms that allow molecules, cells or individuals form particular patterns that often fulfil a very specific function (Jost *et al.*, 2007). Spatial distribution is influenced by two kinds of stimuli: relationships between individuals and relationship between individuals and their environment (Deneubourg *et al.*, 2007).

In group-living species, the spatial distribution of individuals is determined by the presence/absence of conspecifics in the neighbourhood. Indeed, living with conspecifics can induce life history trait modifications of individuals and increase their fitness

compared to single individuals (Prokopy and Reynolds, 1998; Holbrook *et al.*, 2000). This implies that species in which fitness is enhanced by any type of conspecific facilitation might suffer from reduced density (Courchamp *et al.*, 1999). Therefore, in such species, the probability to stay or to choose a place to settle is influenced by the probability of finding conspecifics and to live together. This probability of settling in a place increases with the number of conspecifics to an optimal value, then decreases to avoid overcrowding.

The most frequently mentioned benefits of living close to other conspecifics include a decrease in the probability of being attacked (dilution hypothesis (Pulliam and Caraco, 1984)) (Rivault *et al.*, 1998, Ame *et al.*, 2004, Keough, 1983), as well as easier access to food and mates (Ranta *et al.*, 1993; Wertheim, 2005). It can also provide lower energetic costs as being group together decreases temperature loss or water evaporation (Krause and Ruxton, 2002). On the other hand, living close to other conspecifics also presents several disadvantages such as an easier prey location by predators and an increase of competition for water, food, space and mate (Ranta *et al.*, 1993).

The decision for an individual to settle in a particular place near conspecifics depends on its ability to detect other individuals. How this information is obtained varies according to the sensorial ability of the individual. When the sensory system does not allow access to global information, the evaluation of the number of conspecifics is based on local information such as indirect cues that are representative of the number of conspecifics (Astudillo-Fernandez

et al. 2010). These cues may be the result of environment modifications or of marker production (chemical or physical) by conspecifics.

Another factor known to influence the spatial distribution of individuals in many species is the relatedness between individuals. When the benefits of living near conspecifics are shared with closely related individuals, it potentially increases the indirect fitness of group members (Hamilton, 1964). Living with kin could thus be a strategy to increase inclusive fitness by favouring the development and survival of closely related individuals (Lihoreau and Rivault 2009). Conversely, living with kin induces kin competition. Increasing the inter-individual distance would decrease competition for food and mates between relatives (Charlesworth and Charlesworth, 1987; Lambin *et al.*, 2001; Le Goff *et al.*, 2009). Therefore, the distance between individual results from a trade-off between these the two opposite forces, the benefit and the disadvantages of group living with kin.

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is an interesting biological model to test the ratio of benefits to /disadvantages of living in a group with kin. This species presents many prerequisites that might lead to the emergence of social behaviours: its arrhenotoky, which induces a high kin relation among sisters, or the construction of a common web that favours spatio-temporal overlap of generations (Saito, 1983; Clotuche *et al.*, 2009). It presents indicators of a social organisation such as aggregation on its host plant (Strong *et al.* 1997, Le Goff *et al.* 2009)

and a positive group effect on individual fitness (Le Goff *et al.* 2010). Its haplodiploidy reproduction system combined with a low mobility, favours departures towards heterozygote deficiencies (Saito 1997). Indeed, in the field, various types of genetic structures, from monomorphic and highly inbred (Hinomoto and Takafuji 1994) to polymorphic populations carrying different genetic pools, can be observed in *T. urticae* populations (Carbonnelle *et al.* 2007). Within these populations, an individual can have close contacts and interact with non-related or, on the contrary, with highly related individuals.

In this paper, we study the settlement strategies of *T. urticae* by observing how this mite colonises a new plant. Spider mite migration can be triggered by overcrowding and deterioration of the leaves of the plant host (Wanibushi and Saito, 1983). When local resources are exhausted, mated females move in the same direction, following other females (Yano, 2008). *T. urticae* may reach potential host plants by walking, a primary form of dispersal (e.g., Kondo and Takafuji 1985) and following silk trails left by preceding females. These following females also lay silk trails while walking, resulting in a recruitment process amplified by a positive feedback mechanism (Yano 2008); this recruitment can lead to a collective migration (Yano, 2008) to a new and healthy host plant (Strong *et al.* 1997). However, several questions concerning collective migration remain unanswered: is there vertical recruitment inducing a collective climbing along the stem of the plant in the direction of the top of the plant? Is there horizontal recruitment at a particular level of the plant inducing individuals to choose between one of the leaves of the same

level? How do individuals stop following the vertical recruitment to connect themselves on the horizontal recruitment trace?

In this study, we try to answer these questions on the recruitment process. Indeed, we study the settlement strategies of *T. urticae* by observing the spatial distribution of mites during the colonisation of an entire plant composed of two levels of leaves.

We also test the influence of the level of starvation on the settlement of spider mites. Le Goff *et al.*, (unpublished data) observed a decrease in the attraction to silk by starvation as a mechanism for starved individuals to avoid competition and overcrowded areas. In our type of dispersion context, one question emerges: does the attractive property of the silk also decreases during the invasion process of a large starved group?

Finally, we analysed the impact of the genetic background on the settlement of *T. urticae*. In a previous experiment (Le Goff *et al.*, unpublished data), we studied the aggregation dynamics of *T. urticae* placed on a leaf, in relation with the genetic proximity of individuals. We showed that the level of inbreeding influenced the spatial distribution of small groups of mites during the colonisation of a new leaf (Le Goff *et al.*, unpublished data). Here, we study the influence of genetic on the settlement strategy of larger mixed groups composed of individuals from two different populations and comparing their strategy with the strategy of pure groups.

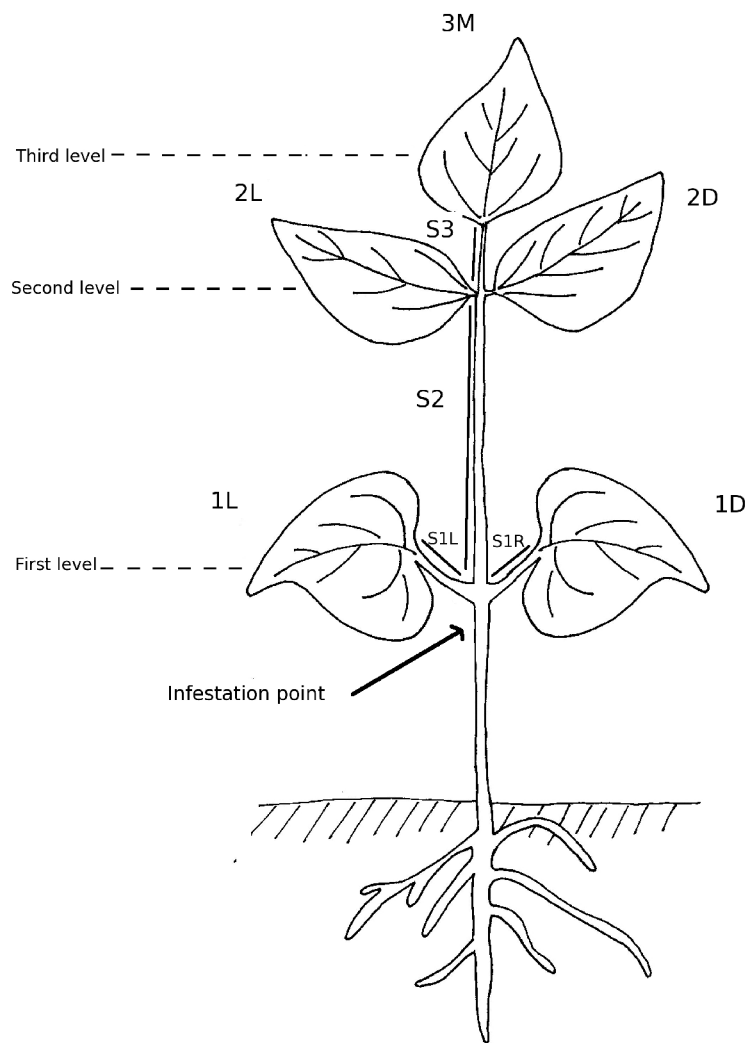


Figure 1: drawing of a standardized bean plant used in our experiments

Material and methods

Two populations of *T. urticae* were used for these experiments: a red form population provided by the Institut National Agronomique, Tunis, Tunisia (Tunis, Dr K. Lebdi-Grissa) and a green form population, which was collected in the green houses of our Laboratory in Louvain-la-Neuve, Belgium. The most accepted taxonomical position does not recognize *T. cinnabarinus* as a valid species as in the World Catalogue of the Spider Mite Family (Bolland *et al.* 1998), which cites it as a synonym of *T. urticae*. The two populations were reared on bean leaves placed on damp cotton (*Phaseolus vulgaris*) in Petri dishes. Breeding stocks were maintained in a climate room at 26°C, 50–60% RH, 16:8 (L:D).

The genetic distance between the two populations used in this experiment has been evaluated in a previous study by the microsatellite technique: both populations were considered inbred (FIS red form population confidence interval (CI): 0.47 (0;32-0.58) and FIS green form population (CI): 0.26 (0.15-0.30) but were genetically different for all tested loci ($F_{ST}=0.47$, Nei distance=0.95) (Le Goff *et al.* unpublished data).

Settlement tests

Sixty females less than 72h old belonging to the same population (Tunisia (red form) or Belgium (green form) or thirty from each of the two populations (mix) were placed one by one in the

centre of a bean leaf disc. Twenty-three replicates were made for each condition. The bean leaf disc was then placed at the base of a bean plant. For all replicates, bean plants were isolated in a cage (25cm*30cm*25cm).

Furthermore, sixty females less than 48h old belonging to the same form (red or green) or thirty from each of the two forms (mix) were placed one by one in the centre of a plastic disc surrounded by water to avoid escape. This plastic disc was then placed 24h later at the base of the bean plant. Therefore, in this case individuals had been starved for 24h before being allowed to colonise a new plant. Twenty-four replicates were made for each condition. For all replicates, bean plants were isolated in a cage (25cm*30cm*25cm).

Standardised bean plants were used for our experiments. The plant architecture consisted of two first levels with two leaves each (1 left (L), 1 right (R), 2L, 2R) and a third level with only one leaf (3M) (Figure 1). The stem dimensions (S1L, S1R, S2, S3) (figure 1) of bean plants were measured (mean sizes: S1L= 3.38cm, S1R= 3.32cm, S2= 10.12, S3=1.72), no difference were observed for S1, S3 and S4 between modalities (normally fed, mixed groups and starved individuals) (Kruskal-Wallis S1=3.42, $P>0.50$; Kruskal-Wallis S3=3.32, $P>0.50$; Kruskal-Wallis S4=0.73, $P>0.50$). Bean plants used for green starved individuals accidentally presented a longer S2 than the others (Kruskal-Wallis S2=19.11, $P<0.01$, Dunn's multiple comparison test: fed red vs. starved green: $P<0.05$; fed mixed vs. starved green: $P<0.01$). However, the linear regression between the S2 length of the bean plant used for starved green populations and the

number of individuals found on the leaf did not show any significant relation (deviation from zero: $F= 0.77$, $P>0.05$). Therefore, this difference in plant dimensions did not influence the settlement strategy of starved green individuals.

Two days after infestation, the five leaves of each plant were collected and placed in a freezer (-20°C) during 1 hour. Then, we took a picture of the upper and under side of each leaf. Finally, we analysed each picture with the Image J software (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, MD, USA, <http://rsb.info.nih.gov/ij/>, 1997–2008), and counted the number of individual on the upper and under side of each leaf. Based on the number of recovered individual on each plant we calculated the mean percentage of recovered individuals that stopped at the first, the second and the third level in each modality ((green vs. mixed vs. red) and (fed vs. starved)).

Data analysis

1) Spatial distribution

The first step of this study was to analyse the spatial distribution of the 60 individuals: were they homogeneously or heterogeneously distributed on the plant? To do so, for each modality (normally fed individuals, fed mixed groups, starved individuals and starved mixed groups), using heterogeneity χ^2 we compared the experimental results to a homogeneous theoretical distribution where the probability to choose between the two leaves of the same level is identical.

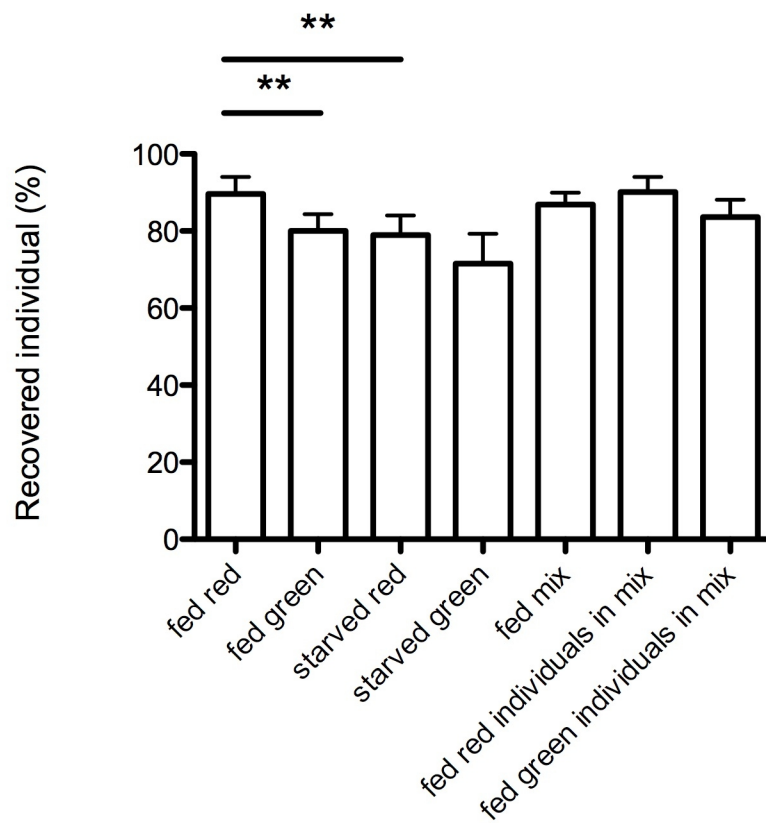


Figure 2: mean percentage of recovered individuals on a bean plant for all tested modalities (red vs. mixed vs. green) and (fed vs. starved). Error bars indicate the 95% confidence interval (CI)

Therefore, with this theoretical distribution, we hypothesised that the left and the right side of the bean plants were the same. We calculated for each modality a probability $P1=P2$ to choose a leaf at the first level (1L or 1R), a probability $P3=P4$ to choose a leaf at the second level (2L or 2R) and a probability $P5$ to stop at the first level (3M). These probabilities were calculated following the below formula

$$P1= P2= 0.5(N1/Nt)$$

$$P3=P4=0.5(N2/Nt)$$

$$P5= N 3/Nt$$

With $N1$, $N2$ and $N3$ the total number of individuals that stopped respectively at the first, second and third level of a modality and NT the total number of recovered individuals in a modality. If our result corresponded to this theoretical distribution, mites were homogeneously distributed. Otherwise, they were heterogeneously distributed. We compared the mean number of individuals recovered between the different modalities (normally fed individuals, fed mixed groups, starved individuals and starved mixed groups) with Kruskal Wallis tests followed by Dunns test.

2) Vertical recruitment: choice between first and second level

If these spider mites were not influenced by other individuals or by plant heterogeneity for the level choice, the number of individuals that stopped at the first or the second level of the plant should be similar to the mean number of individuals found at the first or the second level of the plant. In this case, this proportion should

Table 1: parameters of the χ^2 tests to compare experimental results to expected results for vertical recruitment

			Heterogeneity χ^2 , DF, p-value
Fed	Red population	1 st level	$\chi^2= 220.07, 22, P<0.001$
		2 nd level	$\chi^2= 266.31, 22, P<0.001$
	Mix population	1 st level	$\chi^2= 213.16, 22, P<0.001$
		2 nd level	$\chi^2= 188.63, 22, P<0.001$
	Red individuals in mix population	1 st level	$\chi^2= 199.87, 22, P<0.001$
		2 nd level	$\chi^2= 113.64, 22, P<0.001$
	Green individuals in mix population	1 st level	$\chi^2= 93.90, 22, P<0.001$
		2 nd level	$\chi^2= 76.10, 22, P<0.01$
	Green population	1 st level	$\chi^2= 64.28, 22, P<0.001$
		2 nd level	$\chi^2= 35.55, 22, P<0.05$
Starved	Red population	1 st level	$\chi^2= 120.43, 23, P<0.001$
		2 nd level	$\chi^2= 169.00, 23, P<0.001$
	Mix population	1 st level	$\chi^2= 97.95, 23, P<0.001$
		2 nd level	$\chi^2= 159.11, 23, P<0.001$
	Red individuals in mix population	1 st level	$\chi^2= 98.87, 23, P<0.001$
		2 nd level	$\chi^2= 128.86, 23, P<0.01$
	Green individuals in mix population	1 st level	$\chi^2= 56.73, 23, P<0.001$
		2 nd level	$\chi^2= 47.51, 23, P<0.05$
	Green population	1 st level	$\chi^2= 127.96, 23, P<0.001$
		2 nd level	$\chi^2= 155.43, 23, P<0.01$

follow a binomial distribution with parameters for the first level, n = the number of recovered individuals and p = the mean percentage of recovered individuals that stop at the first level (fed red= 35%, fed green= 62%, fed mixed= 46%, fed red in mixed= 42%, fed green in mixed= 52%, starved red=49%, starved green= 51%, starved mixed= 49%, starved red in mixed= 47%, starved green in mixed= 53%) and for the second level, n = the number of that reach the second level and p =the mean percentage of individuals that stop at the second level (fed red = 44%, fed green= 47%, fed mix= 53%, fed red in mixed= 47%, fed green in mixed= 49%, starved red=45%, starved green =46%, starved mixed= 50%, starved red in mixed= 49%, starved green in mixed=52%). Heterogeneity χ^2 as well as the p-value were calculated to compare experimental results to the binomial distribution. Afterwards, we compared the mean number of individuals found at each level between the different modalities (single and mixed populations; fed or starved individuals) with Kruskal Wallis tests followed by Dunn's tests.

3) Horizontal recruitment: choice between a right or a left leaf

We also tested the probability of choosing a side at the first or the second level, and the possible influence of other individuals and plant heterogeneity on that choice. If these spider mites were not influenced by other individuals or by plant heterogeneity to settle on a leaf, the proportion of individual on each side and at each level should be equal to 1/2. In this case, this proportion was expected to

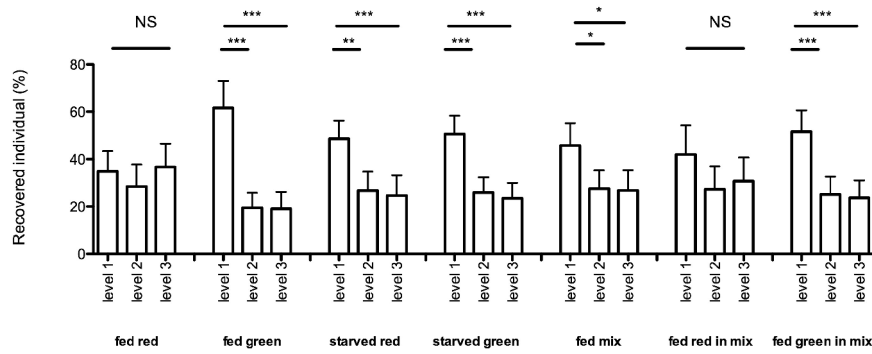


Figure 3: mean percentage of recovered individuals on each plant level and for each modality

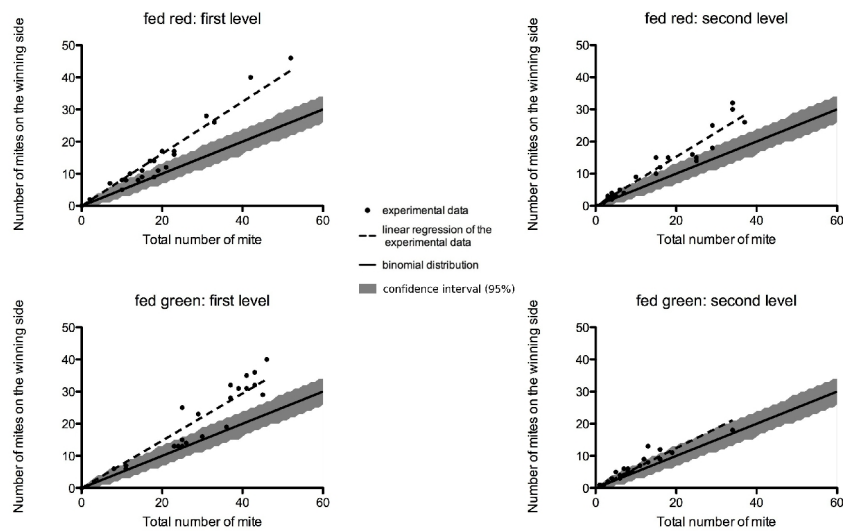


Figure 4: linear regression of the numbers of mites on the winning side vs. the total number of mites at each level of a bean plant for fed red and fed green form populations. Experimental data vs. expected results under the null hypothesis of a binomial model

follow a binomial distribution with parameters n =number of individuals, that stop at the same level and $p=1/2$. From this binomial distribution, we calculated the confidence interval of 95% around the expected value of $1/2$ and heterogeneity χ^2 as well as the p-value were calculated to compare experimental results to the binomial distribution. Linear regressions were also made between the number of mites on the winning side and the number of mites that stopped at a level. Their slopes allowed us to quantify the settlement process and to compare it between populations.

Statistical analyses

Statistical analyses were performed using Microsoft® Excel® 2008 and GraphPad Prism version 5.01 for Mac. GraphPad Software, San Diego, California, USA (<http://www.graphpad.com>). All tests were applied under two-tailed hypotheses and the significance level p was set at 0.05.

Results

1) Spatial distribution

First, by comparing the two populations, we observed a higher mean percentage of recovered individuals on the plant for the red form populations than for the green form populations but this difference was significant only for fed and starved mixed conditions (Kruskall-Wallis (KW)= 45.12, $P<0.001$, Dunn fed red vs. fed green: $P<0.05$, Dunn starved red in mixed vs. starved green in mixed: $P<0.01$, Dunn starved red vs. starved green: $P>0.05$, Dunn fed red vs. fed green: $P>0.05$) (figure 2)).

Table 2: parameters of the linear regression between the number of mites on the winning side and the number of mites that stopped at a level; and heterogeneity χ^2 tests to compare experimental results to expected results for horizontal recruitment

			R ²	Slope=Recruitment process(%) $\pm$ 95% CI	Heterogeneity χ^2 , DF, p-value
Fed	Red population	1 st level	0.93	81 $\pm$ 5	X ² = 145.00, 22, P<0.001
		2 nd level	0.92	76 $\pm$ 6	X ² = 98.72, 22, P<0.001
	Mix population	1 st level	0.9	76 $\pm$ 5	X ² = 66.01, 22, P<0.001
		2 nd level	0.91	69 $\pm$ 5	X ² = 80.81, 22, P<0.001
	Red individuals in mix population	1 st level	0.94	88 $\pm$ 6	X ² = 148.55, 22, P<0.001
		2 nd level	0.82	64 $\pm$ 7	X ² = 49.78, 22 P<0.001
	Green individuals in mix population	1 st level	0.89	71 $\pm$ 4	X ² = 65.01, 22, P<0.001
		2 nd level	0.86	70 $\pm$ 7	X ² = 40.36, 22, P<0.01
	Green population	1 st level	0.86	74 $\pm$ 6	X ² = 151.64, 22, P<0.001
		2 nd level	0.87	62 $\pm$ 6	X ² = 35.55, 22, P<0.05
Starved	Red population	1 st level	0.8	65 $\pm$ 5	X ² = 145.00, 23, P<0.001
		2 nd level	0.92	72 $\pm$ 5	X ² = 81.18, 23, P<0.001
	Mix population	1 st level	0.59	72 $\pm$ 7	X ² = 166.19, 23, P<0.001
		2 nd level	0.79	65 $\pm$ 6	X ² = 55.83, 23, P<0.001
	Red individuals in mix population	1 st level	0.73	75 $\pm$ 7	X ² = 92.12, 23, P<0.001
		2 nd level	0.82	68 $\pm$ 7	X ² = 43.62, 23, P<0.01
	Green individuals in mix population	1 st level	0.66	74 $\pm$ 6	X ² = 102.21, 23, P<0.001
		2 nd level	0.81	71 $\pm$ 7	X ² = 38.63, 23, P<0.05
	Green population	1 st level	0.84	70 $\pm$ 5	X ² = 125.39, 23, P<0.001
		2 nd level	0.92	67 $\pm$ 4	X ² = 48.03, 23, P<0.01

The comparison of fed and starved populations showed that the mean percentages of recovered individuals was higher for fed than for starved individual, but the difference was significant only for the red form (KW=45.12, $P<0.001$, Dunn fed red vs. starved red: $P<0.01$, Dunn fed green vs. starved green: $P>0.05$) (figure 2).

We did not observed any impact of the genetic background on the mean percentage of recovered individuals. Indeed, no differences were observed between red/green individuals in mixed populations and red/green individuals in single population conditions (KW=45.12, $P<0.001$, Dunn red fed vs. red fed individuals in mixed: $P>0.05$, Dunn green fed vs. green fed individuals in mixed: $P>0.05$) (figure 2).

Second, we observed a heterogeneous distribution within the bean plants in all modalities (single and mixed populations; fed or starved individuals) (fed red: $\chi^2= 996.20$, $df=88$, $P<0.001$; fed green: $\chi^2= 655.96$, $df=88$, $P<0.001$; starved red: $\chi^2= 606.66$, $df=84$, $P<0.001$, starved green: $\chi^2= 499.06$, $df= 84$, $P<0.001$; fed mixed: $\chi^2= 913.39$, $df=88$, $P<0.001$; fed red in mixed: $\chi^2= 712.03$, $df=88$, $P<0.001$, fed green in mixed: $\chi^2= 369.70$, $df=88$, $P<0.001$; starved mixed: $\chi^2= 469.96$, $df=84$, $P<0.001$; starved red in mixed: $\chi^2= 396.94$, $df=84$, $P<0.001$, starved green in mixed: $\chi^2= 255.43$, $df=84$, $P<0.001$).

2)) Vertical recruitment: choice between first and second level

In all tested modalities, the individual decision to stop at the

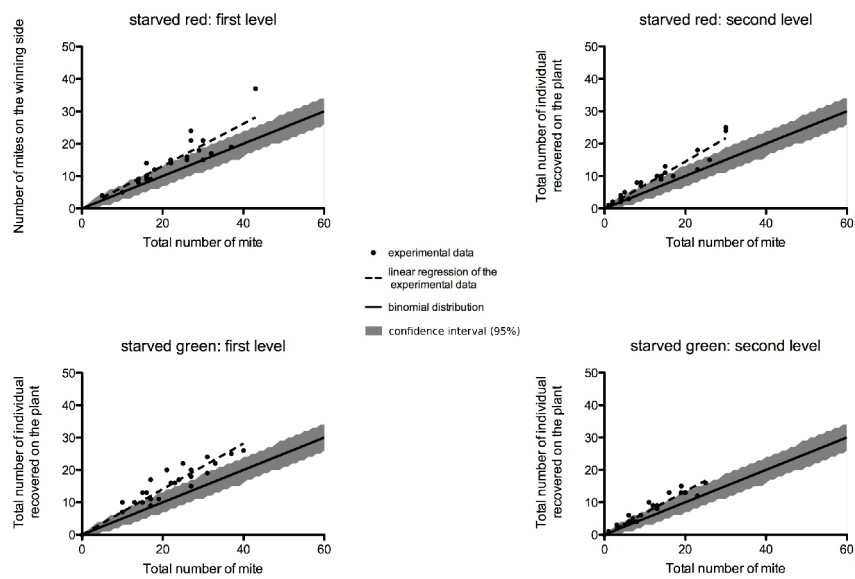


Figure 5: linear regression of the numbers of mites on the winning side vs. the total number of mites at each level of a bean plant for starved red and starved green form populations. Experimental data vs. expected results under the null hypothesis of a binomial model

first or the second level was influenced by other individuals or by plant heterogeneity. Indeed, none of the tested modalities followed the binomial law described in the Materials and Methods section (table 1).

The mean proportion of individuals at each level, for red individuals, the distribution of individuals was 33% at each level of the plants (KW=1.99, $P>0.05$) (figure 3). Green individuals did not followed a homogeneous distribution, as 50% of the individuals were situated at the first level, 25% at the second level and 25% at the third level: (KW=27.51, $P<0.001$, Dunn level 1vs. level 2: $P<0.001$, Dunn level 1vs. level 3: $P<0.001$, Dunn level 2 vs. level 3: $P>0.05$) (figure 3).

For the two starved populations, the distributions of individuals across the levels were 50% situated at the first level, 25% at the second level and 25% at the third level: (starved green: KW=24.63, $P<0.001$, Dunn level 1vs. level 2: $P<0.001$, Dunn level 1vs. level 3: $P<0.001$, Dunn level 2 vs. level 3: $P>0.05$; starved red: KW=17.92, $P<0.001$) (figure 3). No difference was observed for the mean percentages of starved individuals between populations (Kruskal-Wallis (KW)=4.07, $P>0.05$) (figure 3).

For mixed populations, the distribution of individuals was: at the first level: 50%, second level: 25% and third level: 25% (mixed: KW=9.87, $P<0.001$, Dunn level 1vs level 2: $P<0.01$, Dunn level 1vs. level 3: $P<0.001$, Dunn level 2 vs. level 3: $P>0.05$) (figure 3). No difference was observed between red and green individuals in the fed mixed populations and the fed red and green population (fed red

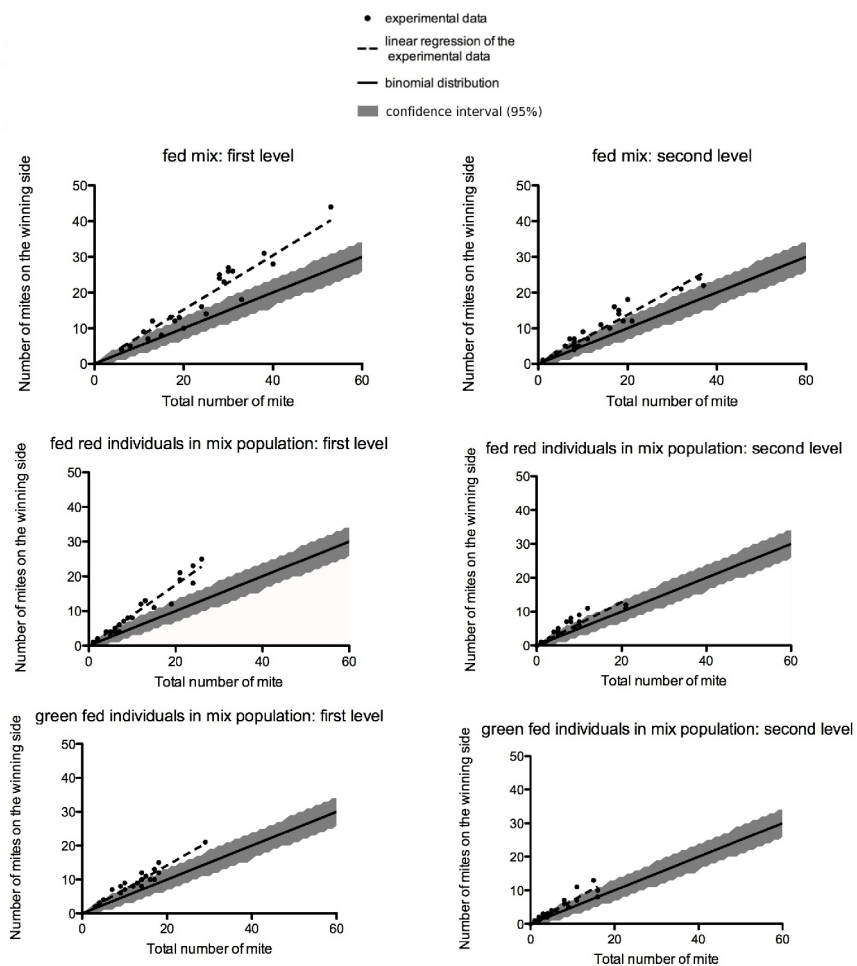


Figure 6: linear regression of the numbers of mites on the winning side vs. the total number of mites at each level of a bean plant for fed mixed populations. Experimental data vs. expected results under the null hypothesis of a binomial model

populations vs. red individuals in fed mixed populations $KW=5.75$, $P>0.05$; fed green populations vs. green individuals in fed mixed populations: $KW=52.46$, $P<0.001$, Dunn level 1: $P>0.05$, Dunn level 2: $P>0.05$, Dunn level 3: $P>0.05$) (figure 3).

Mixed starved populations individuals did not follow a homogeneous distribution across the bean plant levels, as 50% of the individuals were situated at the first level, 25% at the second level and 25% at the third level: ($KW=27.39$, $P<0.001$, Dunn level 1vs. level 2: $P<0.01$, Dunn level 1vs. level 3: $P<0.001$, Dunn level 2 vs. level 3: $P>0.05$) (figure 3). Furthermore, no difference was observed between the red and green individuals in starved mixed populations and the starved red and green population (starved red populations vs. starved red individual in mixed populations $KW=32.16$, $P<0.001$, Dunn level 1: $P>0.05$, Dunn level 2: $P>0.05$, Dunn level 3: $P>0.05$; starved green vs. starved green individuals in mixed populations: $KW=59.33$, $P<0.001$, Dunn level 1: $P>0.05$, Dunn level 2: $P>0.05$, Dunn level 3: $P>0.05$) (figure 3).

3) Horizontal recruitment: choice between a right or a left leaf

For all modalities and for each level, the experimental probabilities to choose a side clearly differed from a binomial distribution, as one side of the leaf was always selected by the mites (table 2) (figures 4-5-6-7). This asymmetric distribution between two presumably identical branches should result from one of two possible

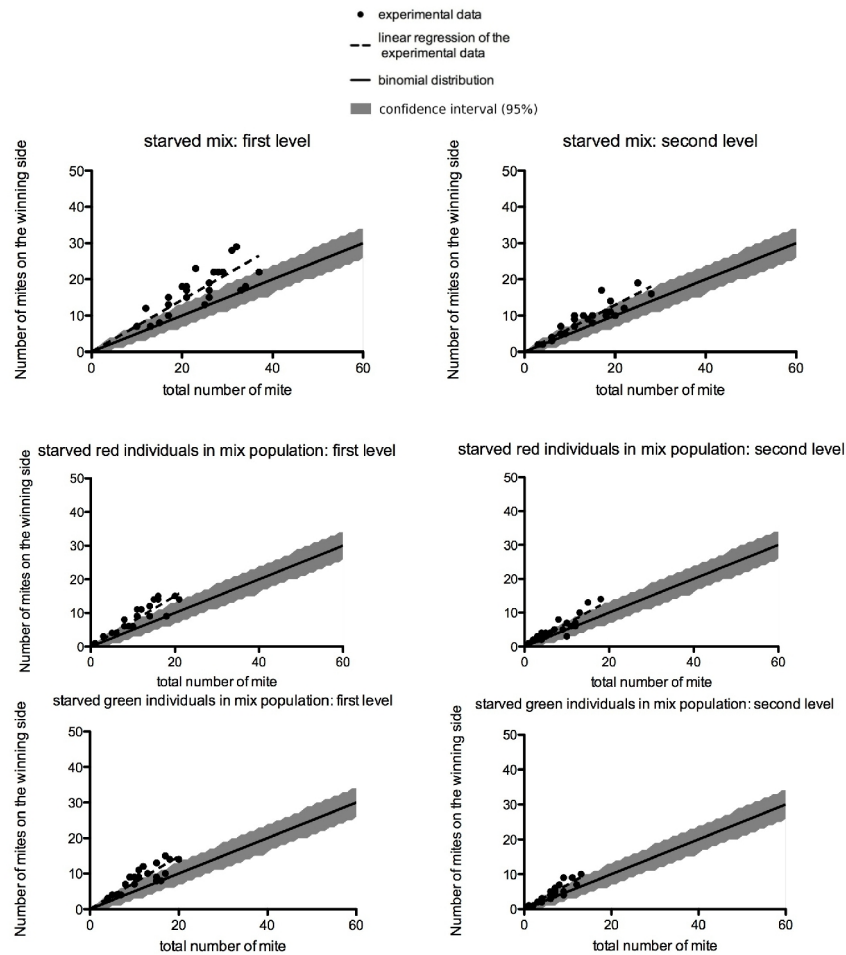


Figure 7: linear regression of the numbers of mites on the winning side vs. the total number of mites at each level of a bean plant for starved mix populations. Experimental data vs. expected results under the null hypothesis of a binomial model

deviations from the binomial situation. Indeed, small differences in the plant stems may induce the choice of one side and mites may also choose in function of the choice of the preceding ones. For the first level, the experimental probabilities to choose a side were similar in the three tested population ($F=1.72$, $P>0.05$) (figure 4). On the contrary, at the second level, the green population had a lower recruitment process than the red population ($F=9.47$, $P<0.01$) (figure 4).

For starved populations, no differences were observed for the recruitment process between the tested populations, either at the first level ($F=1.43$, $P>0.05$) or the second level ($F=2.76$, $P>0.05$) (figure 5). When we compared the recruitment process between starved and fed individuals at the first level, we observed a lower one for red starved populations ($F=16.21$, $P<0.001$) (figures 4-5). On the contrary, the green populations did not present statistical difference between fed and starved individuals ($F=0.70$, $P>0.05$) ($F=16.21$, $P<0.001$) (figures 4-5). For the second level, no difference was observed for any tested populations (red population: $F=0.97$, $P>0.05$, green population: $F=1.96$, $P>0.05$) ($F=16.21$, $P<0.001$) (figures 4-5).

Green and red individuals in the mixed population behaved in the same way as the green and red individuals in single population conditions (first level: red populations vs. red individuals in mixed populations: $F=2.38$, $P>0.05$; green populations vs. green individuals in mixed populations: $F=0.28$, $P>0.05$; second level: red populations vs. red individuals in mixed populations: $F=2.40$, $P>0.05$; green populations vs. green fed individuals in mixed populations: $F=2.68$,

Table 3: summary of results

	Red form population	Impact of starvation	Impact of the genetic background (mix)	Green form population	Impact of starvation	Impact of the genetic background (mix)
Percentage of recovered individual		Decrease	No		No	No
Heterogeneous distribution	Yes			Yes		
Vertical recruitment	Yes	More individuals stop at the first level	No	Yes	No	No
Horizontal recruitment	Yes	Decrease	No	Yes	No	No

$P > 0.05$) (figure 6).

The same trends were obtained for starved individuals under mixed or single population conditions (first level: red populations vs. red individuals in mixed populations: $F = 3.94$, $P > 0.05$; green populations vs. green individuals in mixed populations: $F = 0.89$, $P > 0.05$; second level: red populations vs. red individuals in mixed populations: $F = 0.92$, $P > 0.05$; green populations vs. green fed individuals in mixed populations: $F = 0.88$, $P > 0.05$) (figure 7).

Discussion

In this paper, we investigated the spatial distribution of mites' groups during the infestation of a new plant and the impact of genetic background and of the level of starvation on this spatial distribution. Our results are summarised in table 3.

For all tested modalities, we observed a vertical recruitment process in the settlement strategy of *T. urticae*. This might result from the influence of different factors such as 1) geotropism and/or phototropism (Suski and Naegele, 1968; Fournier *et al.*, 1985). 2) Plant heterogeneities such as vertical folds of the surface along the stem 3) a recruitment process induced by silk laying behaviour.

Concerning the leaf choice, when individuals had to choose between the left and right side, we observed a clear horizontal recruitment process due to individual interaction and/or plant heterogeneities. This results in finding a large number of individuals on the same leaf. Collective migration offers mites the possibility to

form aggregates, which then gives, several advantages to individuals. Indeed, collective migration behaviour might be linked to the advantage for individuals to remain grouped (Rivault *et al.*, 1998; Ame *et al.*, 2004; Le Goff *et al.*, 2009). In general, forming such aggregates has many advantages both for the individual and the group. It also allows the establishment of new colonies by groups of females and therefore the immediate cooperative construction of webs, which effectively protects all members from predators (McMurtry *et al.*, 1970) and from wind and rain (Davis 1952, Linke, 1953). Furthermore, Le Goff *et al.* (2010) described a positive group effect in *T. urticae* on fertility, survival and silk production during colony foundation. It would be therefore reasonable to expect settlers to look for conspecifics. However, these experiments has been done with groups only composed of females. It could be interesting to redo these experiments with groups composed of females, males and juvenile stages to be closer to natural conditions. In addition, another next step of this study could be to test different densities of individuals as it can influence the resistance of the plant and therefore the dynamic of population and the behaviour of *T. urticae* (Rotem and Agrawal, 2003).

Our results also highlight interesting behavioural differences between populations concerning the plant infestation dynamic. During natural infestations, *T. urticae* is supposed to colonise the lowest green leaves (Gilstrap *et al.*, 1980; Logan *et al.*, 1983). Our results confirm this statement only for the green form populations (1st level: 50%, 2nd level: 25% and 3rd level: 25%), but for not the red form populations

(1st level: 33%, 2nd level: 33% and 3rd level: 33%). Our results also highlight interesting behavioural differences between the populations in terms of the probability to choose between the right and the left side. Indeed, at the second level of the plant, the red form elicits stronger recruitment than the green form. We also observed that the red populations were homogeneously distributed across the levels, whereas for the green form populations individuals mainly chose to settle at the first level. This might mean that the recruitment process in *T. urticae* depends of the number of individuals that reach a level. The higher number of mites that arrived at the second level for the red form population than for the green form population might induce a stronger recruitment process. This result is in accordance with those of Yano (2008) who found that *T. urticae* females prefers to follow trails laid by five than by one preceding females.

During our experiments, the total number of individuals was not always recovered after two days; this could be due to mortality or movements off the plant (Barron and Margolies 1991). Indeed, an unfavourable environment, such as a high/low density of conspecifics, a low quality of infested host plants, or a high intensity of local mate competition, may cause greater levels of activity in the mites, resulting in mites jumping/falling off the leaves (Barron and Margolies 1991). However, we suppose that the same mortality rate occurred in the two populations, the fact that we recovered more individuals in the red form population reinforces the idea that this population elicits stronger recruitment than the green form population.

Starvation did not influence the tested populations in the same

way. Actually, only the red form population was influenced by the starvation period in terms of the infestation rate and the recruitment process. Indeed, in this case, significantly fewer starved red individuals were recovered compared to fed red populations and the recruitment process is weaker for red starved individuals than for fed individuals. In 2008, Yano found that a recruitment process can be explained by silk trail following. Furthermore, Le Goff *et al.* (unpublished data) observed a modulation of the attraction to silk by the level of starvation. Therefore, this lower number of red individuals recovered and this weaker recruitment process observed for the red starved individuals in red and mixed populations can be explained by a decrease of silk attraction. Indeed, it would be reasonable to expect starved settlers to avoid overcrowded areas (Aviles and tufino, 1998). Red individuals are found preferentially at the first level (50%) when they are starved instead of 33% when they are fed. This can simply be explained by the fact that starved individuals settle immediately on the first level to feed and do not move far away.

Mixing two populations had no influence on the settlement strategy. Indeed, the results of the mixed groups are always intermediate to those of pure groups and the results of the red and green individuals in mixed populations are statistically similar to those of red and green populations, respectively. This should mean that individuals of *T. urticae* cannot recognise their kin through their silk production and that they recognise their kin through other cues. Testing the attraction of silk between different populations of *T. urticae* would allow us to answer this question and studying the kin

recognition system of *T. urticae* could be an interesting next step in this study. *T. urticae* is a species in which tactile stimuli are very important (Oku *et al.* 2005). Furthermore, Bostanian (1973) described chemoreceptors and mechanosensory/olfactory receptors on the two first walking legs of *T. urticae*. Therefore, kin recognition through tactile contacts could be a way to explain the kin discrimination ability of *T. urticae*.

To sum-up our results, here we show a recruitment process for plant infestation in the two spotted spider-mite *T. urticae*. It allows mites to form large aggregates in the same part of the plant and to benefit from the advantages of group living (dilution effect, group effect, common web building, etc.). In addition, this aggregation behaviour may have an impact on the plant health and the dynamics of populations of *T. urticae*. Furthermore, we provide evidence that this recruitment process can be modulated by the level of starvation, probably to avoid overcrowded areas and competition for food patches. This modulation was observed only in one of the two tested populations. Therefore, this mechanism cannot be generalised to the *T. urticae* species and has to be probably related to the origin or the genetic diversity of the population. However, during experiments with mixed populations, we did not observe any influence of the genetic origin of the conspecifics, on the settlement strategy of *T. urticae*. This should mean that individuals of *T. urticae* do not care about their genetic environment during this period or that it needs more/other stimulations from its conspecifics/environment to discriminate its kin.

Acknowledgements

We are very grateful to Pr. Kaouthar Lebdi who supplied the red form of *Tetranychus urticae*, which was used in our experiments and to George van Impe for the useful discussions about *Tetranychus urticae*. We would like to thank all the team of the “Laboratoire d’écologie et biogéographie” for their help in data analysis. G.J. Le Goff is supported by a Grant from the FRIA (Fonds pour la Formation de la Recherche dans Industrie et l’Agriculture). The authors are also indebted to the National Fund for Scientific Research (FNRS, Belgium) for funding through the Fund for Fundamental and Collective Research (FRFC, convention 2.4622.06). AC Mailleux is financially supported by IRSIB (Institut d’encouragement de la Recherche Scientifique et de l’Innovation de Bruxelles).

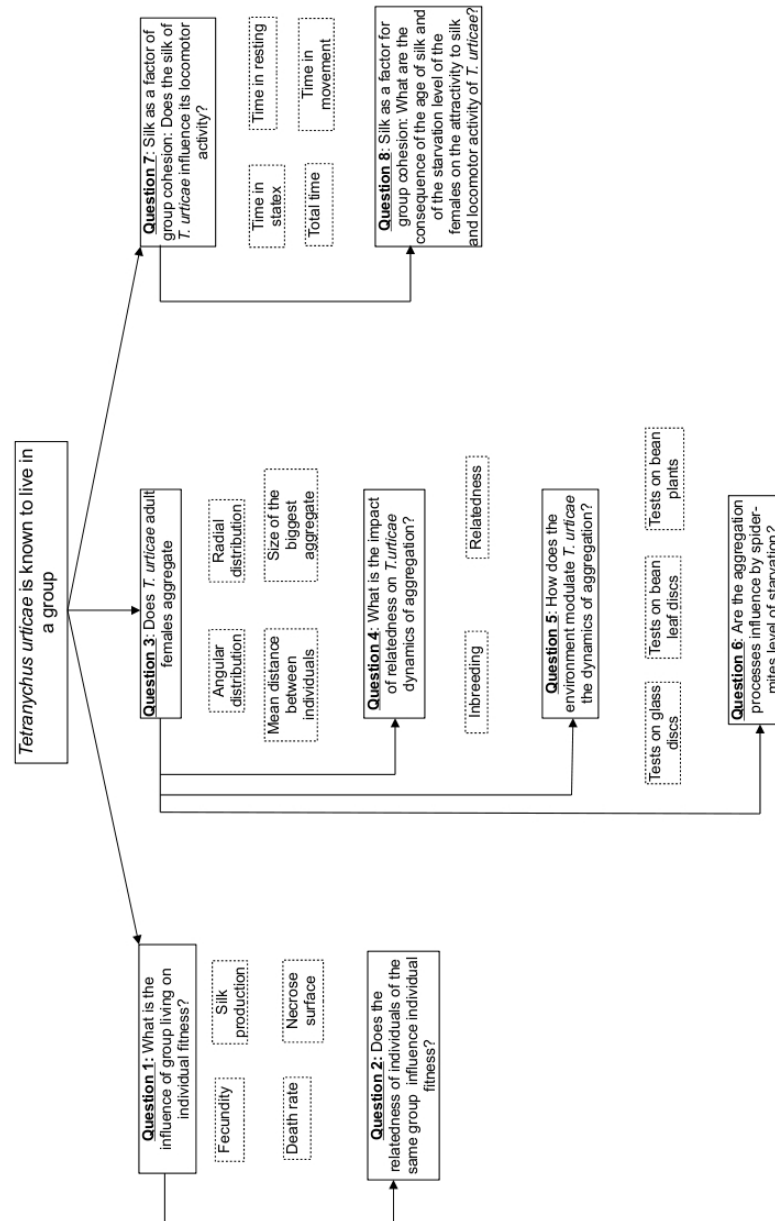


Figure 6: Flow chart of the PhD thesis, showing the different steps of the study

Question 7 and 8 – paper 6: “Silk as a factor of group cohesion: does the silk of *T. urticae* influence its locomotor activity?”, “What are the consequences of the age of silk and of the starvation level of the females on the attractivity to silk and locomotor activity of *T. urticae*?”

Spider mites differ from many social insects by the production and the use of silk to build irregular webs. This silk prevents dispersion of the individuals and ensures the group cohesion during swarming and collective displacements, playing a similar role to the trails of pheromones in ants (Deneubourg and Goss, 1989).

Silk attracts mites, but the influence of starvation on this attraction has never been studied. One can guess that a decrease of food availability should increase the hunger of individuals. Starved individuals are probably more attracted by new space devoid of silk as they represent not yet exploited places. So, we expect a decrease in silk attractivity for starved individuals. In this context, the influence of starvation on mites behaviour was investigated.

The “freshness” of the silk could be a biotic indicator for a female. A trail of silk freshly laid could indicate the close presence of conspecific, whereas an old trail of silk probably lead to a plant that was already damaged. In this context, the influence of the silk age on the behaviour of mite was investigated.

Impact of starvation on silk attractiveness in a weaving mite, *Tetranychus urticae* (Acari: Tetranychidae).

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Submitted in journal of ethology (minor revision)

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Abstract

The two spotted spider mite *Tetranychus urticae* is a silk producer known to live in groups. Its silk production plays an important role in protection against external aggressions (predators, rains, etc.). It is also used during collective migration by ballooning or as a dragline during the individual migration by walking. Until now, the role of silk in enhancing migration has been poorly studied.

In this paper, the influence of the presence of silk on *T. urticae*'s locomotor activity was studied. One virgin female was placed at the centre of a cover glass partially covered by silk. Moving and resting time were studied on the silk or the clean part of the cover glass as a function of the starvation level of mites (fed vs. starved) and the age of the silk (30 vs. 60 vs. 90 vs. 120 vs. 150 min).

The results show that a fed female spends more time on the silk-covered part than on the clean part as long as the silk is fresh (less than 120 min). Thus, the age-related changes in chemical and/or physical properties of the silk influence the spatial distribution of fed mites. Whatever the age of the silk, starved mites spend more time on the clean part of the set-up. Indeed, the silk freshly laid by conspecifics is attractive only to fed mites, starved mites probably prefer sites without silk (and conspecifics). This study shows that the silk influences the spatial distribution of *T. urticae* according to its level of starvation and that silk is probably an indicator of the presence of conspecifics.

Keywords: *Tetranychus urticae*, silk, stigmergy, locomotor activity

Introduction

Arthropods are the only silk weavers of the animal kingdom. Among them, few groups have developed silk secreting glands: Hymenoptera, Lepidoptera, Embioptera, Thysanoptera, some larvae of Diptera, and more particularly Arachnida (Craig 1997; Selden 1989; Shear *et al.* 1989). The numerous functions of silk include web building in spiders, leaf tying and constructing pupal cocoons and safety lines when dislodged from a substrate (Alexander 1961; Fitzgerald 1983; Common 1990). The web can also be used as a mean of locomotion, and many small spiders (Decae 1987), caterpillars (Moore and Hank 2004) and mites (Margolies 1995) use silk to lift themselves off a surface and disperse through anemochory. Finally, silk can be used as a vector of communication, which is essential in the organisation of arthropod societies. For example, in social spiders, a silk-laying behaviour leads to the formation of silk ‘highways’ that ensure group cohesion as well as collective decision-making processes during swarming (Lubin and Robinson 1982; Avilés 2000). In the same way as pheromones are used by social insects, silk can lead to amplification processes and collective decisions.

The phytophagous two-spotted spider mite *Tetranychus urticae* Koch is a ubiquitous species, present worldwide and is considered a major pest of many cultivated plants (more than 200 host plants, Van Impe 1985). In this species, a collective web results from individual silk thread produced passively by walking by all moving individuals (Hazan 1974; Saito 1997; Yano 2008) even on a non-

natural substrate (Clotuche *et al.* 2009). Previous studies have shown the importance of *T. urticae*'s web as a shield to protect the colony against some predators (McMurtry *et al.* 1970). Indeed, it can prevent some generalist predator such as *Amblyseius andersoni* from attacking *T. urticae* while the specialist predator of *T. urticae*, *Phytoseiulus persimilis*, is less hindered by the web unless it has become very dense (Sabelis 1985, Tien *et al.* 2009). The web also protects *T. urticae* from rain (Davis 1952; Linke 1953) and pesticides (Ashley 2003; Davis 1952; Linke 1953). The silk produced by *T. urticae* is a key element to explaining social evolution in this species such as group living (Van Impe 1985; Le Goff *et al.* 2009; Le Goff *et al.* 2010), common web building (Saito 1983) and communication (Yano 2008).

As the spider mite population increases, the damage to the plant increases and mites begin to abandon the damaged plant when all the apical foliage becomes infested and chlorotic inducing starvation in the mite population. Then, starved mites try to reach another potential food patch by passive dispersal by wind in webs enclosing many individuals (Brandenburg and Kennedy 1982), by walking from the leaves of a plant to the nearby leaves or by walking on the ground to reach another plant (Kennedy 1985; Kondo and Takafuji 1985; Krainacker and Carey 1990). Yano (2008) has shown that dispersing mites follow a silk trail left by preceding individuals. Therefore, these trail-following behaviours induce recruitment that can lead to aggregate formation and common web building. In social spiders such as *Anelosimus eximius*, the “pheromonal” function of silk

is known to be influenced by internal factors such as the state of starvation (Bernard and Krafft 2002). In spider mites, the influence of starvation on this trail-following behaviour has never been studied, as the observations on recruitment described above and made by Yano (2008) were done on populations of well-fed *T. urticae*. One can imagine that spider mite recruitment might be influenced by starvation. In this scenario, a decrease in food availability should increase the hunger of individuals and might cause a decrease in attraction for silk and thereby increase the number of mites able to temporarily leave the silk trail. In that context, the influence of the state of satiety on the attractiveness of pre-deposited silk and therefore, on the behaviour of adult female two-spotted spider mites was studied by measuring the impact of the presence/absence of silk on their locomotor activity.

In this paper, the influence of the age of silk was also tested. Indeed, the “freshness” of the silk could be a biotic indicator for mites, as a freshly laid silk trail could indicate the close presence of conspecific, whereas an old trail of silk would probably lead to a plant that was already damaged. In this context, the influence of silk age on mite behaviour was investigated.

Materials and methods

Mites

The mites used in this study belonged to the red form of *T. urticae* (also called *T. cinnabarinus*) and were provided by the Institut National Agronomique (Tunis, Tunisia; Dr. K. Lebdi). The strain was

reared on bean leaves (*Phaseolus vulgaris*), placed on damp cotton and maintained at 26°C, 50–90% RH, 16: 8 (L: D). The mites were reared for >20 generations before the study.

Experimental set-up

Experiments were undertaken in the same environmental conditions as breeding. A virgin female aged less than 24 h was allowed to walk freely on a half microscope cover glass (ø15 mm), whereas the other part of the cover glass was covered with wet paper. The cover glass was previously placed on a wet paper that was deposited on damp cotton to avoid individual from leaving the glass lens. For 30 minutes, the individual was allowed to deposit silk (and possibly pheromones and excrements) by walking on the free half of the cover glass (Saito 1977); no eggs were laid during this period. In the literature, it is already known the mating status of *T. urticae* females may greatly influence its oviposition behaviour with a higher egg production by mated females (Van Impe, 1985). Therefore, by using virgin females to spin silk on the set-up, silk production was homogenised among replicates and egg laying was avoided. Then, the wet paper was removed and the set-up was dried for 30 minutes at 26°C. Therefore, the cover glass was divided into a silk-covered part (SP) and a clean part (CP). A new virgin female less than 48h old (fed or starved for 24 h) was placed at the centre of this cover glass at the SP/CP frontier by using a one-haired brush and its behaviour was video-recorded for 30 minutes. Females were isolated on bean leaf discs at the teleochrysalid stage. They were starved by isolating them

on a cover glass for 24h just after molting, whereas fed females continue to feed on the bean leaf disc for 24h after molting. This starvation period did not induce abnormal mortality: only one starved individuals died during the starvation period. Experiments were performed with five different silk ages: 30 minutes (fed n=35; starved n=33), 60 minutes (fed n=32; starved n=32), 90 minutes (fed n=33; starved n=33), 120 minutes (fed n=29; starved n=31) and 150 minutes old (fed n=31; starved n=28). The silk-covered part was alternatively on the right or on the left side of the set-up between two experiments. This allowed us to avoid any environmental influence which could have attracted tested individuals on the same side of the set-up.

Four behavioural parameters were recorded on each part of the set-up with the software Jwatcher (Copyright © 2000–Daniel T. Blumstein, Christopher S. Evans & Janice C. Daniel): (1) the total time spent on each part; (2) the time in movement; (3) the resting time; and (4) the time in static exploration (“statex”). Statex is a behaviour characterised by individuals staying at the same place but turning on themselves. This statex behaviour was observed during previous experiments when mites were on natural substrate such as bean leaves or plants. Consequently, it cannot be considered as an artefact (online resource 1 and 2). This kind of behaviour had never before been described for this species. The results of time in movement, resting time and time in statex were represented by their mean relative proportion on each part of the set-up. The relative proportion of each behavioural item was calculated by dividing the time spent on one behavioural item (time in movement, in resting or in

statex) on one part of the set-up by the total time spent on that part.

Statistical analysis

To evidence a preference for the silk part or the clean part as a function of the age of silk, we subtracted for both starved and fed individuals the absolute value of the total time spent on the clean part to the absolute value of the total time spent on the silk part. If the mean result was significantly positive, mites spent significantly more time on the silk part. On the contrary, if the mean result was significantly negative, mites spent significantly more time on the clean part. The third possibility was that the mean result was statistically similar to zero, in this case, it meant that the mites did not spend more time neither on the silk part nor the clean part. A two-way ANOVA followed by a Bonferroni post-hoc test was used to test the influence of the substrate and of age of silk on preference of fed and starved individuals.

We normalised the data of the relative proportion of each behavioural item for both fed and starved individuals and for each age of silk by following an arcsine transformation. To simultaneously test the influence of starvation, of the presence/absence of silk and of the age of that silk on the locomotor activity of *T. urticae* (time in statex, resting, and in movement), a three-way MANOVA was made to compare the relative proportion of two behavioural items (resting and movement) among modalities. Indeed, as the sum of the three behavioural items was always 100%, testing only two behavioural items automatically included the result of the third behavioural item.

Tests and graphs were performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com) and Statistica version 9.1 (Statsoft 2010; www.statsoft.fr).

Results

Total time

The level of starvation influenced the total time spent on the silk part (two-way ANOVA, $F=86.94$, $dfn = 1$, $dfd = 307$, $p < 0.001$), while the age of silk had no influence (two-way ANOVA, $F = 1.60$, $dfn = 4$, $dfd = 307$, $p>0.05$). However, a significant interaction was found between the level of starvation and the age of silk ($F = 2.60$, $dfn = 4$, $dfd = 307$, $p < 0.05$). This suggested that the age of silk did not influence fed and starved individuals in the same way.

The age of silk significantly influenced the total time spent by fed individuals on each part of the set up. The total time spent on the silk part minus that spent on the clean part was significantly positive for the three first ages of silk (30 min: lower 95% confidence interval (CI) of mean= 413.60, upper 95% CI of mean= 878.10; 60 min: lower 95% CI of mean=363.90, upper 95% CI of mean= 800.50; 90 min: lower 95% CI of mean=52.59, upper 95% CI of mean= 593.80) (figure 1) and significantly similar to zero for the two last ages of silk (120 min: lower 95% CI of mean=-314.10, upper 95% CI of mean= 335.50; 150 min: lower 95% CI of mean=-93.17, upper 95% CI of mean= 318.30) (figure 1).

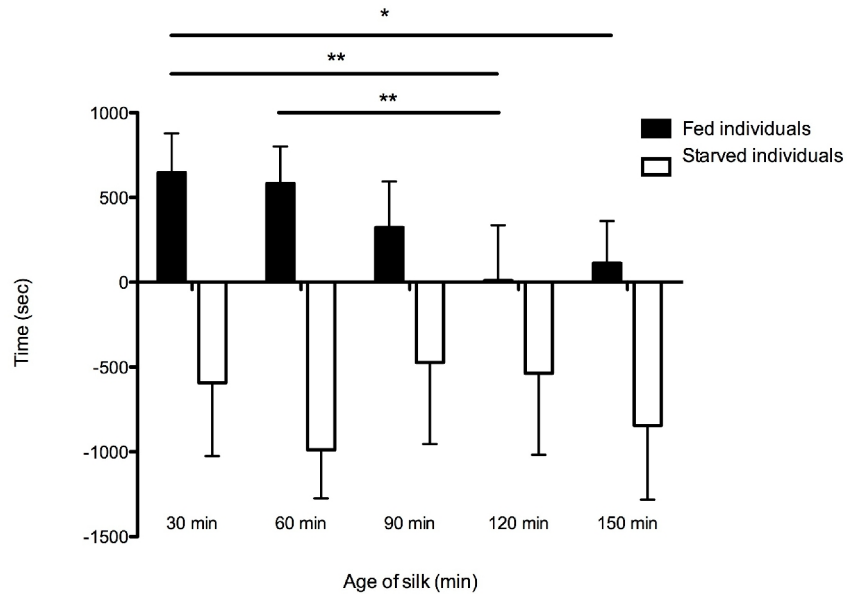


Figure 1: Mean differences between the total time spent on the silk part and the time spent on the clean part for fed and starved individuals for each age of silk. Error bars indicate the confidence interval (95%)

Table 1: Results of the three-way MANOVA comparing the relative proportion of *T. urticae* locomotor activity among modalities

	F	DF	P
(1) State of satiety (Fed, Starved)	78.28	3	<0.001
(2) Substrate (SP, CP)	30.76	3	<0.001
(3) Silk age (30, 60, 90, 120, 150 min)	0.42	12	<1
(1)*(2)	27.68	3	<0.001
(1)*(3)	0.42	12	<1
(2)*(3)	0.49	12	<1
(1)*(2)*(3)	1.37	12	<0.5

For starved individuals, the total time spent on the silk part minus that spent on the clean part was significantly negative for all ages of silk (30 min: lower 95% confidence interval (CI) of mean= -1025, upper 95% CI of mean= -162; 60 min: lower 95% CI of mean=-1275, upper 95% CI of mean= -701.10; 90 min: lower 95% CI of mean=-1017, upper 95% CI of mean= -72.62; 120 min: lower 95% CI of mean=-1019, upper 95% CI of mean= -55.52; 150 min: lower 95% CI of mean=-1282, upper 95% CI of mean= -407.20) (figure 1).

Locomotor activity

Starvation and the presence of silk influenced the locomotor activity while the age of silk did not (tables 1 and 2, figure 2). For the percentage of time spent in movement, fed individuals always spent 75% of their time in movement on all set-ups (figure 2) (table 2). Starved individuals spent more time moving on the silk part (approximately 50%) than on the clean part (approximately 25%), whatever the age of that silk (figure 2) (table 2). Moreover, starved individuals spent less time moving than fed individuals at all ages of silk (figure 2) (table 2).

The percentage of time spent resting by fed individuals was not influenced by the presence of silk whatever the age of that silk. Fed individuals spent approximately 15% of their time resting in all set-ups (figure 2) (table 2). The percentage of time spent resting by starved individuals was influenced the presence of silk as starved

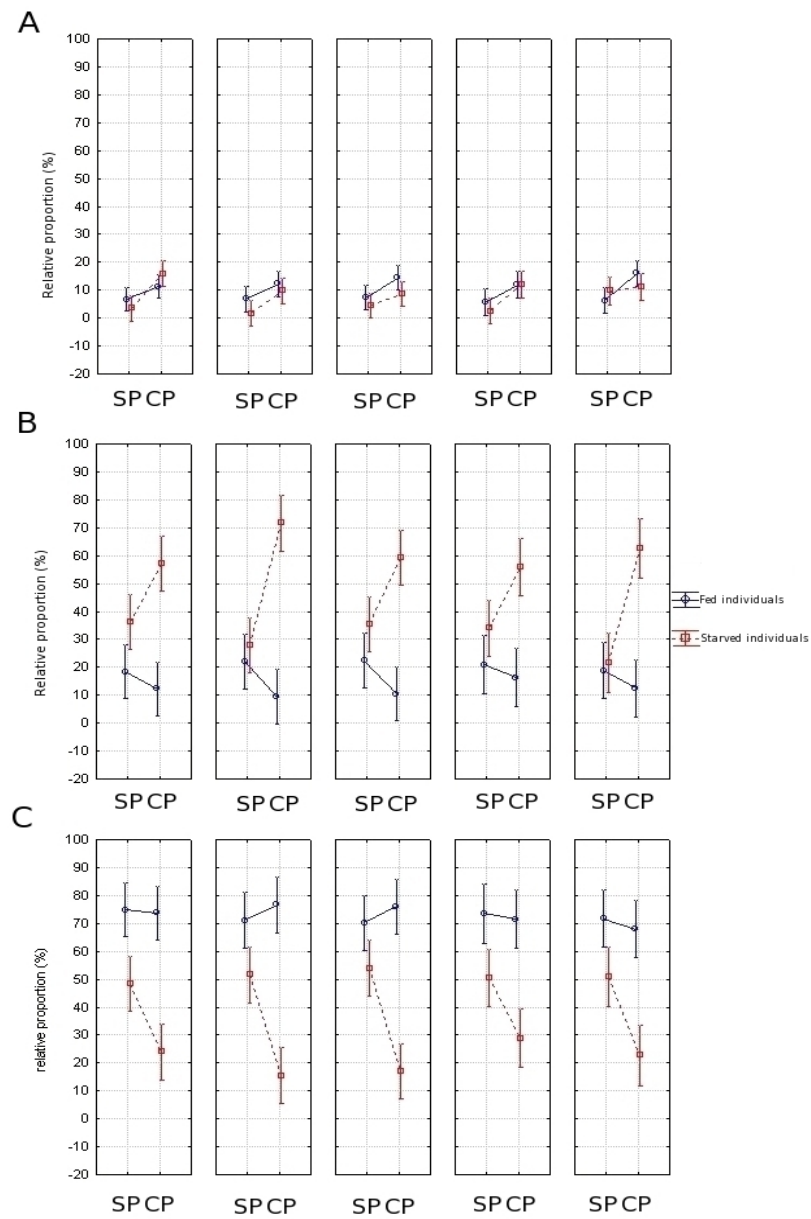


Figure 2: Mean relative proportion of time spent in statex (A), in resting (B) and in movement (C), on the clean part (CP) and on the silk part (SP) for fed and starved individuals for each age of silk. Error bars indicate the confidence interval (95%)

mites spent more time resting on the clean part (approximately 65%) than on the silk part (approximately 35%) (figure 2) (table 2). Starved individuals globally spent more time resting than fed individuals (figure 2) (table 2).

Both fed and starved individuals spent more time in statex on the clean part than on the silk part for all ages of silk (figure 2) (table 2). No difference was found between fed and starved individuals for the time spent in statex (figure 2) (table 2); spider mites spent approximately 10% of their time in statex whatever their starvation level, the presence of silk or the age of that silk (figure 2) (table 2).

Discussion

Our paper is the first showing that the attractiveness of *T. urticae* to its silk can be modified by its state of satiety. Fed individuals of *T. urticae* are more attracted by a silk environment rather than a clean one because they spent more time on the silk-covered part than on the clean part and 80% of this time was in movement. Newly emerged adult females (less than 48h old in our case) of *T. urticae* spent most of their time moving rather than feeding or resting (Bancroft and Margolies 1996). This age of less than 48 h represents the phase during which adult female spider mites are most likely to disperse (Hussey and Parr 1963; Li and Margolies 1993). Following the silk left by preceding individuals would increase the probability of finding conspecifics, food patches or mates.

Silk and pheromones deposited on the set-up represent cues of

Table 2: Mean relative proportion of time spent in statex, in resting and in movement on the clean part (CP) and on the silk part (SP) for fed and starved individuals for each age of silk. Error bars indicate the confidence interval (95%)

		Fed individuals		Starved individuals	
		Silk part	Clean part	Silk part	Clean part
Statex	30 min	6.74±3.11	15.06±4.72	4.51±3.85	15.91±7.10
	60 min	6.83±2.88	16.03±5.16	3.12±2.57	9.78±4.69
	90 min	7.35±3.78	18.65±5.00	5.04±4.60	8.54±3.66
	120 min	5.57±3.58	17.75±4.04	4.07±3.19	11.63±6.84
	150 min	6.40±3.01	19.77±5.36	10.19±6.81	11.19±6.09
Resting	30 min	18.43±6.87	14.35±6.02	30.97±11.28	57.08±12.75
	60 min	22.12±8.35	12.11±5.19	24.37±10.49	71.69±9.37
	90 min	22.41±9.98	13.59±5.26	30.01±12.83	59.42±10.93
	120 min	20.89±7.50	18.69±7.21	29.41±11.12	53.94±13.02
	150 min	18.86±6.58	15.07±5.82	21.01±9.73	62.5±13.06
Movement	30 min	74.83±7.10	62.76±7.31	45.17±11.97	23.98±9.50
	60 min	71.06±7.83	64.61±5.87	46.69±13.10	15.44±5.01
	90 min	70.25±10.33	63.69±5.84	50.11±12.90	16.89±6.02
	120 min	73.54±8.50	60.10±6.59	44.47±12.64	27.99±11.11
	150 min	71.71±8.53	56.92±7.63	45.23±12.26	22.74±10.23

conspecific presence or a trail to follow to find a colony (Yano 2008). This attraction to the silk-covered part can also be due to the possible presence of excrement on that part of the set-up. Indeed, excrement gives many advantages to the individual and to the group such as humidity absorption and predators repulsion (Oku 2008, Le Goff *et al.* 2010). Female mites might deposit additional chemical pheromones that might be bound (or not) to the threads, and that could have acted alone or in combination with silk compounds. This remains to be investigated (Yano 2008). Such attraction through silk pheromones has already been shown in several studies on social spiders such as *Anelosimus eximius* (Bernard and Krafft 2002; Krafft 1970; Robert and Krafft 1981). The communication between these social spiders is complex because it associates chemical communication with physical and visual communication or vibratory communication. The same kind of mechanism might exist in *T. urticae* with a mix of chemical and tactile communication because tactile stimuli are significant during the initial contact of the male with females. Oku *et al.* (2005) reported that adult males of *T. kanzawai* spider mites perceive the mating status of a female by her trails, which also suggests the involvement of trail-bound pheromones. The next step of this study might then be to isolate and identify these active chemical pheromone(s).

The attraction for silk cover part let suggests the existence of a stigmergic mechanism regulating collective web building in *T. urticae*. Stigmergy refers to a class of mechanisms that mediate animal–animal interactions. It has to be supplemented with an additional mechanism

that makes use of these interactions to coordinate and regulate collective building in a particular way (Theraulaz and Bonabeau 1999). A trace left in the environment by an action stimulates the performance of a next action. In this respect, silk attraction, through chemical cues or through the physical path it constitutes, regulates individual interactions and leads to an aggregation. The benefits of settling near conspecifics include reduced search and settlement costs (Fletcher 2006), the use of conspecifics as indicators of habitat quality (Doligez *et al.* 2003) and the fitness increase associated with the Allee effect (Courchamp *et al.*, 1999, Astudillo-Fernandez *et al.*, 2010).

Our results show that starved individuals spent more time on the clean part than the silk-covered part and 60% of this time was resting. This long time spend in resting is not surprising as it would allow individuals to save internal reserves (Margolies and Bancroft 1985) and to decrease metabolism. The attraction of starved mites for the clean part is more surprising: This is probably a mechanism for starved individuals to avoid competition and overcrowded areas and to find new feeding sites. As the social spider, *Anelosimus eximius* (Bernard and Krafft 2002), a fed *T. urticae* is more attracted to a silk-covered part than a clean part, whereas a starved one is more attracted to a clean part than a silk-covered part. On an infested plant, starved individuals are attracted by light and move to the uppermost parts of the plant (Huffaker *et al.* 1969). These starved individuals spin new silk by moving to new sites, the silk attracts fed individuals also spinning silk and so on until the collective web is built and a growing colony of mites settles. When the plant is completely infested, an

increasing number of individuals become starved, climb to the uppermost parts of the plant and the cycle begins again. Such feedback is both functional and energy saving as it speeds up migration from exhausted plants. In that scenario, the modulation of silk attraction by the level of satiety might, at least partly, regulate the population of mites and the process of migration to a new plant.

Silk seems to have the same attractive effect when all individuals are in the same physiological state and triggers a recruitment process that insures group cohesion. This mechanism regulating group cohesion decreases the probability of individuals leaving the colony and might be one of the factors explaining the evolution of a social way of life. Another consequence of this silk avoidance by starved individuals is the expansion of the web around the colony, which increases its protection against external aggression. This web expansion can also lead to a collective dispersal behaviour where a group of individuals forms a silk ball through a recruitment process: by depositing silk threads on their way up to the plant apex, mites favour and amplify the recruitment towards the balls (Clotuche *et al.* 2011) and leave the plant through anemochory (Margolies 1995) or zoochory (Weeks *et al.* 2000) when the plant is overcrowded.

Our results show that older silk is less attractive than silk freshly deposited by fed individuals. This strongly suggests the presence of chemical cues (i.e., which might be oxidised or degraded over time) or the presence of volatiles (Yano 2008). However, with these results, we cannot exclude a physical modification of the silk or a change in its structure. Further experimentation such as identifying

silk-bound pheromones would help us to separate the physical and chemical impacts of silk on *T. urticae*'s behaviour. The age of silk did not influence the behaviour of starved individuals, which suggests that silk could represent a different ecological cue according to the physiological state of individuals. Our results suggest that, before choosing to settle or migrate, fed individuals take account of the physical and chemical information provided by the relative freshness of the silk, whereas starved individuals avoid the silk regardless of its relative freshness.

Static exploration (statex) behaviour in *T. urticae* has been described for the first time in this study. Although there are no significant differences between fed and starved conditions concerning the time spent in statex, it seems that this behaviour is constant and relatively important (approximately 10% of their time for fed and starved individuals on each side of the set-up). *T. urticae* is a species in which tactile stimuli are very important (Oku *et al.* 2005).

Bostanian (1973) described chemoreceptors and mechanosensory/olfactory receptors on the two first walking legs of *T. urticae*. The fact that spider mites spent more time in statex on the clean part than on the silk part suggests that this behaviour is probably a probing behaviour displayed by *T. urticae* to detect several indicators of its environment (silk, conspecifics, new substrates and so on).

The next step of this study would be to test this attraction to the silk and its modulation by the level of starvation in natural conditions (on leaf/plant with mated females).

Acknowledgements

We are very grateful to Pr. Kaouthar Lebdi who supplied the red form of *Tetranychus urticae*, which was used in our experiments and to George van Impe for the useful discussions about *Tetranychus urticae*. We would like to thank all the team of the “Laboratoire d’écologie et biogéographie” for their help in data analysis. G.J. Le Goff is supported by a Grant from the FRIA (Fonds pour la Formation de la Recherche dans Industrie et l’Agriculture). The authors are also indebted to the National Fund for Scientific Research (FNRS, Belgium) for funding through the Fund for Fundamental and Collective Research (FRFC, convention 2.4622.06). AC Mailleux is financially supported by IRSIB (Institut d’encouragement de la Recherche Scientifique et de l’Innovation de Bruxelles).

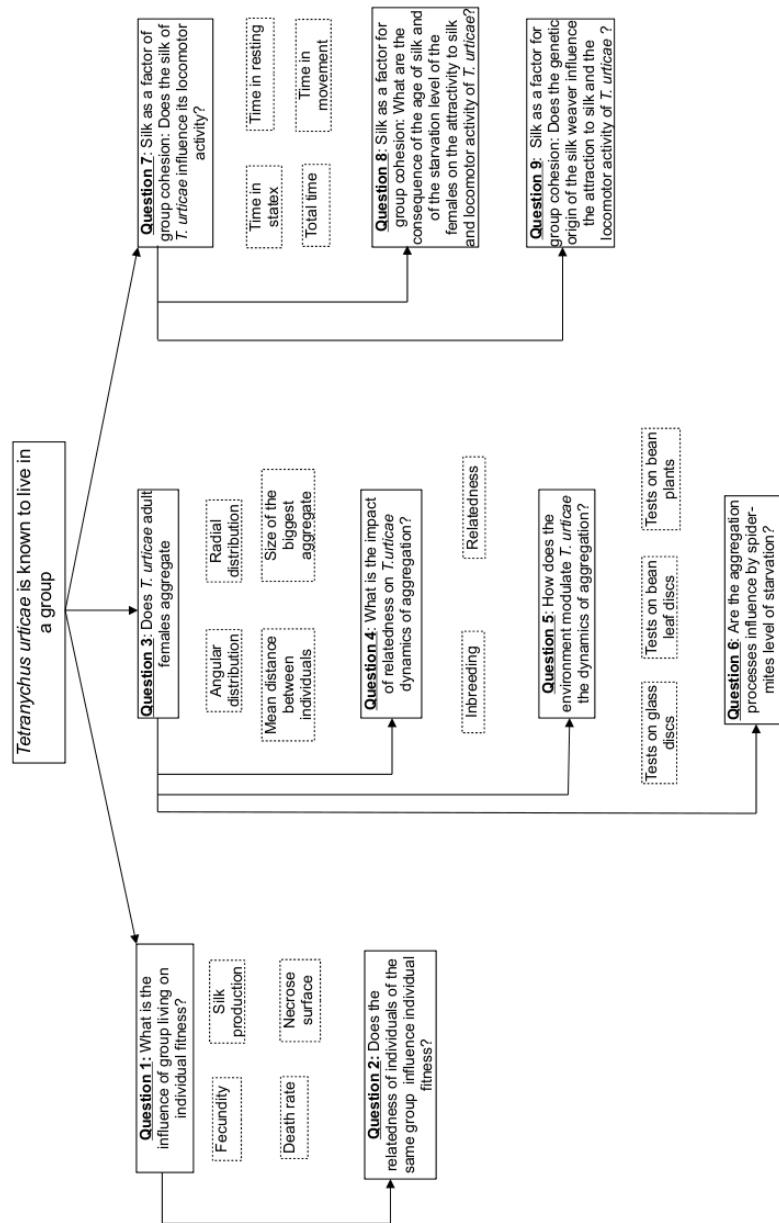


Figure 6: Flow chart of the PhD thesis, showing the different steps of the study

Question 9 – paper 7: Silk as a factor for group cohesion: Does the genetic origin of the silk weaver influence the attraction to silk and the locomotor activity of *T. urticae*?

In social biology, the genetic relatedness plays a key role in the organization and functioning of social groups of many species. Living with kin could thus be a strategy to increase inclusive fitness by favouring development and survival of individuals closely related. According to the optimal foraging theory, individuals attempt to maximize the energy gained and/or minimize the time spent getting a fixed amount of energy (Charnov, 1976).

T. urticae is a group-living species. We showed previously, that an isolated individual requires conspecifics, and more particularly kin to increase its fitness.

We showed also that the presence of silk is an indicator of the presence of conspecifics: finding silk increases the probability to find conspecifics. Therefore, finding silk from kin might decrease the locomotor activities favouring settling and, on the contrary, a place without silk or with non-kin silk may trigger the exploration of the environment (statex and movement in *T. urticae*).

The locomotor activities on sites recovered by silk produced by related and unrelated spider mites in *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract

The two spotted spider mite *Tetranychus urticae* is a silk producer known to live in group in a common silk web that can cover entire plants and protect mites against predators, rain and wind. Silk also plays an important role during collective migration by ballooning or by walking. In this context, we studied the locomotor activity i.e. time in movement, in resting and in static exploration of an individual confronted by clean places and/or places recovered by silk coming from its own population or from another one. Two populations were tested, one from Belgium (green form) and one from Tunisia (red form). The experimental results showed behavioural differences between the two populations: the individuals of the green form spend more time moving and less time resting than individuals of the red form. The presence of silk affected the locomotor activity of *T. urticae*; the red form was able to modify its environment in such a way that both forms spent more time on the place covered by the silk woven by red individuals. This surprising result might have been due to a difference in the quantity and/or quality of silk woven by the two forms. Our hypothesis is that, by spending longer time on the silk woven by one of the two populations (the red form) related and unrelated mites can join their forces to reinforce the silk nest. This might be a strategy to rapidly increase the population of the colony, then more rapidly build a protective web.

Keywords: *Tetranychus urticae*, kin discrimination, silk, locomotor activity

Introduction

Silk is known to be produced by many Arthropod species in diverse phylogenetic groups from spiders or pseudoscorpions to insect including Diptera, Lepidoptera, Hymenoptera and Trichoptera insects (Craig 1997; Selden 1989; Shear *et al.* 1989). There are many kind of silk with a large diversity of properties and chemical constituents (Craig, 1997). Silk has many roles, from web building, protection and thermal regulation to communication between individuals (Bernard and Krafft, 2002; Yano, 2008). Silk thread laid by an individual can also be used as a trail followed by other conspecifics as shown in social spiders and caterpillars (Fitzgerald, 1983; Saffre *et al.*, 1999; Ruf *et al.*, 2001). These silk laying and following behaviours may trigger and amplify a recruitment process by creating a positive feedback mechanism. In social spiders, recruitment processes can be mediated by the use of silk draglines (Saffre *et al.* 1999; Mailleux *et al.*, 2008). For instance, during collective displacements, each individual of the social spider *Anelosimus eximius* contributes to the growth of a webbing structure through the addition of its own dragline. The opportunity for an individual to use a predecessor's dragline as a shortcut causes an amplification process. In this context, silk draglines laid by social spiders can be seen as trail marking and trail following usually associated with eusocial insects (e.g., ants and termites). Therefore, a network of silk can be created and can be directed toward one side that favours group formation and cohesion (Lubin and Robinson, 1982; Aviles, 2000; Jeanson *et al.*, 2004; Mailleux, *et al.*

2008).

T. urticae is phytophagous mite that produces silk from salivary glands and excretes it at the pedipalp level while walking. All developmental stages are concerned (Saito, 1977). Mites live in a common web that can cover entire plants and that protects them against predators (Helle and Sabelis, 1985; Hoy and Smilanick, 1981) or competitors (Van Impe, 1985, van Impe and Hance, 1991), against abiotic agents like humidity (Hazan *et al.*, 1975), wind (Davis, 1952), rain (Linke, 1953) or even acaricides (Davis, 1952; Linke, 1953). As the quantities produced per individual are very low, building a common web requires an important number of conspecifics laying silk threads at the same place and time.

This implies a kind of cooperative behaviour such as common web spinning (Le Goff *et al.* 2010). In spider mites such as *T. urticae*, this silk can also be used as a vector of locomotion and favours group formation and cohesion (Saito, 1977; Yano, 2008). Indeed, females are able to follow the silk trails (on which chemical cues are present) left by preceding females (Yano, 2008). This behaviour induces an amplification process, resulting in aggregation at a new colony.

Previous studies show strong differences in the probability to follow a silk dragline between individuals coming from different populations of the same species in social arachnids (Evans, 1999; Mailleux *et al.* 2008). They show that the probability to follow the silk secreted by individuals coming from another population is lower than the probability to follow the silk secreted by genetically close individuals. Therefore, the presence of individuals coming from

different genetic pool reduces the cohesion of the swarm groups during the settlement process and thus increases the spatial dispersion of spiders (Mailleux *et al.* 2008).

In species dispersing partly or predominantly by walking, locomotor activity can be an indicator of their tendency to leave an unsuitable environment, and/or their ability to find new food resources and/or conspecific aggregates. Here, our aim was to observe and compare the locomotor behaviour of *T. urticae*, i.e. time in movement, in resting and in exploration placed on a site with or without silk. Previous papers (Le Goff *et al.* 2010) showed that, in this species, living in groups increased silk production, fecundity and survival. This implies that individuals can perceive the presence of their conspecifics through direct and/or indirect interactions. (In)direct perception through chemicals (pheromones) or tactile perception of conspecifics might constitute indicators of group size (Prokopy & Duan, 1998; Prokopy & Reynolds, 1998). Our assumption is that the presence of silk should be an indicator of the presence of conspecifics for *T. urticae*, which could increase the probability of finding conspecifics and food. In this scenario, the presence of silk should decrease its locomotor activities while a place without silk may trigger environmental exploration.

Materials and methods

Mites

The red form of *T. urticae* was provided by Dr. Kaouthar Lebdi Grissa (Institut National Agronomique, Tunis, Tunisia), whereas the green form was collected in the green-houses of our laboratory in Louvain-

la-Neuve, Belgium. The most accepted taxonomical position does not recognize *T. cinnabarinus* as a valid species as in the World Catalogue of the Spider Mite Family (Bolland *et al.* 1998), which cites it as asynonym of *T. urticae*. The two populations were reared on bean leaves placed on damp cotton (*Phaseolus vulgaris* L.) in Petri dishes. Breeding stocks were maintained in a climate room at 26°C, 50–60% RH, and 16:8 (L: D).

The genetic distance between the two forms used in this experiment was evaluated in a previous study using microsatellites. Both populations were considered inbred (FIS red form population confidence interval (CI): 0.47 (0.32-0.58) and FIS green form population: 0.26 (0.15-0.30) but were genetically distinct for all tested loci ($F_{ST} = 0.47$, Nei distance=0.95) (Le Goff *et al.*, unpublished data).

Experimental set-up

Experiments were undertaken under the same environmental conditions as rearing. A microscope circular cover glass (ø15 mm) was divided into two equal areas by a piece of wet paper (1 mm in width). A virgin female (called a weaver female) aged less than 24 h from the red or the green population was allowed to walk on the free part of the microscope cover glass. Over 30 minutes, it deposited silk trails (and possibly pheromones and faeces) (Saito 1977); no eggs were laid during that period. Then, the wet paper was removed and the set-up was dried for 30 minutes at 26°C.

We tested three treatments: 1) half glass with silk from the red form (RSP) and the other half free of silk (CP), 2) half glass with silk

from the green form (GSP) and a half free of silk and 3) half glass with silk from the red form (RSP) versus the other half with silk from the green form population (GSP).

For each treatment, a virgin female (called tested female) of less than 24 h from the red form or the green form population was placed at the centre of the cover glass after the set-up has been dried and its behaviour was video-recorded for 30 minutes. Twenty replications were made per treatment.

The silk part was alternated on the right or on the left side of the set-up between two experiments. The same was done for the three treatments to avoid any environmental bias.

Four behavioural parameters were recorded in the tested females: (1) the total time spent on each part; (2) the time in movement; (3) the resting time; and (4) the time in static exploration ("statex"). Static exploration is a behaviour characterised by individuals staying at the same place but turning on themselves, probably to probe their environment (Le Goff unpublished data). The durations of time in movement, resting time and time in statex were represented by their absolute value and by their mean relative proportion on each part of the set-up. The relative proportion was calculated by dividing the time (time in movement, in resting or in statex) spent on one part of the set-up by the total time spent on that part (sum of the times).

Statistical tests

To provide evidence for preference for the silk part or for the clean

part for the three treatments (RSP vs. CP, RSP vs. GSP, GSP vs. CP), we subtracted for both populations the absolute value of the total time spent on the clean part to the absolute value of the total time spent on the silk part. In the third treatment, when the tested individual was confronted to two silk parts (RSP vs. GSP), we subtracted the absolute value of the total time spent on the green silk part to the absolute value of the total time spent on the red silk part. If the mean result was significantly positive, mites spent significantly more time on the silk part. On the contrary, if the mean result was significantly negative, mites spent significantly more time on the clean part. The third possibility was that the mean result was statistically similar to zero, in this case, it meant that the mites did not spend more time either on the silk part or the clean part.

To test the influence of the genetic origin of the weaver female and of the tested female on the locomotor activity of *T. urticae* (time in statex, in resting, and in movement), we performed a two-way MANOVA to compare the relative proportion of two behavioural items (resting and movement) among modalities. Indeed, as the sum of the three behavioural items is always 100%, testing only two behavioural items will automatically include the result of the third behavioural item.

Tests and graphs were performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com) and R version 2.8.0 (© R Foundation for Statistical Computing, 2008). All tests were applied under two-tailed hypothesis and the significance level was set at 0.05.

Results

Red females: The silk woven by red females significantly influenced the total time spent by other red females on each part of the set up. The total times spent on the red silk part minus the times spent on the clean silk part was significantly positive (RSP vs. CP: lower 95% confidence interval (CI) of mean= 9.61, upper 95% CI of mean= 874.40). The total times spent on the red silk part minus the times spent on the green silk part was significantly positive (RSP vs. GSP: lower 95% CI of mean=8.90, upper 95% CI of mean= 876.40). Red females were not influenced by the green silk (GSP vs. CP: lower 95% CI of mean=-463.70, upper 95% CI of mean= 473.30) (figure 1).

Green females: the total time spent on the green silk part with silk from their own population minus the one spent on the clean/red silk part were statistically similar to zero (GSP vs. CP: lower 95% confidence interval (CI) of mean= -606.50, upper 95% CI of mean= 268.30; RSP vs. GSP: lower 95% CI of mean=-291.30, upper 95% CI of mean= 551.10). Contrary to red females, green females seemed to be attracted by the red silk part as the difference of the time spent on the red silk part and the time spent on the clean part was significantly positive (RSP vs. CP: lower 95% CI of mean=5.72, upper 95% CI of mean= 431.50) (figure 1).

Two-way MANOVA showed that the genetic origin of weaver individuals did not influence the relative proportion of each behavioural item either for the red or the green population ($F=0.25$,

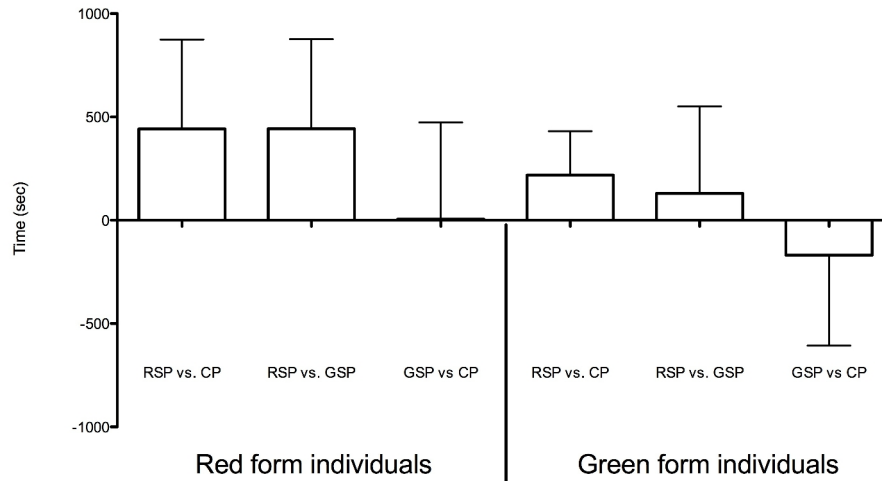


Figure 1: Mean differences between the total time spent on the silk part and the total time spent on the clean part (RSP vs. CP and RSP vs. GSP) and between the total time spent on the red silk part and the total time spent on the green silk part (RSP vs. GSP) for both red and green individuals. Error bars indicate the confidence interval (95%)

Table 1: Mean relative proportion of time spent in statex, resting and in movement on the whole set-up for each modality (RSP vs. CP, RSP vs. GSP, BSP vs. CP) for both Tunisian red form and Belgian green form individuals. RSP= Red form Silk Part, BSP= Green form Silk Part and CP=Clean Part

		Statex (mean± CI)	Resting (mean± CI)	Movement (mean± CI)
Tunisian red form individuals	RSP vs CP	13.35± 2.84	39.65± 10.03	47.01± 9.77
	RSP vs GSP	13.67± 3.57	39.98± 8.96	46.36± 8.06
	GSP vs CP	11.94± 4.10	40.03± 11.89	48.30± 10.29
Belgian green form individuals	RSP vs CP	16.51± 3.30	9.09± 3.18	74.41± 17.63
	RSP vs GSP	13.22± 3.13	26.43± 14.60	60.36± 14.03
	GSP vs CP	15.51± 3.93	25.73± 14.00	58.76± 13.09

df=1, $P=0.91$). Furthermore, the same two way MANOVA also showed a clear difference between the two tested populations ($F=11.12$, df=1, $P<0.001$). Indeed, for the three treatments the red form individuals spent less time in movement and more time resting than individuals of the green form but this difference was statistically significant only for the first treatment (table 1). Red tested females spent approximately 45% in movement, 40% in resting and 15% in statex, whereas green tested females spent approximately 65% in movement, 20% in resting and 15% in statex.

Discussion

T. urticae is a silk producer and its web confers substantial advantages such as dispersal, colony establishment, mate finding, protection against natural enemies and climatic conditions. Furthermore, *T. urticae* presents several aspects of an elementary social organisation such as kin discrimination capability (Roeder *et al.*, 1996). Our study aimed to test whether *T. urticae* could recognise the silk produced from its own population.

First, our study confirms that tested mites spend more time on a place covered with silk (Yano 2008, Le Goff *et al.* (unpublished data). Indeed, both populations spent more time on the part of the set-up covered with the silk laid by the red populations. As expected, the red form spent more time on the silk that its related conspecifics laid. A surprising result was that the tested females of the green form spent more time on the silk part covered by weaver females of the red

population, but not on the silk part covered by weaver mites from its own population. This surprising result might be explained by two non-exclusive hypothesis: differences in the quantity and/or quality of silk or differences in other products (other than the silk) laid by the red populations that could cause different behavioural responses in tested mites.

In 2008, Yano showed that the attraction of *T. urticae* for silk increased with the quantity of silk laid (Yano 2008). This might explain why the tested females from both populations spent more time on the red silk part, as weaver mites from the red form population produce more silk than green form individuals. However, our data showed some differences between the two tested populations. Indeed, the green and the red form present different locomotor activity patterns on similar set-ups and therefore different patterns of silk production. Here, we show that individuals of the green form population move longer and rest lower than individuals of the red form population. If the secretion of the silk was continuous during walking (Saito, 1977), the green form population should produces more silk than the red form population as it walked longer periods. For a similar period of time in movement, an individual walking faster should produce more silk than another individual. In this case, the difference in attractivity observed during our experiments should be linked to other properties of the silk (qualitative and/or quantitative). Time in movement is not the only indicator of locomotor activity that can inform us on the silk production of *T. urticae*. Testing an individual's locomotor activity in each population and more particularly the speed on set-ups without

silk could allow us to quantify the covered distance and therefore to estimate the link between silk production and displacement.

Another hypothesis is that the silk laid by the green form differs qualitatively from the silk laid by the red form and presents physical and/or chemical properties disfavouring displacements. Indeed, we can imagine that, as the secretion of silk is a continuous process (Saito, 1977), a more sedentary mite (red form) might produce thicker silk that is a substrate favouring displacements while a mite more mobile (green form) might produce thinner and stretched silk that complicates displacements. Chemical compounds laid on the silk may also explain why mites prefer to spend more time on the silk laid by the red form. Indeed, the red form seems to present a higher level of sociality with a higher propensity to live in groups (higher silk production, higher survival rate), than the green form, which is penalised by group living (lower number of eggs) (Le Goff *et al.*, unpublished data). One can guess that a more effective chemical communication has been selected in the red form population with a higher quantity or a higher persistence of this pheromone on the silk than in the green form population. Isolating these chemical compounds and testing their attraction on several populations could also be an interesting next step for this study.

In conclusion, we show that one form of mites is able to transform its environment in such a way that both forms spent more time on its silk. This surprising result, if it is confirmed by other studies, might improve the probability of colony survival. As related and unrelated mites spend longer period of time on the silk woven by

one population, they can reinforce the nest and lay eggs. This might be a strategy to rapidly increase the population of the colony and to more rapidly build a protective web. Such a phenomenon has already been observed in social spiders, where the absence of antagonistic behaviour towards non kin can increase the growth rate of the colony and can increase its probability of survival (Aviles and Tufino, 1998; Evans, 1999). In spider mites, this ability of the silk to attract individuals from another population has been observed in only one of the two tested populations. Therefore, this strategy cannot be extrapolated to the whole *T. urticae* species. However, it could be interesting to see if this strategy is a common one among *T. urticae* by testing other populations with the same set-up.

Currently, we do not know which properties of the silk (qualitative and/or quantitative) differs as a function of the weaver population. For example, testing an individual's locomotor activity from each population on set-ups without silk should allow us to test whether mite behaviour is modified by their own silk production. Moreover, studying the kin recognition system of *T. urticae* could be an interesting next step to understand these social interactions. *T. urticae* is a species in which tactile stimuli are very important (Oku *et al.* 2005). Bostanian (1973) described chemoreceptors and mechanosensory/olfactory receptors on the two first walking legs of *T. urticae*. Therefore, tactile contact could be a way to detect and identify other individuals as the cues they have laid, including silk trails.

Acknowledgements

We are very grateful to Pr. Kaouthar Lebdi who supplied the red form of *Tetranychus urticae*, which was used in our experiments and to George van Impe for the useful discussions about *Tetranychus urticae*. We would like to thank all the team of the “Laboratoire d’écologie et biogéographie” for their help in data analysis. G.J. Le Goff is supported by a Grant from the FRIA (Fonds pour la Formation de la Recherche dans Industrie et l’Agriculture). The authors are also indebted to the National Fund for Scientific Research (FNRS, Belgium) for funding through the Fund for Fundamental and Collective Research (FRFC, convention 2.4622.06). AC Mailleux is financially supported by IRSIB (Institut d’encouragement de la Recherche Scientifique et de l’Innovation de Bruxelles).

General discussion

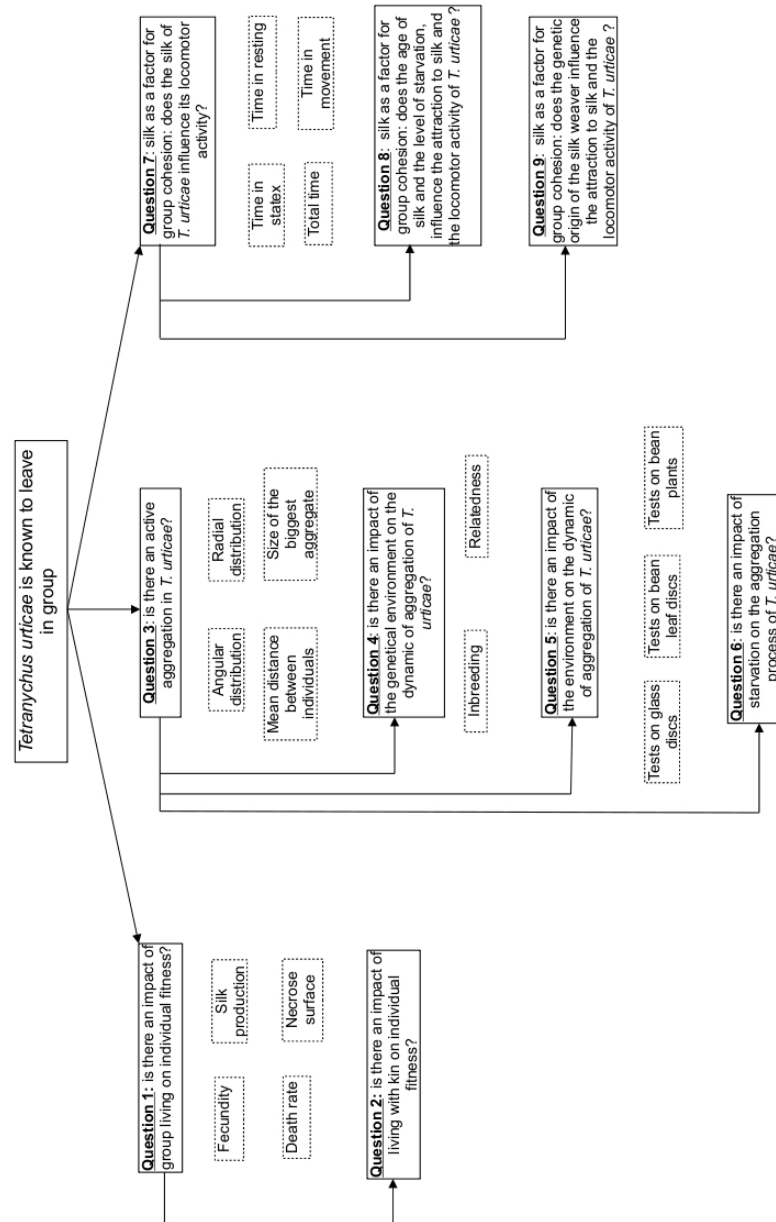


Figure 6: Flow chart of the PhD thesis, showing the different steps of the study

In this PhD thesis, we have studied aggregation and cooperation mechanisms in the phytophagous mite *Tetranychus urticae*. Our main questions were threefold:

- 1) Is there an impact of living in groups on individual fitness when compared to living alone?
- 2) Does *T. urticae* exhibit aggregative behaviour?
- 3) Does silk production of an individual influence the spatial distribution and the locomotor activity of its conspecifics?

These main questions were subdivided in nine subquestions as presented in the flowchart (Figure 6).

We begin this discussion section, by briefly answering these nine subquestions. Then, we summarize the results to answer the three main questions, and finally we discuss the factors that modulate the social organization of *T. urticae* (genetic proximity, starvation, population).

What did we find? The answers of the major questions of this thesis

Question 1: Is there an impact of group living on individuals' fitness?

Under our experimental conditions, living in groups increased silk production, fecundity and survival for the red form of *T. urticae*.

Question 2: Is there an impact of living with (non) kin on individuals' fitness?

The level of genetic relatedness between individuals was varied by testing two different populations of *T. urticae* separately: a red form population (Tunisia) and a green form population (Belgium). Afterwards, the number of eggs and white faecal pellets were used as indicators of individual fitness in groups of ten individuals from the same population or from a mixture of the two populations. This method was designed to test whether interactions between individuals from different populations/genetic pools influence the social organization of *T. urticae*.

We predicted that if the genetic origin did not influence other conspecifics, in groups composed of individuals coming from two different populations, we should obtain intermediate values of number of eggs and white faecal pellets between those of groups composed by individuals from the same population. In our case, when individuals

coming from two different populations were mixed, the mean number of white faecal pellets in mixed groups was statistically similar to the value obtained in the green group (the weakest one). Therefore, living with non kin affected some aspects of the behaviour. Moreover, these results might be evidence of kin recognition in *T. urticae*, because there was a discriminative response for the white faecal pellet production.

Question 3: Is there active aggregation in T. urticae?

The aggregation behaviour of *T. urticae* was tested under several conditions: glass or bean leaf disc, and bean plants. In all situations tested, aggregations of mites were observed. The fact that the spider mites aggregated even in a homogeneous environment (on glass disc) suggests that aggregation results from the interaction between individuals, in addition to a common attraction to an environmental cue.

Question 4: Is there an impact of the genetic environment on the dynamics of aggregation of T. urticae?

The genetic relatedness of *T. urticae* was varied using two methods. The first method consisted of creating inbred lines and therefore, testing the impact of inbreeding on each line in each generation on *T. urticae*'s aggregative behaviour. For the second method, we used the same method as for the impact of living with

(non) kin on individuals' fitness (question 2).

Concerning the impact of inbreeding on the spatial distribution of *T. urticae*, on bean leaf discs after 4 generations of sib-mating, the mean distance between *T. urticae* individuals increased by about 1.6 mm; which is three times the length of an individual.

Concerning experiments with mixed populations, we predicted intermediate values of aggregation between green and red individuals assuming that mites were indifferent to the genetic origin of their conspecifics. In fact, in experiments on glass and bean leaf discs, results of mixed groups were always statistically similar to those of the green groups.

Question 5: Is there an impact of the environment on the dynamic of aggregation of T. urticae?

The aggregation behaviour of *T. urticae* was tested on three different kinds of substrate, from the simplest and most artificial substrate to the most complex and natural one. (1) Our aim was to observe the influence of conspecifics on their spatial distribution in a homogeneous environment (glass discs), (2) in a heterogeneous environment (bean leaf discs with food and leaf heterogeneity (nerves), and finally (3) at the plant scale (whole bean plants).

On bean leaf discs, aggregation was lower than on glass discs. Furthermore, on glass discs, aggregations were more concentrated at the border of the set-up, than on bean leaf discs. A recruitment process was observed during plant infestation but we cannot conclude that this

resulted from the influence of other individuals or to plant heterogeneity (such as different petiole angular directions between the two sides of the plant). Two kinds of recruitment processes were observed: a vertical process leading to choice of level in the plant, and a horizontal process leading to choice of leaf and side of leaf.

Question 6: Is there an impact of starvation on the recruitment process of T. urticae?

The recruitment process was influenced by the level of starvation. However, starvation did not similarly influence all the tested populations. Actually, only the red form population was influenced by the starvation period, as it decreased the infestation rate and the recruitment process.

Question 7: Silk as a factor for group cohesion: does the silk of T. urticae influence its locomotor activity?

T. urticae was more attracted by a site covered with silk than a site without. However, silk did not affect the relative proportion of behavioural components, as these were constant: 75% in movement, 15% in resting and 10% in probing behaviour for both silk and clean substrates.

Question 8: Silk as a factor for group cohesion: does the age and the starvation level influence the attraction to silk and the locomotor activity of T. urticae?

Silk deposited 2 hours ago was less attractive to well fed individuals than freshly deposited silk. Starved individuals seemed to avoid silk: whatever the age of the silk, starved mites spent more time on clean parts of the substrate than the silk-covered parts. Regardless of the fact that starved individuals showed clear attraction to clean substrates, the relative proportion of each behavioural component was constant; 25% in movement, 65% in resting and 10% in probing behaviour for both silk and clean substrates.

Question 9: Silk as a factor for group cohesion: does the genetic origin of the silk weaver influence the attraction to silk and the locomotor activity of T. urticae?

The red form was able to modify its environment (by adding silk and probably pheromones) in such a way that it became more attractive for both forms, whereas the silk of the green form did not attract either of the two populations.

Advantage of group living for *T. urticae*

In many vertebrates and invertebrates showing some level of sociality, individuals reared in isolation exhibit differences in trait expression compared to those reared in groups of two or more (Prokopy and Duan, 1998; Prokopy and Reynolds, 1998; Rotem & Agrawal, 2003). For example, in the German cockroach, *Blattella germanica* (L.), females in groups had substantially larger oocytes than isolated females (Holbrook *et al.*, 2000).

Individuals from the red form population of *T. urticae* benefit from the presence of conspecifics, for instance through environmental modifications produced by an increase in silk and production of white faecal pellets. This was associated with a higher investment in egg production (number and volume). Producing a web is likely to entail a cost; mites that arrive on a leaf already covered with a protective web are expected to refrain from producing it (Oku *et al.*, 2009). These mites that spend less energy producing a web may then allocate more resources to other fitness-enhancing activities, such as laying eggs (Oku *et al.*, 2009).

Take home message:

T. urticae benefits from group living with an increase of its fitness parameters: fecundity, survival, faecal pellets, and silk production

Conditions for *T. urticae*'s aggregation

Aggregation is one of the most basic social features emerging from many different activities in social insects, spiders and mites (Deneubourg *et al.*, 2002). Indeed, the spatial distribution determines the level of interactions between individuals, and more generally the structure as well as the organization of populations (Deneubourg *et al.* 2007). Forming aggregates implies increased local population density compared to the density in the surrounding environment (Rivault *et al.* 1998; Ame *et al.* 2004). Aggregation of individuals may result from attraction between individuals (Allee 1931; Okubo 1980; Parrish and Edelstein-Keshet 1999, Camazine *et al.*, 2001) and/or attraction between individuals and environmental heterogeneity (Fraenkel and Gunn, 1961; Ceuster, 1977; Dambach and Goehlen, 1999; Depickere, 2004, Deneubourg *et al.*, 2007). The spatial distribution of individuals is thus very sensitive to environmental and social factors such as food resources or the presence of related individuals.

During experiments where mites were placed on glass discs, the absence of food forced individuals to disperse (Kondo and Takafuji 1985) and aggregations were formed mainly at the border of the set-up. This may indicate a high propensity of *T. urticae* to trace irregularities of the substrate (e.g. by thigmotaxis) which might result in increasing the probability to meet other individuals and to form aggregates. A similar mechanism probably triggers the movement of mites from an old exhausted plant eaten by a colony of *T. urticae* to a fresh and unexploited plant. The individuals follow the irregularity of

the leaves (nerves, border, silk) and form silk balls at the top of the plant or of a leaf (Clotuche *et al.* 2011), which results in group dispersal through anemochory or zoochory (Huffaker *et al.*, 1969, Kennedy and Smitley, 1985; Yano, 2004).

The low level of aggregation of mites placed on bean leaf discs may arise from avoidance of other individuals and therefore, to a mechanism limiting the competition for food and space between relatives (Lambin *et al.* 2001, Charlesworth and Charlesworth 1987, Stow and Beattie 2008; Ridley and Sutherland 2002). Furthermore, the presence of food probably decreases the probability of moving and therefore, also decreases the probability to meet conspecifics and slow down the dynamics of aggregation.

Our results clearly indicate that a recruitment process occurs in *T. urticae* and is influenced by plant heterogeneity and/or the presence of conspecifics (probably through silk production). Therefore, two factors influencing the level of settlement in *T. urticae* are geotropism/phototropism and recruitment process. Consequently, a large amount of individuals is found on the same leaf. We suppose that collective migration offers them the possibility to form aggregates, which gives several advantages to individuals (Rivault *et al.*, 1998; Ame *et al.*, 2004). In general, forming such aggregates has many advantages both for the individual and the group (see introduction). It also gives the opportunity to establish new colonies and therefore, to collectively and rapidly construct large webs efficiently protecting all members from predators (McMurtry *et al.*, 1970), wind and rain (Davis 1952, Linke 1953).

Take home message:

1) The aggregation of *T. urticae* is influenced by inter-attractions between conspecifics.

2) The aggregation of *T. urticae* depends on environmental conditions:

- Absence of food: high level of aggregation
- Presence of food: low level of aggregation

There is probably a trade off in *T. urticae* between the benefits of aggregation and the competition for food patches.

3) A recruitment process is observed in *T. urticae* that can be due to plant heterogeneity and/or the presence of conspecifics.

4) In natural conditions, on a plant, the choice of the settlement place probably depends of two main factors: the geo/phototropisms and/or some recruitment processes.

Silk as a factor for group cohesion

The silk produced by Tetranychidae is a key element to explain the social evolution of some silk-weaving species. Previous studies have shown the importance of the web as a shield to protect the colony (McMurtry *et al.* 1970, Davis 1952; Linke 1953, Ashley 2003). Our hypothesis in this study was that the silk produced by *T. urticae* is a factor of group cohesion and therefore plays a great role in its social evolution. Yano (2008) showed that the silk is probably a vector for chemical communication among *T. urticae* individuals. Therefore, a combination of the physiological, ecological, and social

conditions may influence the behaviour of *T. urticae* toward silk threads.

We observed that *T. urticae* was more attracted to a substrate covered with silk than by a silk-free substrate. Silk and pheromones represent cues of conspecific presence and/or a trail to follow to find a colony (Yano 2008). Our assumption is that a stigmergic mechanism is involved in the *T. urticae* regulation of the collective web. Stigmergy refers to a class of mechanisms that mediate animal–animal interactions (Theraulaz and Bonabeau 1999). The principle is that the trace left in the environment by an action stimulates the performance of a next action, by the same or a different agent. The stigmergic effect of the silk from *T. urticae* can result from chemical cues or the physical path and may lead to aggregation.

Take home message:

The silk production of *T. urticae* plays a role in the aggregation behaviour and its group cohesion probably through a stigmergic mechanism.

Influence of the genetic proximity

The genetic relatedness between the individuals of a group seems to play a great role in the social organization of *T. urticae*. In our case, four generations of inbreeding increased the interindividual distances to about 1.6 mm, corresponding to three times the size of an

individual. The differences between generations suggest that, in the field, inbreeding should increase the tendency to leave the patch or, at least, should increase the inter-individual distance. What could be the benefit of such avoidance of close relatives? Increasing inter-individual distance may decrease competition for food, mates and space between relatives (Lambin *et al.* 2001, Charlesworth and Charlesworth 1987). The trend to follow the leaf border also increases with inbred generations. In the field, this increased thigmotaxis might lead mites to leave the leaf, follow the branches, and disperse throughout the plant.

When we mixed individuals from two different populations, we expected that, without any influence of the genetic environment, the values of the factors measuring aggregation and group effect should be intermediate between those of groups composed of individuals from the same population. On the contrary, results of mixed groups were always statistically similar to those of the green groups for aggregation and faecal pellet production. In fact, by mixing the two populations, the probability to encounter a non-relative increased and decrease the effect of relatedness between red individuals. This result might also be due to the coexistence of two different locomotor or aggregation strategies of the red and green form populations. The green population might influence and trigger the displacement of the red population, resulting in aggregation and a group effect similar to the one observed in individuals of the green population.

Although *T. urticae* is able of kin discrimination (Roeder *et*

al., 1996), no segregation between the two populations under test occurs in mixed population experiments (glass/bean leaf and bean plant). In a similar context, in the cockroaches *Blatella germanica*, a low interpopulation attraction can lead to the formation of a mixed aggregate similar to a one-population aggregate (Amé *et al.* 2004). Possibly, there is a trade-off between the degree of relatedness of individuals in a group and the ratio of benefits to costs it brings. Living closely and reproducing in an endogamous population may increase individual fitness (see below). On the contrary, it can also have negative impacts on the fitness of relatives because of inbreeding (Helle, 1965). Furthermore mating with mites from other populations may increase genetic diversity.

Take home message:

Genetic proximity influences the spatial distribution of *T. urticae* and therefore also influences the group effect.

There is probably a trade-off between the degree of relatedness of individuals in a group and the ratio benefits/cost it brings.

A kin recognition ability may occur in *T. urticae*.

Influence of starvation

Starved individuals from the red form population seem to avoid silk. Our assumption is that starved mites leave the silk-infested part to avoid competition and to find new feeding sites. These individuals spin silk in these new sites and attract fed individuals spinning silk themselves which lead to a snowball effect that lasts until the complete plant is infested. In that scenario, the modulation of silk attraction by the level of satiation might, at least partly, regulate the process of migration on a new plant.

The silk seems to have an attractive effect when all individuals are well fed, and triggers a recruitment process that maintains group cohesion. These mechanisms of group cohesion observed in *T. urticae* when they are all well-fed decrease the probability of individual dispersal and might be one of the factors explaining its evolution to a social way of life.

Changes in the attractiveness of the silk with respect to starvation seem to have consequences in the recruitment process, as we observed a weaker recruitment for starved individuals than for fed ones. A decrease of silk attraction (or even a silk repulsion?) could decrease the silk trail following behaviour observed in *T. urticae* (Yano, 2008) and therefore could decrease aggregation. Indeed, it is reasonable to expect starved settlers to avoid overcrowded areas (Aviles and Tufino, 1998).

Starvation does not similarly influence all tested populations. Actually, the attraction to silk and the recruitment process are

influenced by the starvation period only in the red form population. Therefore, this strategy of avoiding competition through the presence of silk cannot be extrapolated to the species *T. urticae* as a whole.

Take home message:

Starvation influences the spatial distribution of *T. urticae*. By avoiding places covered with silk, individuals avoid competition for food patches.

This observation can not be generalized for the whole *T. urticae* species as it has been observed in one of the two tested population.

Population differences

In addition to the study of the social behaviour of *T. urticae*, this PhD thesis highlights striking inter-population differences in spatial distribution, group effect, and attraction to silk. To explain such differences between the two populations, we can refer to local selection theory. Indeed, as natural selection should favour the genotypes that are best able to use the information in their environment (Darwin, 1859), individuals of different local populations may have been subject to different local selection regimes (Maeda 2005). The green form is found in temperate zones (Belgium in our case), whereas the red form is Mediterranean and tropical (Tunisia in our case). Therefore, different parameters of the mites environment,

such as the plant nutrient content, or microclimate on certain parts of a host plant, might contribute to mite aggregations (Barron and Margolies, 1991) and reproductive strategies (Bonato and Gutierrez, 1999). Therefore, different local populations of *T. urticae* exposed to different local selection pressures might exhibit inter-population variations in their social behaviours and life-history strategies. Testing other green and red form populations would allow to confirm or to invalidate this hypothesis.

From an evolutionary point of view, these inter-population differences can be explained by different levels of genetic diversity within the groups. Indeed, there is a high level of inbreeding in both populations tested. This high level of inbreeding can be found commonly in the wild as a virgin female can found a colony by mating with her sons. In the field, highly inbred populations were found for spider mites (Hinomoto and Takafuji 1994), and can sometimes result in monomorphic populations (Carbonnelle *et al.*, 2007). Individuals of the red form seem to have a higher propensity to live in groups as they benefit from group-living (higher white faecal pellet/silk production, higher survival rate), whereas individuals of the green form pay a cost for group living (lower number of eggs). One possible reason is that the red form has a higher FIS; meaning that red form individuals are closer relatives than green individuals. According to Hamilton (1964), living with closely related individuals, increases indirect fitness of group members by sharing the benefits of group living. Therefore, the lower level of genetic diversity of the red form population can be a factor inducing a higher level of sociality. On the contrary, the green

form presents a higher level of genetic diversity and therefore a lower probability to exhibit social behaviours.

Finally, the specific status of the green and the red form of *T. urticae* females is not clear. Indeed, according to some authors, both forms should be considered the same species (Dupont 1979, Gotoh *et al.*, 1993, Navajas 1998), whereas other works suggest a differentiation (Goka *et al.* 1996; Hance *et al.* 1998; Zhang and Jacobson 2000; Sugawara *et al.* 2002). A possible evolution of these two forms toward two different species might also explain the many differences between the two forms/populations.

Take home message:

Differences between the red and the green form have three main explanations:

- local selection
- the level of inbreeding
- the specific status

About the method

Spatial distribution characterization

Concerning the characterization of *T. urticae* spatial distribution, several indicators were used (i.e. angular distribution, radial distribution, mean distance between individuals and size of the biggest aggregate). In the third chapter (Spatial distribution and

inbreeding in *T. urticae*), to quantify the spatial distribution of *T. urticae*, we used the mean distance between individuals, as an indicator of the inter-individual spatial proximity. In the fourth chapter (Where and with whom do I aggregate? The dilemma of *Tetranychus urticae*), we used the size of the largest aggregate.

The first step to quantify the spatial distribution of a group of individuals is to calculate the mean distance between individuals. It is a basic indicator of the inter-individual spatial proximity that takes into account the entire tested group. However, with this indicator, it is difficult to imagine how individuals are really distributed in their environment and is therefore, a generalist indicator of the spatial distribution. Therefore, we used the size of the largest aggregate as an indicator of the pattern of distribution. Aggregate size is an indicator more appropriate for the spatial distribution of spider mites than the mean distance between individuals and is commonly used to characterize the spatial distribution of eusocial (Depickere *et al.*, 2004, Sempo *et al.*, 2006) and non-eusocial insects (Jeanson *et al.*, 2005). Indeed, it shows that individuals are preferentially closer to some individuals than to others. This means that aggregation will only benefit part of the group. The calculation of this indicator needs to set a threshold distance that is chosen in function of the physiological and /or morphological criteria. In our case, this threshold distance was the maximal distance that allows an individual to detect another using tactile sensing. This threshold was commonly used as reference in the works studying aggregation (Depickere *et al.*, 2004, Sempo *et al.*, 2006, Jeanson *et al.*, 2005). Moreover, it gave to the best agreement

between naked-eye observation and automated analysis of video frames.

These two indicators represent two different steps of a spatial distribution quantification and they present both different drawbacks and advantages. The characterization of a spatial distribution is more completely described with several indicators.

Faecal pellet production as a fitness indicator?

Although *T. urticae*'s faecal pellet number was cited an indicator of silk production (Hazan *et al.*, 1971), this increase of faecal pellet production can be a disadvantage as it can increase the probability to be predated or parasitized (Poiani, 1992). It is common for predators and parasitoids to use cues from waste materials to locate their hosts or prey items (Weiss, 2006). However, in the case of *T. urticae*, faecal pellet production might present different advantages for both the individual and the colony such as:

- Humidity absorption (Hazan *et al.*, 1971)
- Reinforcement of the web structure (Le Goff *et al.*, 2010)
- Predator avoidance (Oku, 2008)

Conclusion and perspectives

This thesis contributes to the definition of the social status of

Tetranychus urticae, to the understanding of group-living mechanisms present in this species, and to their impact on individual fitness. We studied different aspects of the sociality of *T. urticae* e.g. spatial distribution, group effect, and finally communication among individuals. We now have a better understanding of *T. urticae* sociality and specifically more information on the influence of conspecifics, the starvation state, and the environment in its social organization. This study has revealed several secrets of the social life of this spider-mite but the main part remains a black box.

In this thesis, our research focused on the adult females of *T. urticae* mainly because they constitute the main part of a population (Saito, 2009). Furthermore, in our experiments, we mainly used virgin females as they behave with less variability than mated females. Therefore, by using virgin females, we tried to homogenize the behaviour of tested individuals, to avoid egg-laying among replicates and to avoid the impact of egg presence on *T. urticae* behaviour. However, the next step of this work should be to study the role played by others individuals of the colony to complete our knowledge of *T. urticae* social organization. For example, we might study the behaviour of mated females as they have higher egg production (Van Impe, 1985) and enter diapause sooner than virgin females (Popov and Veerman, 1996). We might also search for the possible influence of the mated females on the virgin ones. The study of the spatial distribution of eggs might also show a protective behaviour of females and a hypothetical female cooperation in their protection. In this context, the questions should be: Do females aggregate their eggs in

the same place or does each female lay eggs under her own web? Does the probability to aggregate eggs differ with respect to the status of females? Are there inter-individual differences in the persistence of aggregating eggs over time and generation (specialisation and division of work)? Furthermore, testing the role of males in the social organization of *T. urticae* could also be an interesting next step for this study.

Another interesting point to study is the role of faecal pellets in *T. urticae* society. Indeed, social arthropods face particular challenges because their feeding behaviour and/or site fidelity makes them vulnerable to problems associated with waste accumulation (Poiani, 1992), but successful animal societies have solved the problem of waste control. In 14 group-living (under web nests) Tetranychidae species of the genera *Eotetranychus*, *Oligonychus*, and *Schizotetranychus*, waste management behaviours occur (Saito, 1995). They appear to avoid soiling their living space or nests by defecating only at specific sites (Saito, 1983). Another way to manage waste would be to use it instead of eliminating it. For example, Oku (2008) discovered a defensive role of excreta in *T. kanzawai*. This mite uses its webs as a place for excretion and this study suggests that *T. kanzawai* deposits yellow pellets on webs to repel predators and therefore, to reduce the risk of predation. *T. urticae* also deposits its pellets on its web, therefore testing how the predators of *T. urticae* might be influenced by their pellets would be an interesting future study. In addition, studying the role (physical and chemical) of *T. urticae*'s pellets in their colony will help us to have a better

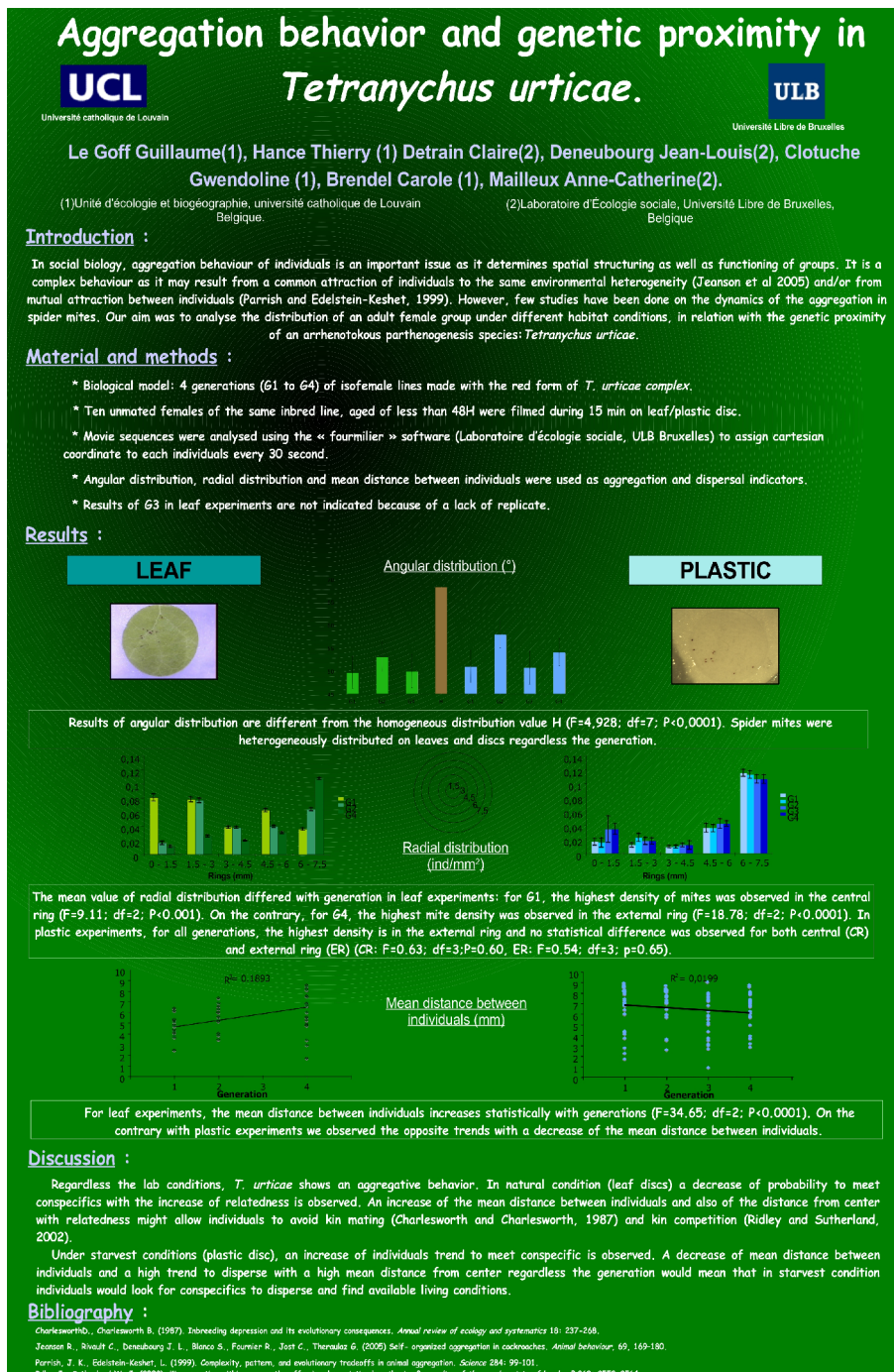
understanding of its social organization.

Another continuation of this project could also be the integration of this social parameters in management of this pest to protect cultural crops. Indeed, social behaviours are behind spatial distribution, dispersal movement, and invasions by the pest and, more generally, rule most of their life history traits. Social behaviours usually influence the immediate result of a control measure as individuals living in groups may initiate the compensative responses to the treatment more rapidly than solitary species. In turn, upsetting normal behavioural mechanisms of a social pest species (e.g., lure with aggregative/ dispersal pheromone) might be used as means of control. Therefore, studying social behaviour has great relevance to control a group living pest (Myllymäki 1975). The control of mites in greenhouses and fields can be arduous and complicated as they are covered with dense webs that protect them from acaricides. Furthermore, *T. urticae* is difficult to manage because of its ability to quickly develop resistance to acaricides (Van Leeuwen *et al.*, 2005). Mites are also difficult to eradicate because of the large number of eggs produced by individuals. One proposed technique might be the use of traps or baits loaded with mite pheromones that could attract adults, nymphs and larvae and to hold them in a place without a protecting web. A positive group effect evidenced in *T. urticae* strongly suggests that, if this method of control is implemented at a low frequency, it could fail because it should increase the local density and therefore, the rate of fecundity, resulting in larger populations that would be harder to control. From a biological control point of view,

the use of dispersing pheromones might be more efficient (Dicke *et al.*, 1986) except in greenhouses. Regular treatments of the crops to prevent its arrival or the maintenance of populations under economically acceptable thresholds might help to control these mites.

An important goal of biological control would be to obtain information on the impact of predators on the social organization of *T. urticae*. This may improve its biological control and might be the next steps of our study.

Annexes



Living in group enhances fertility, survival and web production in *Tetranychus urticae*



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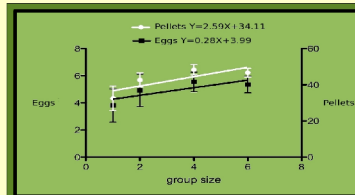
Living in groups modifies the physiology and/or the behaviour of individuals compared to isolated ones. This phenomenon, called "group/Allee effect" has been poorly studied despite its importance in the animal kingdom. *Tetranychus urticae* (Acari: Tetranychidae) is a worldwide pest of many agricultural crops. This species is characterised by a simple social organisation and displays some forms of cooperative behaviour such as aggregation and common web spinning. To evaluate the potential cost and benefits of such a group effect, we investigated the effect of group living on the parameters of life history such as fecundity, death rate and pellet production (=web production indicator).

MATERIALS AND METHODS

The fecundity, the survival and the white faecal pellet production (web production indicator) per individual were measured after five days. Virgin females were placed on bean leaf discs either **isolated** or **in groups** (groups composed of 2-6 individuals). **Density of individual per leaf surface unit was kept constant.**



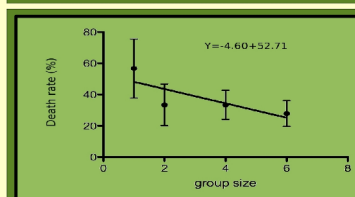
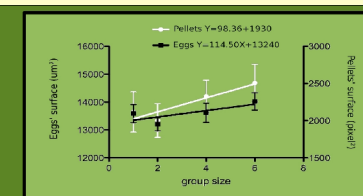
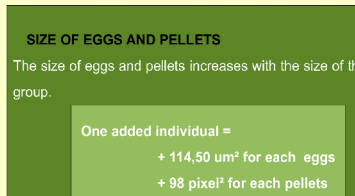
RESULTS



NUMBER OF EGGS AND PELLETS

The number of eggs and pellets increases with the size of the group.

One added individual =
+ 0.28 eggs per alive individual
+ 2.59 pellets per alive individual



DEATH RATE

The cumulative death rate decreases as the size of the group increased.

One added individual = - 4.60% of death rate

CONCLUSION

In *Tetranychus urticae*, living in groups increases fecundity, silk production and survival. This supports the view that group living triggers physiological changes in such a way that metabolism can become more efficient. This implies **grouped individuals make a better use of body reserves, survive longer and stabilize their environment**.

- more silk= more protection against external aggression (weather, predators, pesticides)
- more white faecal pellets might reduce variation of relative humidity, repel predators, stabilize the web structure

We assume that, under natural conditions with a higher group size, these group effects might have a stronger impact on the dynamics of the *T. urticae* population. However, in overcrowded conditions, the presence of conspecifics could have a negative impact on its population dynamics (negative group effect).

Modulation de l'attraction pour la soie chez *Tetranychus urticae* par le niveau de satiété et l'âge de la soie



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Tetranychus urticae est un acarien phytophage présentant certaines caractéristiques d'une **organisation sociale** (comportement d'agrégation, reconnaissance de parenté). La **soie** produite par cet acarien pendant ses déplacements, joue un rôle important dans son organisation sociale, notamment pour la construction d'une **toile commune**. C'est dans ce contexte que nous avons étudié l'influence de la soie sur son **activité locomotrice**.

MATERIELS ET METHODES

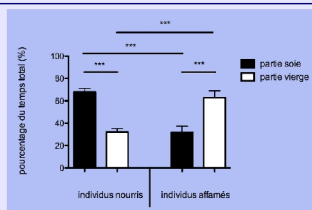
Une femelle de moins de 48h a été placée sur une lamelle de verre divisée en deux parties égales: **soie** vs **vierge**. Nous avons mesuré: le temps **total** ainsi que le temps passé en **mouvement** et au **repos** sur chacune des deux parties durant 30 minutes.

Avec ce protocole, nous avons testé l'influence du **niveau de satiété** (**nourris** vs **affamé**) et **de l'âge de la soie** (30 à 150 min) sur l'activité locomotrice.



Femelle rouge de *T. urticae*

RESULTATS



Attraction à de la soie fraîche (30 min)

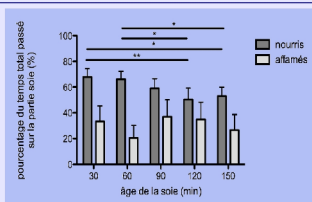
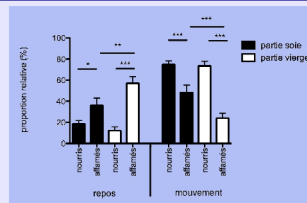
Individus nourris: plus de temps sur la partie soie

Individus affamés: plus de temps sur la partie vierge

Influence de la soie fraîche sur l'activité locomotrice (repos, mouvement)

Individus nourris: aucune influence de la soie sur l'activité locomotrice

Individus affamés: plus de temps au repos et moins de temps en mouvement sur la partie vierge



Influence de la fraîcheur de la soie sur son attraction

Individus nourris: attraction pour la soie de moins de 2h

Individus affamés: attirés par la partie vierge quel que soit la fraîcheur de la soie

DISCUSSION

• Une femelle nourrie passe plus de temps sur une soie fraîche (moins de 2h). La **distribution de *T. urticae* est donc régulée par des indices chimiques qui se dégradent avec l'âge de la soie.**

• La **toile commune** pourrait être le résultat d'un **mécanisme stigmergique**: les individus attirés par la soie des autres tisseraient leur toile à un même endroit.

• Une femelle affamée passe plus de temps sur la partie vierge, quel que soit l'âge de la soie, **le niveau de satiété va donc moduler cette attraction pour la soie.**

• La **soie est donc un indicateur de présence de conspécifiques**: fuir ou accepter la compétition en fonction de son état de satiété.

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